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Multi-modal Assessment of Newborns at Risk of Neonatal Hypoxic Ischaemic Encephalopathy – The MONItOr Study

Thesis presented by

Aisling Garvey MB BCh BAO MRCPI

https://orcid.org/0000-0002-8443-3246

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Department of Paediatrics and Child Health

Head of School/Department: Prof Deirdre Murray

Supervisors:

Prof Eugene Dempsey

Prof Deirdre Murray

Prof Geraldine Boylan

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Table of Contents

List of Figures

List of Tables

Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.

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Aisling Garvey

Date

Abstract

Background

Hypoxic ischaemic encephalopathy (HIE) is the leading cause of acquired brain injury in term infants. At present, therapeutic hypothermia (TH) is the only approved therapy for infants with moderate-severe HIE. However, it must be commenced before 6 hours of age resulting in a clinical challenge to resuscitate, stabilize, identify and stratify infants in this narrow timeframe. Furthermore, a significant proportion of infants with mild HIE will have neurodevelopmental impairment. Improved, timely identification of infants at risk of brain injury is required.

The aim of this study was to improve our knowledge of the early physiology of infants with HIE by describing the evolution of electroencephalography (EEG), near-infrared spectroscopy (NIRS) and non-invasive cardiac output monitoring (NICOM) in infants with all grades of HIE and to determine whether these markers may be helpful in the identification of infants at risk of brain injury.

Methods

This prospective observational study was set in a tertiary neonatal unit (November 2017-March 2020). Infants with all grades of HIE had multi-modal monitoring, including EEG, NIRS and NICOM, commenced after delivery and continued for up to 84 hours. All infants had an MRI performed in the first week of life. Healthy term controls were recruited after delivery and had NICOM monitoring at 6 and 24 hours of age.

In this thesis, I also included infants recruited previously as part of four historic prospective cohorts that had early EEG monitoring. These infants were combined with infants with mild HIE from the current prospective cohort to examine the difference in EEG features between infants with mild HIE and healthy term controls.

Results

Eighty-two infants were recruited in the prospective cohort (30 mild HIE, 25 moderate, 6 severe, 21 controls) and 60 infants were included from the historic cohorts.

This study identified significant differences between EEG features of infants with mild HIE and controls in the first 6 hours after birth. Seventy-two percent of infants with mild HIE had some abnormal features on their continuous EEG and quantitative analysis revealed significant differences in spectral shape between the groups.

In our cohort, cSO_2 increased and FTOE decreased over the first 24 hours in all grades of HIE regardless of TH status. Compared to the moderate group, infants with mild HIE had significantly higher cSO_2 at 6 hours (p=0.003), 9 hours (p=0.009) and 12 hours (p=0.032) and lower FTOE at 6 hours (p=0.016) and 9 hours (0.029). Beyond 18 hours, no differences were seen between the groups.

NICOM was assessed in infants with HIE and compared with controls. Infants with mild HIE had a significantly higher heart rate at 6 hours of age compared with controls (p=0.034). Infants with moderate HIE undergoing TH had a significantly lower cardiac output compared with mild HIE (p=0.046) and control groups (p=0.040). Heart rate was significantly reduced (p<0.001) but stroke volume was maintained and gradually increased from 6-72 hours despite TH.

Finally, we assessed the ability of EEG, NIRS and NICOM to predict short-term outcome (abnormal MRI +/- death in the first week of life). At 6 hours, none of the EEG, NIRS or NICOM measures predicted short-term outcome. At 12 hours of age, both qualitative and quantitative EEG features significantly predicted abnormal short-term outcome.

Conclusion

Identification of infants at risk of brain injury immediately after birth is challenging. Objective, early biomarkers are required. This is the first study to combine EEG, NIRS and NICOM in infants with all grades of HIE. Multi-modal monitoring is feasible and this thesis provides novel insights into the underlying physiology and evolution of injury in infants with HIE. Furthermore, it reaffirms the importance of early continuous EEG in HIE.

Abbreviations

HIE	Hypoxic Ischaemic Encephalopathy
APH	Antepartum Haemorrhage
ATP	Adenosine Triphosphate
DNGM	Deep Nuclear Grey Matter
TH	Therapeutic Hypothermia
EPO	Erythropoietin
EEG	Electroencephalogram
aEEG	Amplitude-integrated Electroencephalogram
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
SpO_2	Pulse Oximetry Oxygen Saturation
HR	Heart Rate
CP	Cerebral Palsy
RCT	Randomised Control Trial
BD	Base Deficit
NICU	Neonatal Intensive Care Unit
NIRS	Near-infrared Spectroscopy
SWC	Sleep-wake Cycling
SaO_2	Arterial Oxygen Saturation
rSO_2	Regional Tissue Oxygenation
cNIRS	Cerebral Near-infrared Spectroscopy
cSO_2	Cerebral Oxygenation
TOI	Tissue Oxygen Index

Hb	Haemoglobin
FOE	Fractional Oxygen Extraction
FTOE	Fractional Tissue Oxygen Extraction
BP	Blood Pressure
CPP	Cerebral Perfusion Pressure
MAPopt	Optimal Mean Arterial Pressure
CO	Cardiac Output
LVO	Left Ventricular Output
RVO	Right Ventricular Output
PDA	Patent Ductus Arteriosus
PFO	Patent Foramen Ovale
NICOM	Non-invasive Cardiac Output Monitoring
EC	Electrical Cardiometry
RBC	Red Blood Cells
ECG	Electrocardiogram
BR	Bioreactance
SV	Stroke Volume
HRV	Heart Rate Variability
DWI	Diffusion Weighted Imaging
PLIC	Posterior Limb of the Internal Capsule
NAA	N-acetyl aspartate

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Publications

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Oral Presentations

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1. Introduction

1.1. Overview and Pathophysiology of HIE

1.1.1. Overview

Asphyxia relates to a period of impaired gas exchange. Its subsequent progression to hypoxia, hypercarbia and metabolic acidosis is largely determined by the duration and extent of the lack of perfusion of the organ in question. Birth or perinatal asphyxia is a period of asphyxia in the perinatal period, often secondary to interruption in placental blood flow.

Perinatal asphyxia accounts for 6 per 1,000 live births. (1) Hypoxic Ischaemic Encephalopathy (HIE) refers to neonatal encephalopathy that occurs secondary to a period of asphyxia. (2, 3) HIE may be caused by any interruption in blood flow and/or oxygen supply to the brain of the foetus. HIE can be caused by antenatal insults secondary to placental or maternal issues such as infection, maternal hypertension or pre-eclampsia, or due to cord issues such as a nuchal cord or a knot in the cord. Peripartum events include placental abruption, shoulder dystocia, significant antepartum haemorrhage (APH) or cord accident and post-natal events include secondary apnoea. The severity of the subsequent condition and clinical features vary depending on the duration and severity of the sentinel event. (4)

Globally HIE accounts for 1-3 per 1,000 live births. (4) Despite significant advancements in antenatal and neonatal care, this figure remains static and infants who survive carry a significant risk of neurodevelopmental disability. (5) HIE is the leading cause of acquired brain injury in term infants (4) and is also one of the leading causes of disease burden worldwide. (6)

This chapter will discuss the pathophysiology of HIE and the assessment of an infant at risk of HIE along with currently available treatments and monitoring modalities.

1.1.2. Pathophysiology

Hypoxia relates to the insufficient amount of oxygen in the cells. In this state, cells are deprived of an adequate oxygen supply required to maintain normal metabolic processes. In utero, blood supply from the placenta supports homeostasis in the foetus in a relatively hypoxic environment. (7) The foetus can tolerate brief periods of mild hypoxia without developing encephalopathy or injury. (8, 9) During periods of reduced oxygen concentration, the foetus initially adapts to preferably maintain blood supply to critical organs such as the brain, heart and adrenal glands at the expense of peripheral organs. (10, 11, 12, 13, 14) Heart rate initially decreases but this is followed by an increase in both heart rate and blood pressure along with vasoconstriction of less important organs such as the gut, kidneys and skin. (9, 15) This succeeds in maintaining an optimum delivery of oxygen to the brain for a period of time.

If the hypoxia is prolonged or severe, the foetus responds in two ways. Firstly, the cells attempt to reduce energy consumption by reducing non-essential activity in a stepwise manner ultimately resulting in the suppression of neuronal activity if the hypoxia is severe. Secondly, there is a switch to anaerobic metabolism and a subsequent increase in lactic acid production and metabolic acidosis. In optimal conditions, glucose is the preferred substrate for energy metabolism as it is oxidised to produce 38 adenosine triphosphate (ATP) molecules. Without an adequate supply of oxygen, this process cannot be completed. Instead, a switch to anaerobic metabolism occurs in an attempt to maintain a sufficient energy supply. However, anaerobic glycolysis is inefficient as it can only produce two ATP molecules in addition to lactate. For this reason, energy sources quickly become depleted as energy demand exceeds supply with a corresponding increase in lactic acid.

Ischaemia relates to reduced blood flow and primarily results in decreased oxygen and glucose delivery. Suboptimal levels of oxygen drives a switch to anaerobic metabolism, compounded by the lack of substrate available to maintain an alternate energy supply and a reduction in lactate clearance.

When hypoxia and ischaemia occur together, ATP stores deplete rapidly with an accumulation of lactate. The initial protective increase in blood pressure and heart rate cannot be sustained and so hypotension and bradycardia ensue. (16) Pre-clinical studies demonstrate that the duration and severity of the hypotension is strongly correlated with neuronal loss and is more important than hypoxia alone. Significant injury is seen in foetal lambs exposed to prolonged severe asphyxia associated with one or more episodes of associated hypotension. (17, 18)

Within the brain itself, perfusion is not uniform and in times of hypoxia and ischaemia, blood is redistributed to protect areas with the greatest metabolic demand: the deep nuclear grey matter (DNGM) and brain stem. (19, 20, 21) The white matter therefore has limited ability to compensate and the junction points or borders between the three main cerebral arteries, the so-called “watershed” areas, are particularly vulnerable. This pattern of watershed injury is therefore typically seen in partial prolonged episodes of moderate-severe hypoxia ischaemia. (22) It is postulated that in acute, sub-total asphyxia, there is insufficient time for the brain to instigate this protective mechanism. (22, 23) Therefore, in severe abrupt insults, DNGM injury is the predominant pattern of injury seen.

Interestingly, and somewhat counterintuitively, it is not the hypoxia or ischaemia that causes the cell injury or death, rather it initiates a sequence of events that ultimately leads to cell injury and death. HIE is an evolving process which may be appreciated by considering the overlapping phases of acute hypoxic ischaemic (HI) injury, the latent phase, secondary phase and later tertiary phase.(Figure 1.1)

The acute HI injury refers to the initial insult or sentinel event of hypoxia and ischaemia as discussed above. Energy stores are rapidly depleted resulting in failure of the ATP-dependent transport pumps. Depolarisation of the cell membrane follows with a subsequent influx of calcium and sodium ions and thus water resulting in cellular oedema and necrosis. (24) The increase in intracellular calcium causes an increase in excitatory amino acids, free radical production and nitric oxide which

results in further neuronal injury and cellular DNA damage, which leads to apoptosis. (25, 26)

Following the ischaemic event, if circulation is restored, reperfusion of the cells resumes and cellular metabolism may begin to recover. Intracellular pH and phosphorus metabolites return to a normal level but brain activity remains suppressed. (27, 28) This is known as the latent phase and later coined the "therapeutic window". This latent phase typically lasts 6-12 hours however, its duration is inversely proportional to the severity of the initial insult. (29, 30) Therapeutic hypothermia (TH) targets this phase by intercepting the neurotoxic cascade of the secondary energy failure and will be discussed in detail later (Section 1.3). (30, 31)

A secondary energy failure occurs 6-48 hours after the initial insult. (32) Dysfunction of the mitochondria, inflammation and cytotoxic damage leads to the triggering of further apoptotic cascades despite a now stable pH. (33) The severity of this secondary energy failure correlates with long-term neurodevelopmental outcome. (27, 34) Several therapies targeting this phase are currently under investigation. The most promising include Allopurinol and Melatonin. The overarching mechanism of action for both include scavenging of free radicals thus preventing subsequent excitotoxic damage. Melatonin may have additional benefits in neuroplasticity by stimulating cell differentiation and proliferation. (35) Studies are currently underway to determine whether these may be helpful adjunctive therapies to TH.

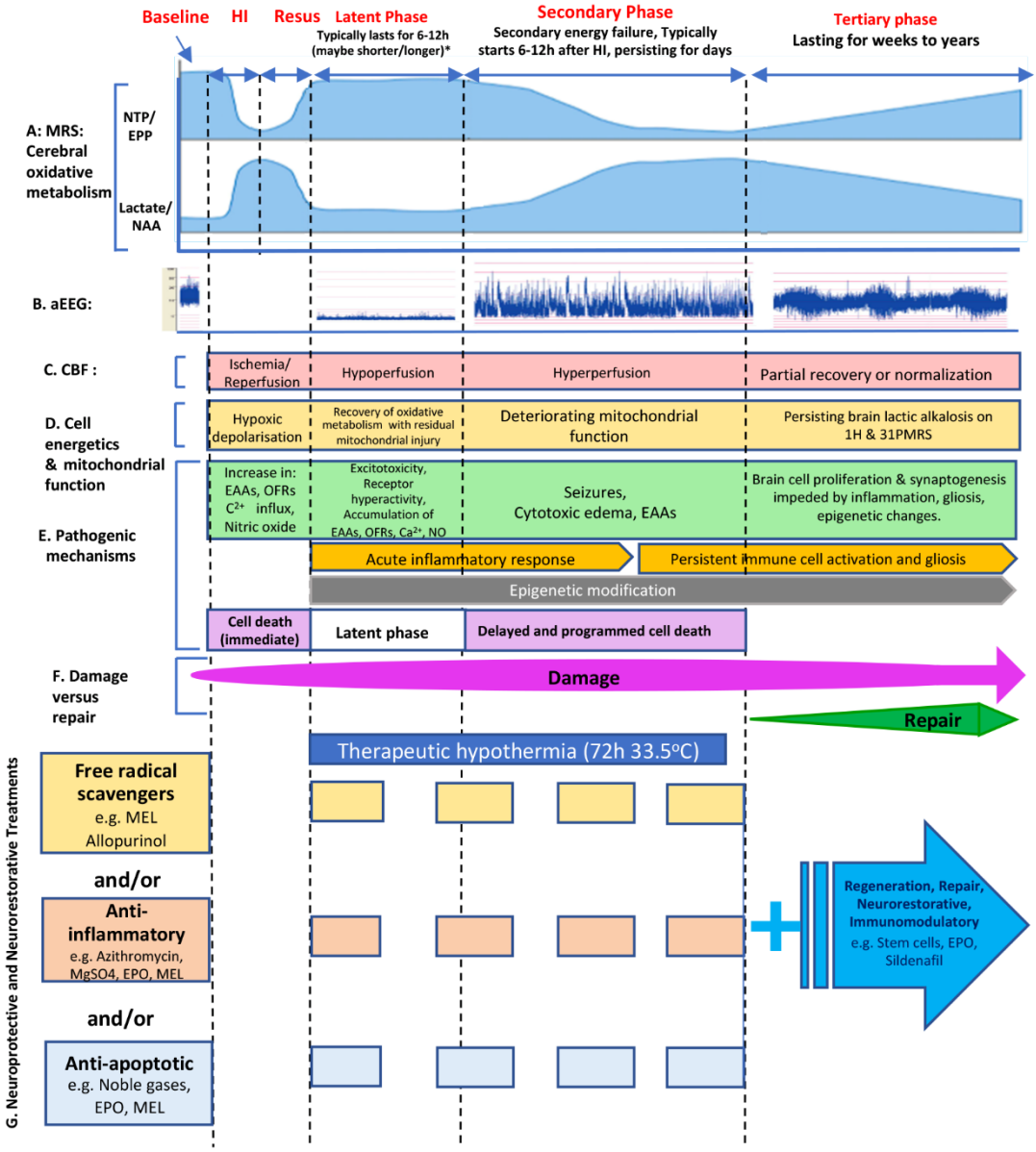


Figure 1.1: Schematic overview of the pathophysiology of HIE. Reproduced from Hassell et al. 2015 (36)

The tertiary phase describes a period of astrogliosis, progressive cell death and remodeling of the injured brain. (37) The evolution and sequela of the original injury may be evident for weeks to years after the acute phase and include, but are not limited to, altered gliosis and oligodendrocyte maturity, epigenetic modifications and persistent inflammation which may lead to a pre-disposition to seizures and sensitisation to further injury. (38) Clinicians have long been aware of the potential for rehabilitation in this tertiary phase to improve outcome through improved plasticity of the developing brain, however it is only recently that research focus has shifted to potential therapies targeting this phase. Erythropoietin (EPO) has anti-inflammatory, anti-apoptotic, anti-oxidative and neuro-restorative effects. (35) A recent systematic review has demonstrated a trend towards lower morbidity and mortality compared to placebo but not when compared with TH. (39) Pre-clinical models have shown that both TH and EPO independently improve outcome in HIE with TH being more effective. (40) The HEAL Trial recruited 500 infants receiving TH for HIE and randomised them to receive EPO or placebo. This study found no difference in the risk of death or neurodevelopmental impairment between the groups. This may be explained by their overlapping mechanism of neuroprotection. (41) Stem cell therapies, specifically human umbilical cord blood mononuclear cells and mesenchymal stromal cells, also target the tertiary phase. Stem cells are postulated to have anti-inflammatory properties along with increasing neurotrophic factors. They promote angiogenesis and the migration of T-cells to areas of injury. (35) Animal studies have demonstrated improved amplitude-integrated electroencephalogram (aEEG) recovery, magnetic resonance spectroscopy (MRS) markers and oligodendrocyte survival. (42) Several Phase I and Phase II trials are currently underway to determine the long-term efficacy of this therapy. (35)

As outlined above, HIE is a clinically evolving process, the management of which clearly depends on the severity and timing of the initial injury. HIE is typically classified into three categories: mild, moderate and severe. The original classification by Sarnat et al. assigned these grades based on clinical examination in combination with electroencephalogram (EEG) grading over the first 24-72 hours of age. TH is currently the only available interventional therapy and it is only advised for infants

with moderate and severe HIE if commenced before 6 hours of age. As a result, assignment of grade must now be evaluated very soon after birth (within the 6-hour therapeutic window). Current classification remains reliant on clinical examination, albeit now in a confined and clinically challenging timeframe. Practical issues surrounding the availability and access to EEG means that this classification is also often performed without the help of neurophysiological monitoring. These two issues result in significant methodological alterations to the original Sarnat classification system yet it continues to form the basis of all HIE management today. Current assessment methods, treatment and monitoring tools for infants with HIE will be discussed in the following sections of this chapter.

1.2. Clinical Assessments

In the clinical assessment of an infant following perinatal asphyxia, one of the most important assessments is the documentation of the infant's condition at birth and response to initial resuscitation. The oldest and most commonly used method of evaluating the newborn is the Apgar score. Early biochemical tests succeed in highlighting infants at risk of brain injury however, clinical neurological examination remains the gold standard for identifying and categorising infants with HIE.

1.2.1. Apgar Scores

It is almost 70 years since Dr Virginia Apgar first introduced the idea of objectively grading infants after delivery. (43) The score was initially developed as a means to compare different interventions in obstetric care and maternal medications as well as resuscitative practices in the newborn in an objective and standardized manner. (44) Prior to their introduction, newborns received little attention in the delivery suite. Dr Apgar proposed that "nine months' observation of the mother surely warrants one minutes' observation of the baby". (45)

Dr Apgar based her assessment around important non-invasive and easily elicited parameters used in anaesthetics to monitor patients during surgery. Five signs were assessed at 1 minute of life and they remain the cornerstones in our assessment of newborn transition today. (46) They include heart rate, respiratory effort, reflex irritability/response, muscle tone and colour. Each parameter is graded from 0 to 2 to allow for a cumulative score of 0-10, with 10 indicating that the infant was "in the best possible condition". (43) Following work by Drage et al. which demonstrated that an Apgar score at 5 minutes was more predictive of neonatal mortality, a 5 minute score was added. (47) Apgar scores are now universally recognised as a method of reporting the physiological status of a newborn after delivery and their subsequent response to resuscitation if required.

Apgar scores are generally divided into low (0-3), intermediate (4-6) and normal (7-10) and are assessed at 1 minute of life, 5 minutes of life and every 5 minutes thereafter until the Apgar score is >7. Apgar scores can be influenced by obstetric factors such as mode of delivery, maternal analgesia, parity and age, and smoking as well as neonatal factors such as the presence of malformations, neurological or cardiorespiratory conditions. (48, 49, 50)

The majority of the measures included in the Apgar score are subjective and as such are prone to inter-rater variability. (51) Perception of colour, for example, varies between clinicians and relates poorly to pulse oximetry oxygen saturation (SpO_2). (52, 53) One study assessed 27 clinicians and found that the mean SpO_2 in which infants were determined "pink" varied from 10-100%. (52) In fact, heart rate (HR), the only objective measure in the Apgar score, is also associated with significant inter- and intra-observer variability. Auscultation and palpation may underestimate HR by 21 and 14 bpm respectively and one study of 29 healthcare staff found that only 30% correctly identified a heart rate as being less than 60 bpm by auscultation. (51, 54, 55) This variability may result in scores differing by up to 2.4 points which is an important consideration when assessing infants for eligibility for inclusion in studies and interventions. (51)

Low Apgar scores are associated with infant mortality, cerebral palsy (CP), Attention Deficit Hyperactivity Disorder (ADHD) and seizures. (56, 57) Birth asphyxia is the most common cause of a low Apgar score and although it was never intended to be used to assess for perinatal asphyxia, it formed an important part of the inclusion criteria for all of the TH randomised control trials (RCT). Its use as a screening tool was assessed by Hogan et al. who matched 183 infants with Apgar scores <7 at 5 minutes of life with 183 control infants with Apgar scores of 9-10. In this group, 70% of those with an Apgar score of <4 at 5 minutes of life developed HIE compared with 14% of those with an Apgar of 4-6 and 0% of infants with an Apgar score of 9-10. (58) However, low Apgar scores in isolation are not indicative of an acute hypoxic event or subsequent poor outcome. Ruth et al. found that 80% of infants born with an Apgar score of ≤7 at 5 minutes will have a normal outcome (59) and Natarajan et al. reported that 20% of infants with an Apgar score of 0 at 10 minutes will have intact survival at school age. (60)

A limitation of the original Apgar score is that it does not account for the level of intervention or resuscitation that an infant requires. To overcome this, other groups have attempted to build on Dr Apgar's original scoring system to improve prediction of adverse neonatal outcome. (61, 62, 63) The Specified-Apgar score records the infant's condition regardless of resuscitative measures, for example, a pink colour with or without oxygen administration. (61) The Expanded-Apgar additionally documents the interventions that the infant receives, for example, continuous positive airway pressure, and the Combined-Apgar includes both scores. (62, 63) Dalili et al. compared all four scores and found that the Combined-Apgar score had the highest sensitivity (97%) and specificity (99%) in predicting infants with perinatal asphyxia. (64) In addition, a Combined-Apgar score of < 10 was associated with HIE ($p=0.02$) but it was unable to predict the severity or grade of HIE. (64) Although, the conventional Apgar score had the lowest sensitivity (81%) and specificity (81%), it remains the most widely used score in newborn evaluation. Apgar scores are not perfect in isolation but they certainly do succeed in achieving Dr Apgar's goal of focusing attention on the newborn after delivery and considering the potential of HIE as a diagnosis when an infant is born in poor condition.

1.2.2. Acid-base status

There is currently no gold standard test for the diagnosis or detection of encephalopathy but it is generally accepted that a low pH (<7.10) or an increased base deficit (>12mmol) are associated with adverse outcome. (65, 66, 67) Although they are not sensitive or specific for outcome prediction, (68, 69) they are key features of perinatal asphyxia. Perinatal asphyxia may occur as a result of a single significant episode of hypoxia, for example following placental abruption, multiple intermittent short episodes during labour or following chronic hypoxia. (70) Perinatal asphyxia results in hypercarbia and thus a respiratory acidosis develops. If the asphyxia is prolonged, there is a switch to anaerobic metabolism and metabolic acidosis ensues. pH and base deficit (BD) are predominantly used as screening tools for encephalopathy and are included in the scoring systems for commencing TH. (71)

pH measurements are widely accessible and can be measured from the foetal scalp during labour giving an early estimation of the infant's well-being. Low cord pH is significantly associated with neonatal morbidity and mortality (66) and infants with a pH of <7.00 have a significantly higher risk of multi-organ morbidity and poor outcome. (72, 73) A recent study by Kelly et al. demonstrated a dose-dependent relationship between degree of acidosis and adverse outcome. At pH levels of 6.9-6.99, 6.8-6.89 and <6.8, combined outcome of death or CP was 3%, 10% and 40% respectively. (74)

However pH has been shown to be both a poor predictor of HIE and a poor discriminator of severity of encephalopathy. (75, 76, 77) One study showed that of 103 infants with a pH <7.16, only 19.4% had low Apgar scores (<7 at 5 minutes) and only 1 had evidence of perinatal asphyxia. (75) Another showed that approximately one third of infants with brain injury due to asphyxia had no evidence of acidosis on arterial cord pH. (77)

BD is also used as a marker of acidaemia. Defined by the amount of base that must be added to fully oxygenated blood to obtain a normal pH of 7.40 (ensuring normothermia and normocapnia), it is calculated using the Henderson-Hasselbalch

equation. Its use is controversial however as studies have shown that BD in isolation is not a predictor of poor outcome; rather it reflects the level of acidaemia as infants with a larger BD also have a lower pH (78, 79) and BD itself is calculated using the pH value, albeit corrected for hypercapnia. In addition, using different gas analysers to measure the same samples can lead to different levels of metabolic acidosis (80) and there can be a discrepancy in calculations used. (81)

Other groups have suggested that lactate may be a better surrogate for injury. Poor outcome occurs as a result of anaerobic metabolism and subsequent metabolic acidosis and not respiratory acidosis alone. (82, 83, 84, 85) Due to its low transport across the placenta, levels measured are almost entirely of foetal origin. (86, 87) Animal models demonstrate that increases in lactate may precede alterations in pH and are associated with a decreased rate of survival and an increase in brain injury. (88) This finding has been confirmed in both term and preterm studies. (89, 90) Umbilical arterial lactate concentrations correlate with brain lactate and every 1.0mmol/L increase in umbilical artery lactate results in a 0.02 increase in brain lactate. (91) This may explain how lactate has shown good ability to predict low Apgar scores and short-term neonatal morbidity however, whether this translates to outcome prediction is unclear. (90, 92) Studies have also shown that lactate levels are unable to differentiate between grade of encephalopathy with mild, moderate and severe grades having a similar median and interquartile range (IQR) (mild 11.6 mmol/L [IQR 10.3-14.5], moderate 11.0 mmol/L [9.4-14.6], severe 11.6 mmol/L [9.6-14.6]). (93) A systematic review by Salvanos et al. reported that although a high lactate had low specificity and low positive predictive value, a low lactate level was reassuring for a good outcome. (94)

Clearly currently available biochemical tests are neither sensitive nor specific to diagnose or grade severity of encephalopathy. Using these parameters in combination may improve the predictive accuracy. (95) They do, however, serve an important purpose as an alert system for clinicians to identify infants who may be at high risk and require further assessment.

1.2.3. Clinical Examination

Prechtl et al. were one of the first groups to try to classify infants following obstetric complications. (96, 97) They recognised that adverse perinatal events had a significant effect on the neurological status and development of the infant. They realised that long-term outcome was dependent on whether infants demonstrated a certain combination of neurologic features. From this, they developed a neurological examination to categorise infants into three broad groups; hyperexcitable syndrome, apathetic syndrome and hemisyndrome, and these groups correlated with risk of adverse outcome at 2-4 years of age. (96, 97) Now known as Prechtl's Assessment of General Movements, this tool is not specific to infants with HIE and can be implemented for the assessment of both term and preterm infants from birth. (98, 99) In high-risk infants, it has a sensitivity of 76% and a specificity of 82% for the prediction of CP. (100) The assessment requires observation of the infant's movements by certified assessors and is therefore expensive and time-consuming thus hindering its widespread acceptance into clinical care. Work is underway to assess the use of artificial intelligence technology as a potential approach to Prechtl's assessment. (101, 102)

Almost 10 years after Prechtl's initial classification, Harvey and Margaret Sarnat attempted to further define these categories by examining 21 infants repeatedly over the first week of life and combining their clinical features with EEG assessment. (2) They too agreed on three broad stages (Stages 1, 2 and 3) but instead of categorising infants into one grade, they recognised that they were a continuum with infants passing from one stage to another and back again. As stage 1 can last for up to 24 hours, worse grade of encephalopathy may not be evident until 48 hours of age.

Robertson and Finer subsequently included 49 infants in their prospective study. They used the Sarnat score to examine infants over the first 7 days of life and infants were assigned to a grade based on their worst score seen over that first week. Infants returned for follow-up at 3-4 years of age. (103) Although their grading significantly correlated with outcome ($p=0.026$), it is important to remember that at this time,

clinical grade was assigned over the first week of life. This study was completed in a time where no therapeutic options were available, and thus the assignment of grade was important only for prognostication and could take place at discharge. With widespread introduction of TH, the determination of grade needs to occur within the first 6 hours after birth, to allow timely introduction of neuroprotection. Hence, the predictive ability of neurological examination will be altered. With so much importance lying on this early examination, alterative and more detailed neurological examination scales have been developed.

In 1985, Levene et al. sought to describe the incidence and severity of post-asphyxial encephalopathy. (104) They grouped infants into three grades of clinical severity based on the clinical syndromes of HIE described by Fenichel. (105) The modified Levene Classification (Table 1.1) and the modified Sarnat score (Table 1.2) are routinely used in clinical practice today. In essence, they are a simplified Sarnat score using only aspects of clinical observation and examination. (1, 106) When performed at 24 hours, the Levene's Modification score has been shown to significantly correlate with outcome (p=0.001). (107)

Table 1.1: Modified Levene Scoring System.

Grade 1 (Mild)	**Grade 2 (Moderate)**	**Grade 3 (Severe)**
Irritable, "hyperalert"	Lethargy	Comatose
Mild Hypotonia	Marked hypotonia	Severe hypotonia
Poor suck	Unable to suck Seizures	Unable to sustain spontaneous respirations Prolonged seizures

Table 1.2: Modified Sarnat Scoring System.

	Stage 1 (Mild)	**Stage 2 (Moderate)**	**Stage 3 (Severe)**
1. Level of Consciousness	Hyperalert	Lethargic	Stuporous or Comatose
2. Spontaneous Activity	Active	Decreased	Absent
3. Posture	Mild distal flexion	Strong distal flexion, complete extension or "frog-legged" position	Decerebrate
4. Tone	Hypertonic, jittery	Hypotonia (axial and/or limb)	Flaccid
5. Primitive reflexes			
Suck	Weak	Weak/absent	Absent
Moro	Strong, low threshold	Incomplete, high threshold	Absent
6. Autonomic Function			
Pupils	Mydriasis	Miosis	Variable, fixed, dilated, unresponsive to light
Heart Rate	Normal, tachycardia	Bradycardia	Variable, irregular
Respiratory Rate	Regular, spontaneous breathing	Periodic, irregular breathing effort	Apnoeic
7. Seizures	None	Common: focal or multi-focal	Uncommon

Another classification system in routine use is the Thompson score. (108) This was first developed at Groote Schuur Hospital, South Africa in the 1990s. It is similar to the Sarnat classification but provides a linear scoring system, rather than a categorical "grade". Initially developed for ease of access in developing countries, it claims to be as accurate as the Sarnat score but easier and quicker to use. The score consists of nine clinical signs, each scored from 0-3. The cumulative score gives an indication of grade with higher scores (>14) signifying greater degree of injury and scores <10 indicating mild encephalopathy. Once again, this score was developed to examine infants on a daily basis, not within the first hours of life and was found to be most predictive of outcome on days 3-4. (108, 109)

Table 1.3: Thompson Scoring System.

	0	**1**	**2**	**3**	**Day 1**	**Day 2**
Tone	Normal	Hypertonia	Hypotonia	Flaccid		
Consciousness	Normal	Hyperalert, stare	Lethargic	Comatose		
Fits	Normal	Infrequent, < 3/day	Frequent, > 2/day			
Posture	Normal	Fisting, cycling	Strong distal flexion	Decerebrate		
Moro	Normal	Partial	Absent			
Grasp	Normal	Poor	Absent			
Suck	Normal	Poor	Absent +/- bites			
Respiration	Normal	Hyperventilation	Brief apnoea	Intermittent positive pressure ventilation (Apnoea)		
Fontanelle	Normal	Full not tense	Tense			
				Score per day		

Each of these scores were originally developed to predict outcome in a pre-TH era, not to guide intervention in the first 6 hours after birth. While they remain useful in counselling parents as to prognosis, 24 hours or beyond is much too late to initiate TH or alternative therapies.

Subtle differences also exist between the individual scoring methods. Each of the TH RCTs utilised a scoring system to determine eligibility for inclusion. Based largely on the above-mentioned classification systems, they have been integrated into clinical practice in most neonatal units. The two most common classification systems in practice are the NICHD and TOBY Classification systems. (71, 110) Although both aim to identify infants with moderate and severe HIE, subtle differences exist between the two resulting in some infants qualifying for TH with one but not the other. (111)

It is very challenging to predict outcome so soon after birth. Clinical examination may be complicated by medications given for sedation, muscle paralysis or seizure control, and can vary depending on whether or not the infant is intubated and the extent of the initial resuscitation required. In addition, repeated handling can lead to alterations in muscle tone and level of alertness. (112) Furthermore, clinical examination is subjective. DuPont et al. found that as many as 20% of infants thought to have mild encephalopathy were misclassified within the first hours of life and therefore did not receive TH. (113) Alternate objective methods of grading encephalopathy in the first hours of life are required.

1.2.4. Grades of HIE

Although many scoring tools exist, the overarching aim of each is to objectively stratify the severity of the underlying encephalopathy. Regardless of the score used, HIE is typically classified into three categories: mild, moderate and severe. These categories are not independent of one another, rather they are a continuum.

1.2.4.1. Mild HIE

Mild HIE accounts for almost 40% of all HIE cases. (114) Infants with mild HIE are noted to be hyper-alert or irritable. They may demonstrate mild distal flexion of their limbs. They may have a weak suck and have mydriasis and tachycardia. They may have a strong Moro reflex easy to elicit at a low threshold. Muscle tone can be normal with normal spontaneous activity and normal respirations.

Historically, infants with mild HIE were considered to have a normal outcome. Robertson and Finer in 1985 concluded that the 66 infants with mild HIE in their cohort all had normal outcomes at 3.5 years. (103) However, on closer examination of their methodology, they defined neurodevelopmental disability as a score of 3 standard deviations below the mean which encompassed cerebral palsy, profound cognitive impairment, severe refractory seizures or impaired vision or hearing. More recently, a systematic review and meta-analysis reported that 25% of infants with mild HIE had an abnormal neurodevelopmental outcome at 2 years. (115) The PRIME study reported higher rates of neurodevelopmental impairment (40%) particularly in the language and cognitive domains. (116) Finder et al. found that infants with mild HIE had cognitive scores lower than those of healthy term controls and similar to infants receiving TH for moderate HIE. (117) In a cohort of 89 infants with all grades of HIE receiving TH, 48 infants with mild HIE were included. (118) 38% of the mild group had hypoxic ischaemic injury on their magnetic resonance imaging (MRI) and no differences were identified in the rate of moderate/severe MRI abnormalities between the mild, moderate or severe HIE groups. This rate of injury has also been documented in an uncooled cohort. (119) It is now clear that up to 25% of infants with mild HIE will have an abnormal motor or emotional/behavioural outcome, (115) although this may not become apparent until school-going age or beyond. (120)

Infants with mild HIE are a largely understudied population, predominantly due to their, incorrectly, perceived lack of neurodevelopmental impairment. The majority of research and trials to date have focused on infants with moderate and severe HIE. (5, 71, 110) Infants with mild HIE were not included in RCTs of TH. (5) As they were not deemed to be at risk for adverse outcome, their risk-benefit ratio did not support

their inclusion into the initial trials. To this end, many clinical and EEG grading systems have combined infants with mild HIE with "normal" infants and have focused instead on delineating between mild and moderate for the purpose of intervention. (71, 110, 121) As such, there is no consensus as to the definition of mild HIE, particularly in the first 6 hours after birth. Rather it may be considered as a diagnosis of exclusion as it is generally accepted that infants with an abnormal neurological examination, but who do not meet criteria for moderate or severe HIE, are classified as mild. (122)

As evidence emerges regarding the level of disability in infants with mild HIE, so too does the question of whether they would benefit from TH. Pre-clinical studies have demonstrated that shorter insults (<10 minutes of ischaemia) are related to selective hippocampal injury. (123) In addition, neuronal injury appears to occur more slowly in shorter, less severe periods of HI. (124) In adult rodents, early initiation of hypothermia after brief ischaemia was highly protective. (125) Gagne-Loranger et al. examined a group of infants who had been referred to their centre for cooling. (119) Thirteen infants out of the 108 receiving TH had an end diagnosis of mild HIE, of which 31% had magnetic resonance imaging (MRI) evidence of brain injury. In the 59 infants who did not undergo TH, 50 had mild HIE of which 40% had MRI injury. The MARBLE study included 47 infants with mild HIE; 32 of whom received TH and 15 who did not. (126) They found that infants with mild HIE who received TH had significantly less MRI injury, specifically lower white matter injury (50% v 87%, p=0.02) and improved MRS (p=0.001), compared with those who did not despite having lower 10 minute Apgar scores. Interestingly, there was no difference in their neurodevelopmental outcomes at 2 years. Two meta-analysis pooled participants from the TH RCTs with a final diagnosis of mild HIE who inadvertently received TH and failed to show a significant benefit. (115, 127)

Despite this, there has been a large drift in clinical practice with many centres now offering TH to infants with mild HIE. Kracer et al. showed that in California, the majority of centres offer TH to infants with mild HIE, with a 17% increase in the number of infants with mild HIE cooled between 2010 and 2012 alone. (128) The

TOBY register in the UK found that 18% of infants cooled had mild HIE. (129) Clinical examination is subjective and a certain proportion of this may be attributable to misclassification and evolution of the encephalopathy itself. DuPont et al. found that as many as 20% of infants thought to have mild encephalopathy were misclassified within the first hours of life. (113) However, a recent UK survey of cooling centres found that 75% offered TH to infants with mild HIE with many citing concerns regarding misdiagnosis, risk of neurodevelopmental impairment and medico-legal litigation as influencing factors. (130)

Clearly an RCT is warranted however, research in this area poses many challenges. (131) Logistically, the ultimate level of disability in infants with mild HIE may be subtle and not evident until school age or beyond, requiring long-term follow-up, thus rendering studies in this population slow and expensive, and requiring larger numbers to power the study correctly. The most pressing issue however, is that no clear definition of mild HIE exists and our current methods of grading HIE are challenging and subjective. (113) Alternate objective methods of identifying infants with mild HIE in the first hours of life are required. As a first step, improved understanding of the evolution of injury in infants with mild HIE is crucial and may be helpful in identifying potentially useful predictors of outcome.

1.2.4.2. Moderate HIE

Approximately 40% of infants with HIE will fall into the moderate HIE group. (114) These infants are lethargic with decreased spontaneous activity. They have mild central hypotonia and demonstrate strong distal flexion. Their suck may be weak or absent and their Moro reflex may be weak or incomplete. When examining their autonomic function, they may be bradycardic, have miosis and demonstrate periodic breathing. Seizures may occur in this group and if present, they tend to occur between 12 and 72 hours of age. These infants meet current criteria for intervention with TH.

Prediction of outcome may be challenging in this group, as their outcome may be heterogeneous. In the pre-TH era, no therapeutic interventions were available and

infants instead received supportive management. Initially considered to have a 32-50% incidence of adverse outcome, it became clear that certain impairments may not become evident until much later. (132, 133) A population-based birth-cohort in Sweden followed a group of 43 infants with moderate HIE to age 15-19 years and found that 30% had cerebral palsy, the majority of whom had associated cognitive and hearing impairments, and a further 51% had cognitive impairment without CP. (134)

With the advent of TH, outcome in this group has significantly improved. A meta-analysis of the TH trials found a significant reduction in death or major disability in infants with moderate HIE (RR 0.68 (95% CI 0.56 to 0.84)) with a number needed to treat to benefit (NNTB) of 6. (5) Despite this, approximately 21% will continue to have moderate-severe disability or death. (135)

1.2.4.3. Severe HIE

Severe HIE is the least common but most devastating. These infants are stuporous or comatose. Their muscle tone is significantly decreased. They are flaccid and may demonstrate decerebrate posturing of their limbs. They have an absent suck and Moro. Their heart rate is variable, they tend to be apnoeic and pupils can be unequal, fixed, dilated or show poor light reflex. Seizures are common in this group. This group also display evidence of multi-organ dysfunction and may have signs of acute renal and liver failure.

Infants with severe HIE tend to die in the neonatal period or if they survive, have significant motor impairments. (132, 133) A meta-analysis of five of the initial TH RCTs found a significant reduction in mortality in infants with severe HIE (RR 0.77 (95%CI 0.64 to 0.93)) with a NNTB of 6 however no difference was found in morbidity rates. (5) Recent studies have confirmed that despite TH, a 65% risk of death or moderate-severe disability persists in this group. (135)

1.3. Therapeutic Hypothermia

Therapeutic Hypothermia (TH) has revolutionised management of infants with HIE. Prior to its introduction, infants received supportive intensive care which focused on providing respiratory and nutritional support along with maintaining normoglycaemia and normocapnia and providing treatment for seizures as required. (136, 137) Now a therapy exists which has been shown to significantly reduce the incidence of death and severe disability. (5)

The exact mechanism of TH is unknown but animal studies demonstrated a reduction in brain acidosis in piglets undergoing hypothermia following HI injury. (138) TH attenuates the consumption of ATP stores however, its effect on cerebral metabolism does not fully explain its neuroprotective effect. (139, 140) TH also slows the production of excitatory amino acids. (141) By blocking or slowing down the secondary energy failure, the inflammatory cascade is blunted. Decreased oxygen consumption and free radical production leads to a reduction in apoptosis and inflammation and allows the cells time to recover. (5, 142, 143, 144, 145, 146, 147, 148) Piglet models suggested that infants with moderate rather than severe HIE, had the most potential for neuroprotection. (149) TH should ideally be commenced within 6 hours of the original insult to be beneficial. Pre-clinical studies have shown that it is not effective if commenced beyond 9 hours post-injury and may be harmful if commenced after 12 hours. (30, 150) A reduction in deep brain temperature to less than 35^{o}C is required for neuroprotection and thermal simulations proposed that for this to be achieved, an infant's core temperature must be lowered to less than 35^{o}C. (151)

Four pilot neonatal studies concluded that mild hypothermia in infants with moderate and severe HIE was feasible and safe. (152, 153, 154, 155) Eleven individual RCTs followed, examining the use of both selective head cooling and whole body hypothermia in infants with moderate to severe HIE. (71, 110, 153, 154, 156, 157, 158, 159, 160, 161, 162) Inclusion criteria varied slightly between the studies; different pH and lactate cut offs were used and three studies used EEG or aEEG

features as part of their inclusion criteria. Individually, these studies demonstrated a neuroprotective effect, and a subsequent systematic review and meta-analysis by Jacobs et al. confirmed a clinical and statistically significant reduction in death and major neurodevelopmental outcome at 18 months of age in infants receiving TH (RR 0.75 (95% CI 0.68-0.83)) with a NNTB of 7 (95% CI 5-10). (5)

Selective head cooling was initially proposed as a method of selectively reducing brain temperature while avoiding the potential systemic side effects of whole body cooling. (163) However, as mentioned, simulation studies demonstrated that a reduction in core temperature was required to obtain a reduction in cerebral temperature. (151) Sub-group analysis of the TH trials, found that while there was a trend towards lower rates of mortality and neurodevelopmental impairment in the head cooling trials, a significant improvement in these outcomes was only seen in the whole body cooling trials. (5)

Groups have since sought to optimise TH treatment by examining the effect of deeper or longer cooling periods or slower rewarming on outcome however these did not result in an improvement in mortality or morbidity. (164, 165, 166)

Current clinical TH in the neonatal unit (NICU) involves lowering an infant's core temperature to 33-34 ^{o}C for 72 hours followed by slow rewarming over 12 hours. It is indicated for infants with moderate and severe grades of HIE. To be effective, it must be initiated within 6 hours of birth and even earlier commencement of TH (less than 3 hours) results in further improvement in motor outcome; "Time is brain". (143, 167)

As discussed, the use of TH in infants with mild HIE is a contentious issue. There is a growing body of evidence that infants with mild HIE have significant levels of disability on follow-up. (116, 117, 120, 168) Preclinical models of TH suggest better outcomes in milder injury (169) and retrospective analysis of infants inadvertently recruited as part of TH trials suggest some benefit to offering TH to this mild HIE

group. (126) For this and other reasons outlined above, there has been a significant therapeutic drift in centres offering TH to infants with mild HIE. (128, 129, 170)

However, TH is not without risks. Cardiovascular side effects reported include sinus bradycardia (RR 11.59 (95%CI 4.95-27.17)), hypotension (RR 1.00 (95%CI 0.92-1.09)), and cardiac arrhythmias (RR 0.55 (95%CI 0.12-2.56)) although they are, for the most part, transient and resolve post rewarming. (5). Although less common, prolonged QT interval and pulmonary hypertension have also been documented. (5) Haematological side effects include thrombocytopenia (RR 1.21 (95%CI 1.05-1.40)), leucopenia (RR 2.4 (95CI 0.85-6.79) and coagulopathy (RR 1.1 (95%CI 0.93-1.29)). (5) Shivering and discomfort may also be associated with low core temperatures requiring sedation. (5, 155) Subcutaneous fat necrosis has been reported in approximately 1% of infants with moderate-severe HIE receiving TH. (171) TH also results in a delay in initiating feeds and breastfeeding. A survey of UK Neonatologists revealed that only 59% of units would initiate enteral feeds during TH and rewarming and when introduced, although expressed breastmilk was preferred, the volume of milk was significantly lower when compared with milk volumes started after TH. (172) Another important consideration is the potential effects on bonding which may result from separation of the infant from its parents. Improved evidence on the effect of TH in infants with mild HIE and potential benefits are therefore required before incorporating TH for mild HIE into routine clinical practice.

Since the introduction of TH as standard of care, the incidence of death or severe disability in infants with HIE has decreased significantly. However, it is important to remember that 40% of infants still have neurological disability at 18-24 months and some learning or behavioural difficulties may not become evident until school-going age or beyond. (110, 115, 134, 168) TH has improved but not eliminated poor outcome and this may be for many reasons.

A main confounding factor in TH treatment is that it must be commenced within 6 hours of injury. (5, 30) In clinical practice, timing of injury is typically considered to be time of birth however, the insult may have occurred minutes, hours or days prior

to delivery. In these cases, the therapeutic window has closed and the secondary energy failure is already underway.

Secondly, the encephalopathy may be complicated by other confounders such as infection, metabolic conditions or other underlying conditions. TH is only indicated for neonatal encephalopathy due to hypoxic ischaemia and may not be as effective in cases due to a different underlying pathology. (173, 174)

Furthermore, our selection criteria of infants for TH is not without its limitations. Markers such as Apgar score, lactate and pH addressed above may provide clues of an hypoxic ischaemic event but are only useful in extremes. (76) The decision to initiate therapeutic hypothermia or not is ultimately based on clinical examination which has been shown to be subjective, has great inter-observer variability and evolves. In the TH trials, many infants with an ultimate diagnosis of mild encephalopathy were enrolled in error due to misclassification in the first 6 hours. (113)

Our current cooling regime is near optimum yet significant levels of disability persists. Focus has now turned to improved objective identification of infants at risk of adverse outcome and adjunctive therapies, which may have additional benefits when used in conjunction with TH. (35, 164, 165, 166) Adjunct therapies targeting the different phases of injury have been discussed previously. Pre-clinical models are currently investigating different combinations of these therapies. Pang et al. used different combinations of melatonin, erythropoietin and hypothermia in their piglet model. (175) They found that “double therapy” (hypothermia plus melatonin/erythropoietin) resulted in improved aEEG recovery. TH plus melatonin was also associated with improved lactate/N-acetyl aspartate levels and TH plus erythropoietin resulted in improved oligodendrocyte survival. “Triple therapy” (TH plus melatonin plus erythropoietin) did not have added benefit; in fact aEEG recovery was slower in this group. They concluded that staggered administration may provide better protection. Nonomura et al. administered magnesium sulphate every day for 3 days and erythropoietin every other day for 2 weeks along with standard TH

therapy in 9 infants with moderate-severe HIE and concluded that combination therapy is feasible in infants with HIE however further research is warranted. (176)

1.4. Monitoring

The importance of objectively monitoring infants with HIE has long been realised and even predates the original Sarnat classification. Newer technologies have allowed for more detailed, timely, non-invasive monitoring of the infant at the bedside. Current commercially available devices include EEG, near-infrared spectroscopy (NIRS) and non-invasive cardiac output monitoring.

1.4.1. Neurophysiology

1.4.1.1. Electroencephalography

The recording of brain activity from electrodes on the scalp was first described by Hans Berger in the 1920s. At this time he noted that the activity changed depending on the "functional status of the brain". (177) Almost 100 years later, EEG remains the gold standard for assessing background brain activity and in the detection of seizure activity.

1.4.1.1.1. Physiology

EEG records the summated electrical activity of thousands of neurons at the cortical surface. Neurons at the cortical surface (apical pyramidal cells) are generally arranged perpendicular to the cerebral surface. Neurotransmitters move across the synaptic cleft and bind to post-synaptic membrane receptors. This results in the opening of ion-gated channels and an influx of ions into the cell. Excitatory neurotransmitters leads to an influx of positive ions (excitatory post-synaptic potential) and inhibitory neurotransmitters results in an influx of negative ions (inhibitory post-synaptic potential). The influx of ions results in a change in extracellular charge. (178)

Further along the axon, these ions leave the neuron and return to the extracellular space altering the charge at this point. This results in differences in charge at different

points along the neuron, also known as a dipole. Electrical current flows from positive to negative charge, therefore the direction of the current alters depending on the post-synaptic potentials.

EEG measures the summation of post-synaptic activity (excitatory and inhibitory) from a large number of pyramidal cells, which is displayed as a voltage difference between two electrodes. EEG waves vary in frequency, amplitude and shape. Frequencies measured in EEG include Delta (<4Hz), Theta (4-7Hz), Alpha (8-13Hz), Beta (13-30Hz) and Gamma (>31Hz) frequencies.

Continuous, multi-channel EEG (cEEG) uses a number of electrodes placed on the scalp according to the international 10-20 system. (179) This system ensures standardisation of electrode placement. An imaginary line is drawn from the bridge of the nose (nasion) to the occiput (inion) and a second line from left to right joining the pre-auricular point on each side. These lines are then divided into 10 and 20% increments, which gives the 10-20 system its name. Each electrode is labelled by both a letter and a number. Letters refer to the area of placement, i.e. F refers to the frontal area, P is the parietal area, T is temporal and O is occipital. The numbers refer to the distance from certain reference points. Electrodes along the midline are denoted with a “z” which stands for zero. Numbers increase as you move laterally with odd numbers on the left and even numbers on the right.

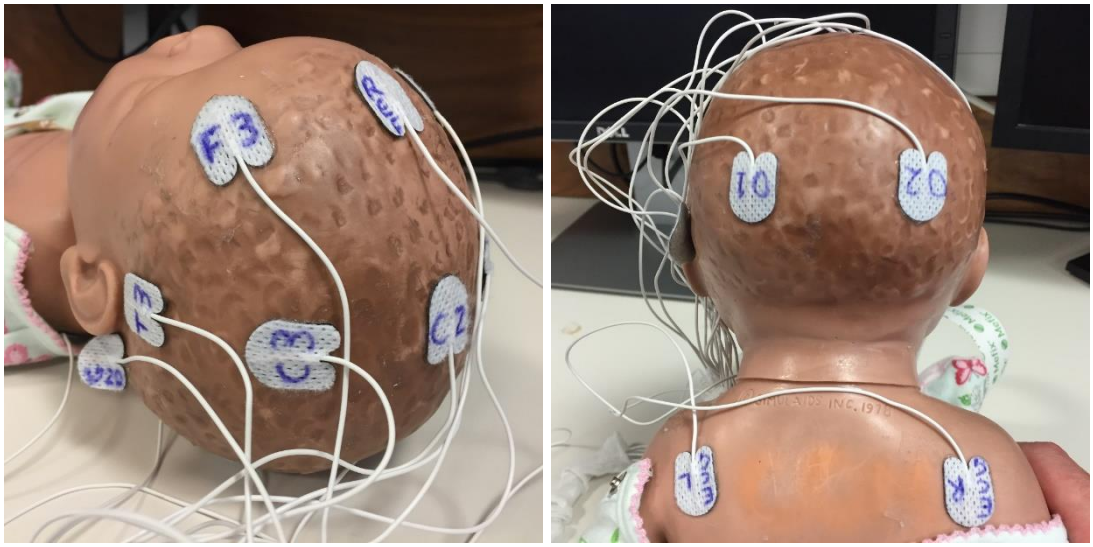


Figure 1.2: Placement of EEG electrodes in our cohort according to the 10-20 system.

A montage describes how the EEG is displayed. Electrodes are initially recorded using a differential amplifier, which takes two inputs and displays the difference between the two. Therefore, each channel of EEG compares the difference in electrical potential between two electrodes. The three main types of montages include referential, bipolar and common average reference.

A referential montage compares each electrode with the same single reference electrode. This method has limitations as the reference electrode itself may be active which may interfere or obscure the signal.

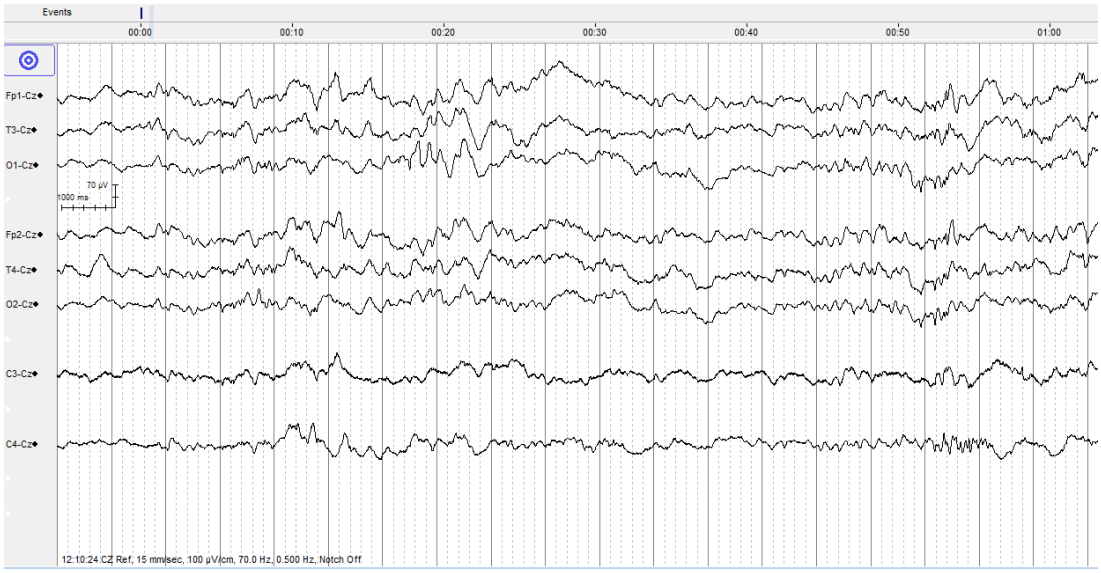


Figure 1.3: EEG demonstrating a referential montage.
Here the Cz electrode acts as the reference against which all other electrodes are compared.

A bipolar montage compares the difference between two adjacent electrodes. This montage is advantageous as it allows for improved localisation of a signal. A limitation of this montage is that signals of opposing sign may cancel each other out.

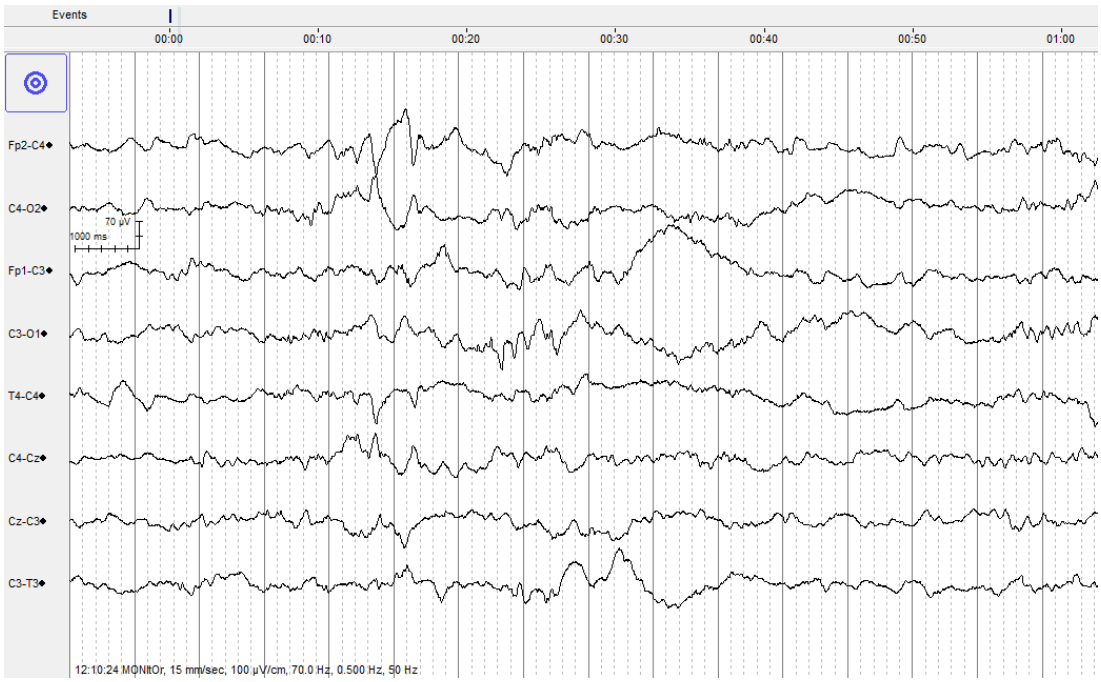


Figure 1.4: EEG demonstrating a bipolar montage.
A bipolar montage displays the difference between two adjacent electrodes.

A common average reference montage compares one electrode to the average of all other electrodes. This montage is advantageous as it provides a constant reference across all electrodes.

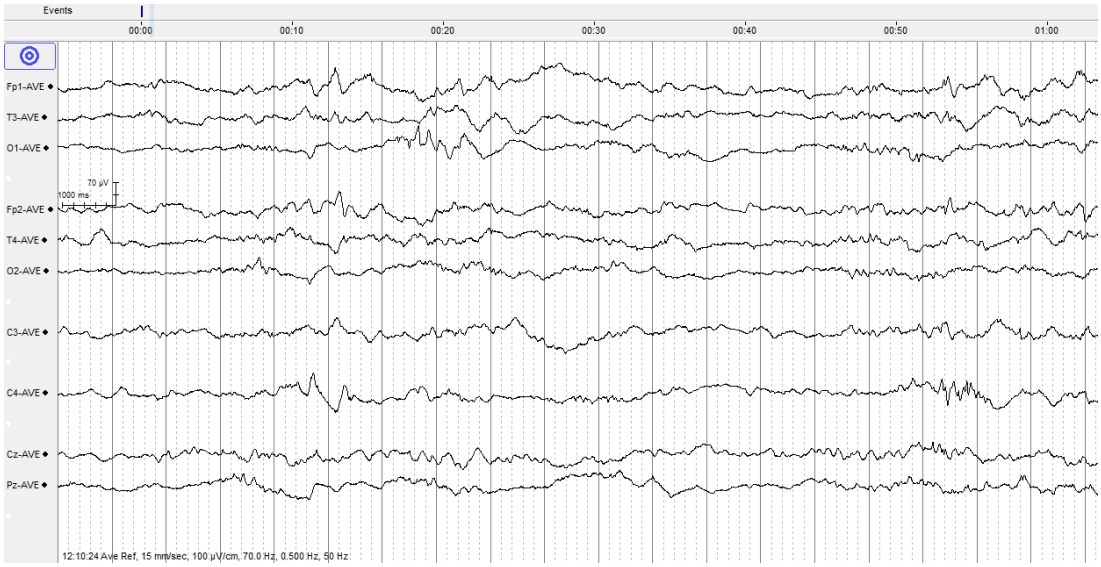


Figure 1.5: EEG demonstrating a common average montage.
This montage compares individual electrodes to the average (AVE) of all other electrodes.

As the dipoles and post-synaptic potentials are dynamic and in a constant state of change, cEEG has high temporal resolution in the order of milliseconds and therefore provides a real-time recording of electrical brain activity in a continuous, non-invasive manner. (76, 180, 181) It is applied at the cot side and can be viewed in real-time or stored for later objective analysis.

A significant challenge of cEEG however is the requirement of trained personnel in its both application and interpretation. Many units now use aEEG as a means to overcome this. aEEG is derived from the cEEG where the amplitude of the raw EEG is compressed, filtered and processed over time. The aEEG can be generated from single or double channels using generally the central or frontocentral electrodes.

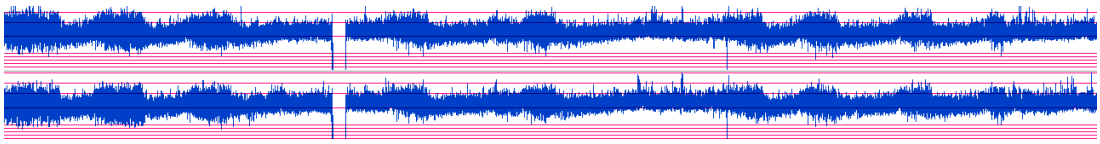


Figure 1.6: aEEG trace.
Top row displays F4-C4 channel. Bottom row displays F3-C3 channel.

1.4.1.1.2. EEG and HIE

EEG has always played a pivotal role in the monitoring of infants with encephalopathy. (76, 121, 182, 183) Sarnat et al. recognised its value and incorporated EEG findings into their clinical grading system. (2) The TOBY trial listed aEEG as an inclusion criteria. (184) EEG is used in the grading and diagnosis of encephalopathy, monitoring its evolution and prediction of outcome in infants with HIE.

Assessment of aEEG is generally classified based on either background activity/amplitude (185) or pattern recognition. (186) Although considered to be easier to apply and interpret, inter-observer reliability is suboptimal. (187, 188) It is also user dependent and studies have shown that many clinicians are not confident in their own ability to interpret aEEG. (189, 190) Nonetheless, aEEG is very helpful in assessing sleep-wake cycling (SWC) and background activity. In the pre-TH era, aEEG as early as 3-6 hours after birth was most predictive of outcome. (180, 191, 192, 193) For this reason, it was used to guide inclusion for TH in the TOBY trial. (184) However, with the introduction of TH the predictive ability of aEEG for outcome has been delayed to 48 hours (194, 195, 196, 197) and a recent meta-analysis has shown that an abnormal aEEG before 6 hours of age does not always indicate adverse outcome. (198) aEEG may also be useful in the detection of seizures however care is needed as seizures generated from areas of the brain other than the frontocentral regions may be missed. Artefact may also significantly affect the ability to interpret both seizures and background activity. (192, 199) Discrepancies have been reported between aEEG and cEEG (200) and this is an important consideration when assigning grade of HIE. Evans et al. found that if aEEG alone was used to determine grade of HIE, 17% of

infants requiring TH would be missed and 43% of infants with no evidence of HIE or mild HIE would be cooled inappropriately. (200) For these reasons, cEEG remains the gold standard method of monitoring brain activity in infants with HIE.

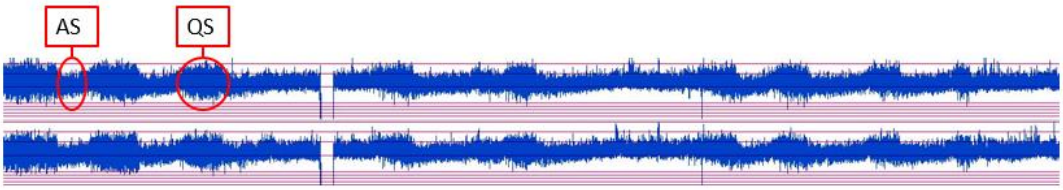


Figure 1.7: Example of sleep wake cycling on aEEG.
Active sleep (AS) and quiet sleep (QS) are highlighted in the red circles. This trace represents 12 hours of recording.

Classification of the cEEG by grade of HIE was first described by Sarnat et al. (2) Since then, over 16 different grading systems have been described in the literature to grade the cEEG of infants with HIE. (201, 202, 203, 204) Fundamentally similar, they are all based on visually examining the voltage and continuity and include, to varying degrees, features such as amplitude, SWC, synchrony, symmetry and seizures. More recently, attempts have been made to standardise the definitions of terms used when assessing and reporting an EEG. (205, 206)

Despite the variation in methodology, many of these studies demonstrated a strong correlation between EEG and clinical grade of HIE. (121, 182, 207, 208) This correlation was strongest at extremes of grade. In the prediction of outcome, both cEEG and aEEG are also helpful at extremes. Infants with normal or mildly abnormal trends generally have normal outcomes, (182, 202, 208, 209, 210, 211) whereas those with a severely abnormal trace tend to do poorly. (2, 183, 202, 208, 209, 210, 212, 213) Prediction becomes more difficult with moderately abnormal traces as outcome in this group varies. (2, 209) Despite TH, cEEG remains the best tool for grading and predicting outcome (121) and has the highest predictive value for MRI injury and long-term outcome at 36 and 48 hours of age. (214) Although the majority of these grading schemes were developed in the pre TH era and as such were not specifically designed to assess the ability of early EEG (<6 hours) to predict outcome,

the worst grade of encephalopathy can usually be seen in the first 6 hours after birth highlighting its potential as an early objective grading tool. (121, 201, 215) Specific details of the scoring system used in this study have been published previously and are outlined below with examples of each grade. (121)(Table 1.4, Figure 1.8 - Figure 1.12)

Table 1.4: EEG classification system used in this thesis.

Grade	Findings	Description
0	Normal EEG Findings	Continuous background pattern with normal physiological features such as anterior slow waves
1	Normal/Mild Findings	Continuous background pattern with slightly abnormal activity (e.g. mild asymmetry, mild voltage depression, or poorly defined sleep-wake cycle)
2	Moderate abnormalities	Discontinuous activity with an interburst interval of <10 seconds, no clear sleep-wake cycle, or clear asymmetry or asynchrony
3	Major abnormalities	Discontinuous activity with an interburst interval of 10-60 seconds, severe attenuation of background patterns, or no sleep-wake cycle.
4	Inactive EEG findings	Background activity of < 10uV or severe discontinuity with an interburst interval of >60 seconds

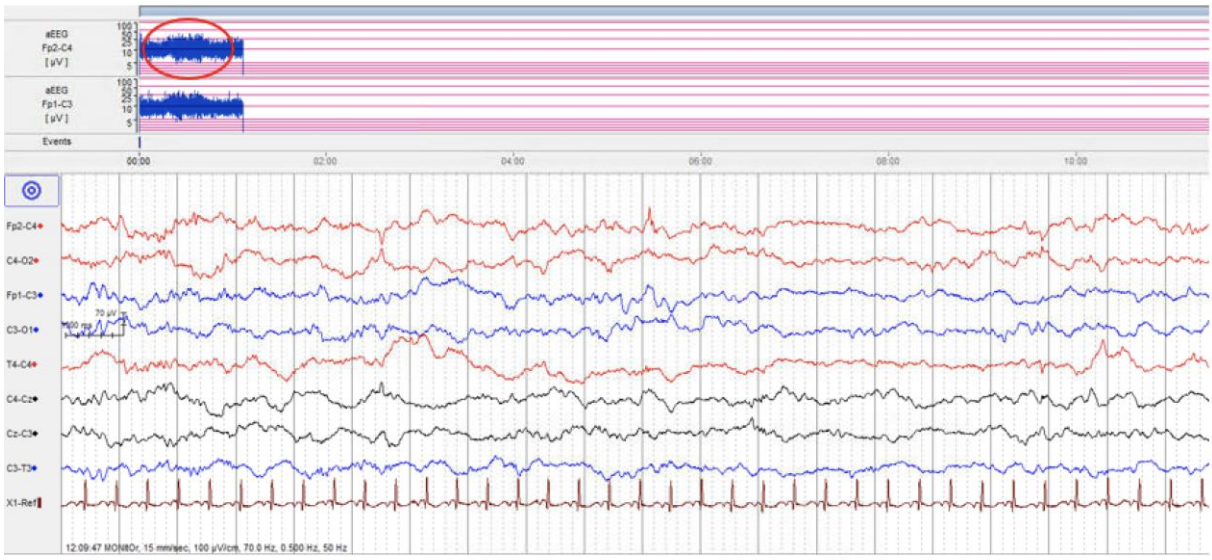


Figure 1.8: Qualitative EEG grading: example of EEG Grade 0.
Continuous multi-channel EEG demonstrates continuous, mixed-frequency activity with sleep wake cycling (highlighted with red circle).

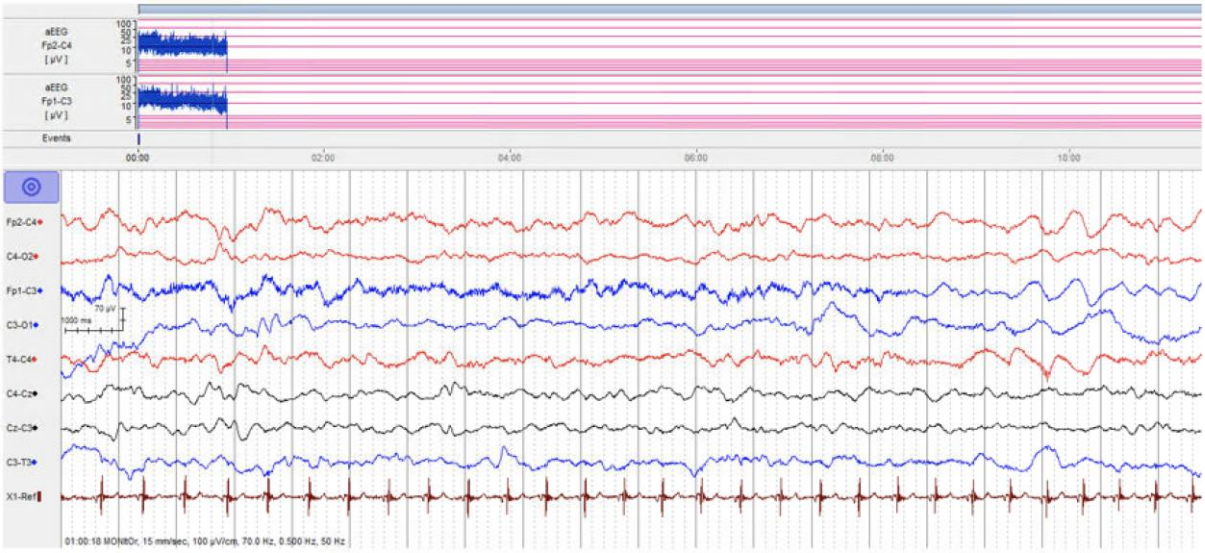


Figure 1.9: Qualitative EEG grading: example of EEG Grade 1.
Continuous, multi-channel EEG demonstrates continuous activity however there is evidence of mild voltage depression. Poorly defined sleep wake cycling on aEEG trace.

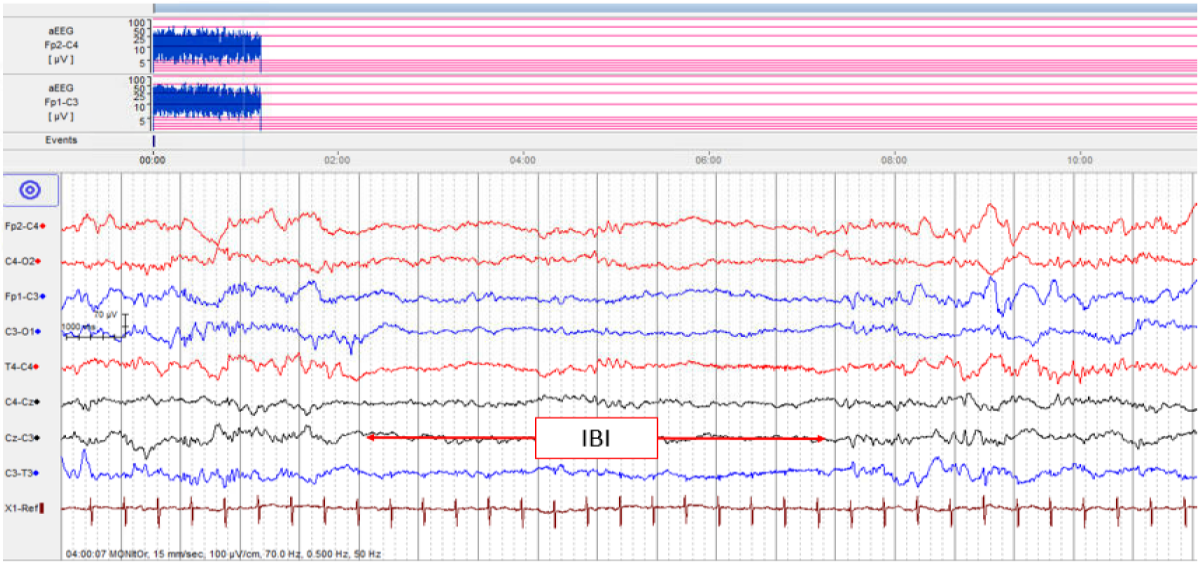


Figure 1.10: Qualitative EEG grading: example of EEG Grade 2.
Continuous, multi-channel EEG demonstrates discontinuous activity with interburst interval <10 seconds (red arrows). No sleep wake cycling.

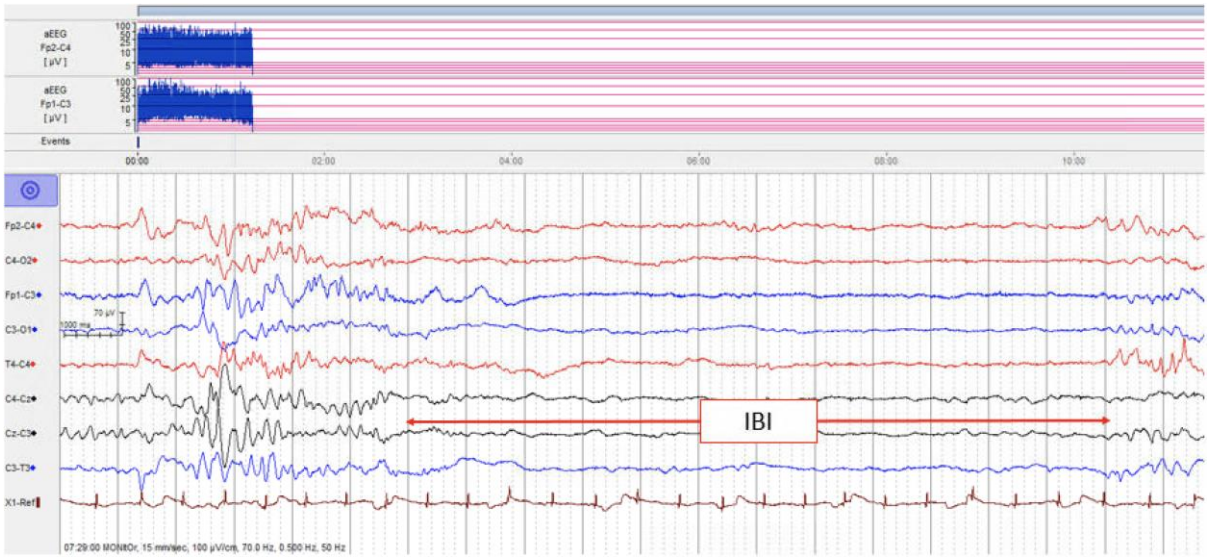


Figure 1.11: Qualitative EEG grading: example of EEG Grade 3.
Continuous, multi-channel EEG demonstrates discontinuous activity with interburst interval 10-60 seconds (red arrows). No sleep wake cycling.

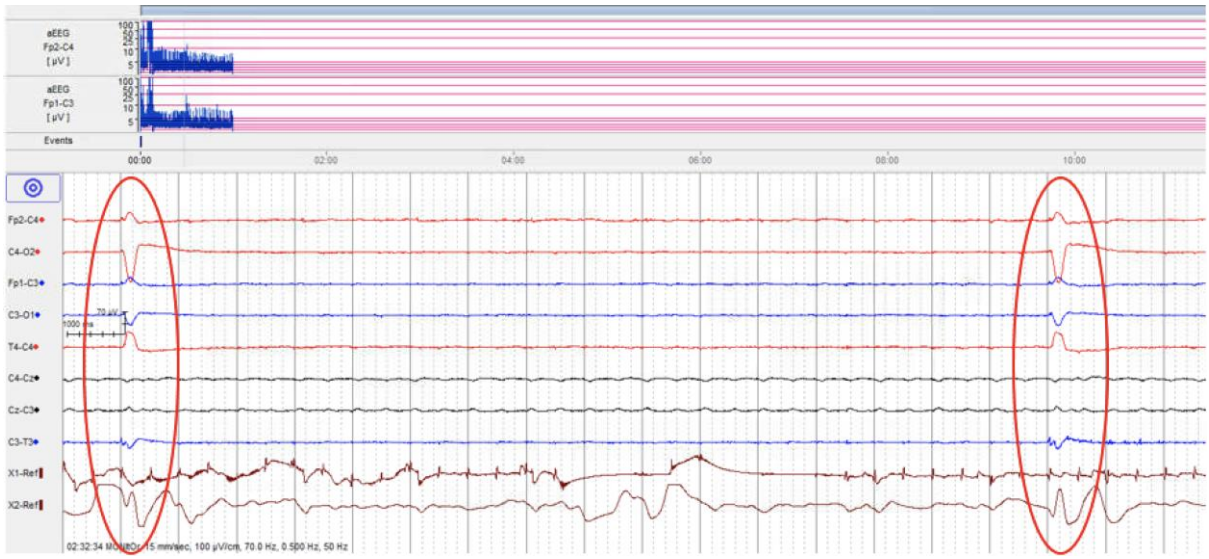


Figure 1.12: Qualitative EEG grading: example of EEG Grade 4.
Continuous, multi-channel EEG demonstrates background activity <10 µV. Some respiratory artefact noted (red circles).

While the grade of the EEG is helpful in outcome prediction, the evolution of the EEG over time is also important. Many studies have shown that a normal cEEG is associated with a normal outcome or if abnormal at the outset, the sooner the

pattern returns to normal or the sooner SWC returns, the better the prognosis. (121, 182, 193)

EEG is also important in the detection of seizures. Despite the introduction of TH, HIE continues to be one of the leading causes of seizures in term neonates. (216, 217) Recognition of seizures in this population is challenging as infants undergoing TH are often sedated, seizures are frequently electrographic only and treatment with medication may cause an uncoupling of clinical signs. (218, 219, 220, 221) Increasing seizure burden is significantly associated with adverse neurodevelopmental outcome independent of underlying injury, and early treatment of seizures had been shown to significantly reduce seizure burden. (222, 223, 224, 225, 226, 227) Early detection is therefore crucial. Seizures can be identified on aEEG, however aEEG may miss short duration seizures (< 30 seconds duration), low amplitude seizures or focal seizures generated from areas of the brain other than those being monitored. (199, 228, 229, 230) For these reasons, cEEG remains gold standard for seizure detection and classification. (231, 232) Due to the necessity of expert input into its interpretation, automated seizure detection algorithms have been developed to aid physicians in the recognition of seizures at the cot side. (233, 234, 235)

Early EEG, particularly in infants with mild HIE, is largely understudied and is an important gap in the literature that needs to be addressed. EEG features of infants with mild HIE have not, as of yet, been clearly defined with many grading systems instead combining normal and mild HIE EEG findings into one classification. (2, 182, 207) The definition of what constitutes a normal EEG also needs to be re-examined. For example, using the pattern classification of aEEG, discontinuous normal voltage is considered a normal trace despite evidence that the absence of SWC is associated with poor neurodevelopmental outcome. (236) Focus previously had been on delineating between mild and moderate HIE for the purpose of intervention. This was reinforced by the fact that a normal or mildly abnormal EEG within 6 hours of birth predicted a normal 2 year outcome. (121) However, follow-up of the same cohort to 5 years revealed that infants with a mildly abnormal EEG had higher rates of disability compared with healthy term controls, highlighting the importance of long-term

follow-up in this population. (120) Therefore, in order to truly assess its ability as an early predictive tool, we must first clarify what EEG features are most useful when identifying infants with mild HIE and describe the features that delineate these infants from both moderate HIE and healthy term infants.

Certainly, cEEG is not without logistical challenges and is not widely available in all neonatal units predominantly due to the necessity of expert personnel for its interpretation. aEEG has been proposed as an alternative however, it too has significant limitations as discussed above. Quantitative EEG analysis may provide a solution as it generates an objective and reproducible description of the EEG without the necessity for expert interpretation. Quantitative EEG also has the ability to detect subtle features which may not be easily identified on visual assessment alone. (237) Quantitative EEG analysis provides a mathematical summary of the EEG signal. Multiple features of amplitude, frequency and inter-hemispheric connectivity are used to describe the neonatal EEG. A standardised definition of the quantitative EEG features is important as it allows for reproducibility and therefore, comparison across studies. Our group has previously published an open source computer code NEURAL (**N**eonatal **E**eg feat**UR**e set in m**A**t**L**ab) software package defining the quantitative EEG features sets used in our analysis. (237) We group features as time-domain features, frequency-domain features and features to describe connectivity between the hemispheres.

Time-domain features include the power, or amplitude, of the EEG. Range-EEG is similar to aEEG however, as different EEG/aEEG machines use different algorithms to generate the aEEG signal, range-EEG provides a standardized alternative.

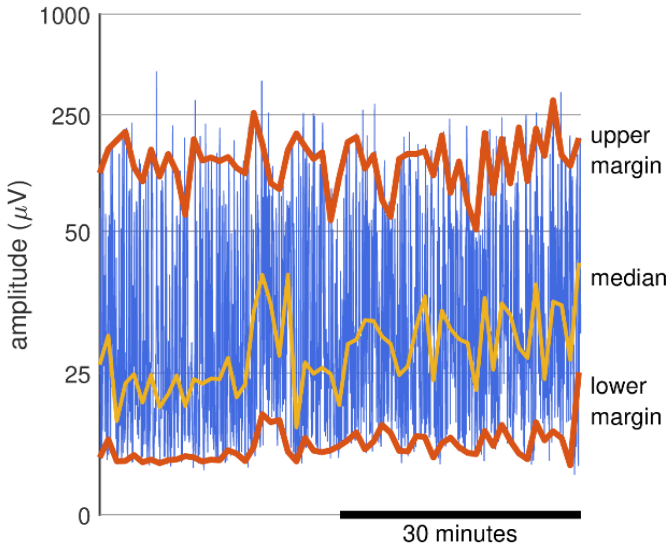


Figure 1.13: Quantitative EEG analysis: range-EEG trace.
Range EEG with median highlighted in orange and lower- and upper-margins highlighted in red. Reproduced from O'Toole et al. 2019. (238)

Frequency-domain features include features of spectral power and shape. The EEG channel is filtered into four frequency bands.

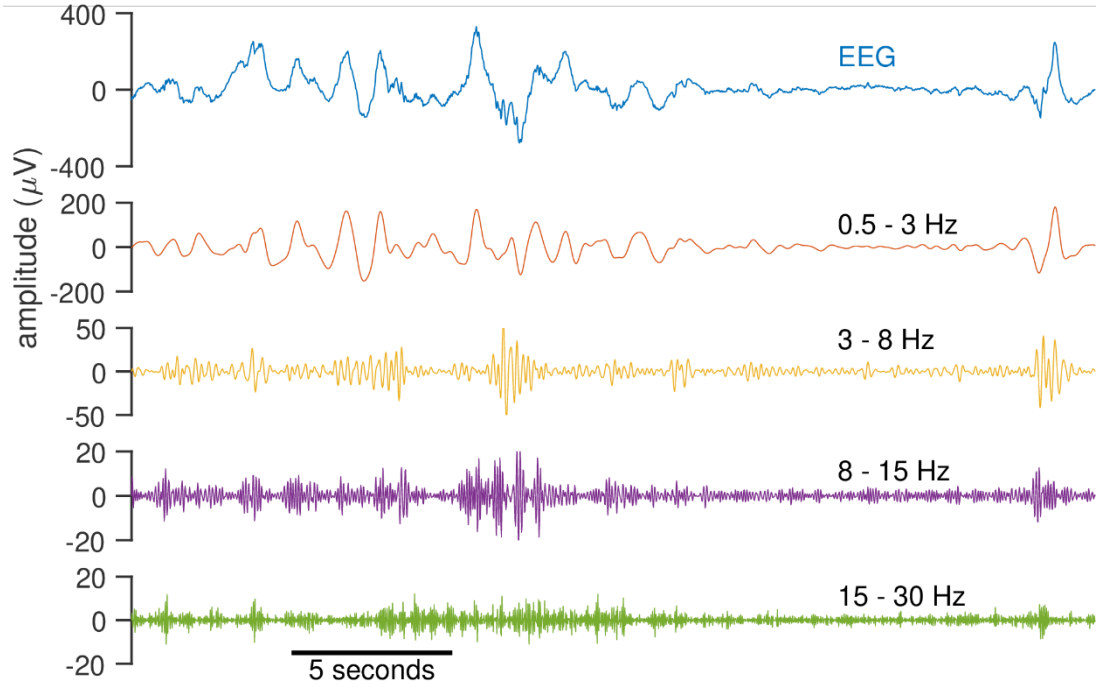


Figure 1.14: Quantitative EEG analysis: EEG split into four frequency bands.
Top row displays one channel of EEG. This is then filtered into different frequency bands below. Reproduced from O'Toole et al. 2019. (238)

Spectral power is a measure of amplitude in the different frequency bands. Relative spectral power is the power in each band relative to each other. Spectral edge frequency is a frequency threshold below which 95% of the power frequencies reside. Features used to describe spectral shape include spectral flatness (a measure of spectral entropy) and spectral difference (a measure of difference in spectral shape over time).

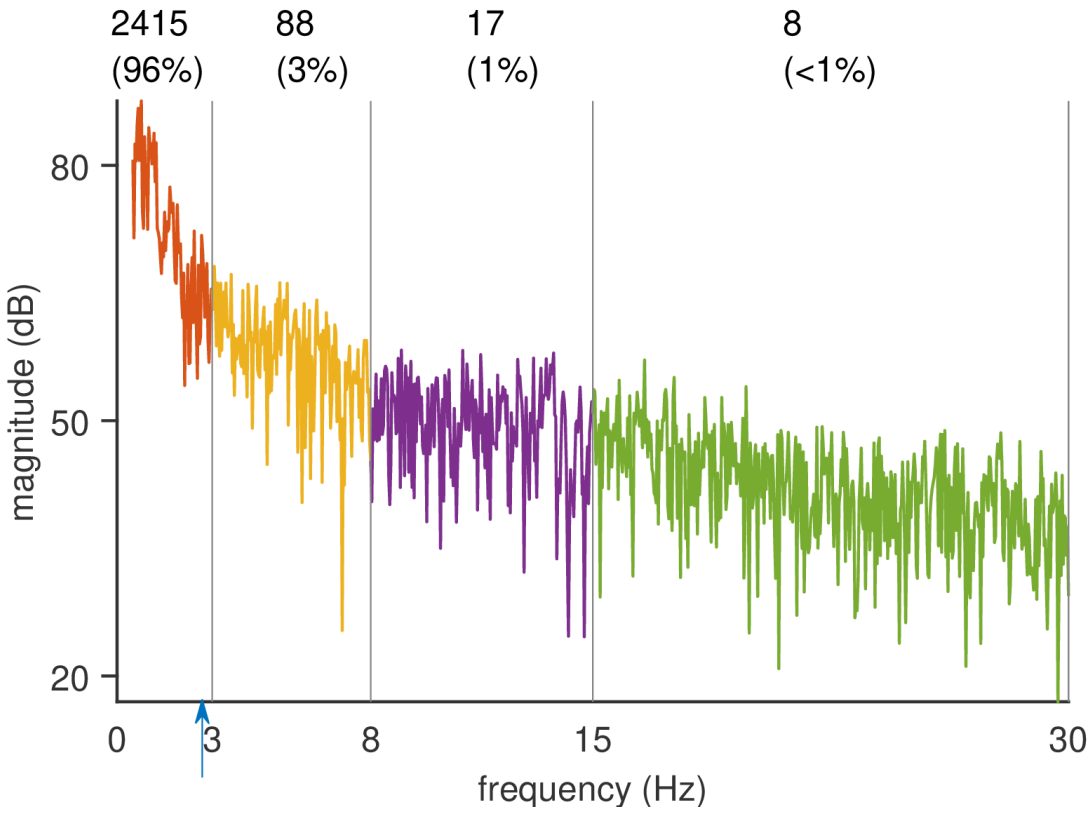


Figure 1.15: Quantitative EEG analysis: spectrum of one channel of EEG in decibels. For each frequency band, spectral power values are displayed at the top of the figure (μV^2) and relative-spectral power as %. Blue arrow marks the spectral edge frequency. Adapted from O'Toole et al. 2019. (238)

Features used to describe connectivity include correlation and coherence between the hemispheres.

Care is needed in quantitative EEG analysis, as it is very sensitive to artefacts. Artefacts must be identified and minimised prior to analysis. Improved automated artefact detection is required, until then, this remains a manual task. Nonetheless, the advantage of quantitative EEG is that it is automated and is therefore easily scaled to analyse multiple EEGs. Many groups are in the process of developing automated EEG analysis programmes. (233, 239, 240, 241, 242) As mentioned previously, seizure detection algorithms have proven very useful in the detection of seizures and many aEEG systems have incorporated background analysis and seizure detection algorithms into their devices. (233) If helpful and clinically meaningful features are identified in infants with HIE, quantitative EEG analysis may also pave the way for automated analysis, which would ultimately provide continuous 24/7 reporting of the EEG at the bedside in the Neonatal Unit.

1.4.1.2. Near-infrared Spectroscopy

NIRS allows for continuous, non-invasive monitoring of tissue oxygenation in critically ill infants in the NICU. First used in adult care in 1977, (243) NIRS has been in use now for almost 40 years, but has grown in popularity over the past decade. (244) It provides an indirect assessment of end-organ oxygenation status and thus, indirectly, perfusion. The simplicity of its application and the availability of a continuous measure of end-organ perfusion at the bedside makes it appealing to use in clinical practice.

NIRS is based on the transparency of biological tissues to light, specifically the near infrared region of light (700-1000nm). When light is passed through a tissue by an emitting electrode, it is either absorbed, scattered or reflected by the tissue. The reflected light is detected by a detector that is usually parallel to the emitting electrode. Multiple detecting receptors are used to reduce the influence of scattering, as the amount of scattering is assumed equal at each detector. If the distance between the emitting and receiving electrodes, the amount of scattering and the wavelength and angle of incidence of the light is known, the amount of light absorbed by the tissue can be calculated.

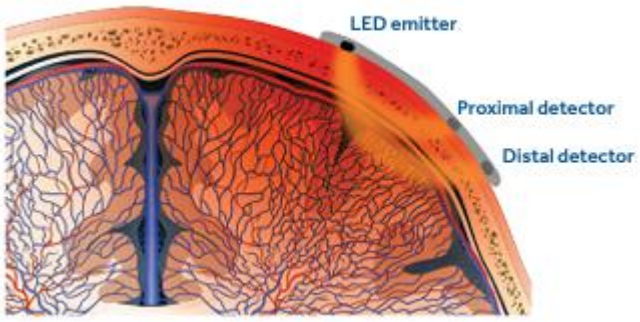


Figure 1.16: Principles of the INVOS® near-infrared spectroscopy technique. Reproduced from Medtronic (USA). (245)

Neonates have relatively thin overlying skin and bone allowing for easier penetration of light into the underlying tissue. As skin, muscle and bone remain relatively stable, changes in values must be due to changes in the underlying tissue. Light is absorbed by chromophores i.e. tissue components such as haemoglobin. Oxygenated and deoxygenated haemoglobin have different absorption patterns therefore giving information about absorption characteristics and components of the underlying tissue thus creating the potential for its application as a useful monitoring tool.

NIRS is different to traditional pulse oximeters, which differentiate between the pulsating arterial blood and the slow-moving venous blood to determine peripheral oxygen saturation, which correlates well with and thus acts as a surrogate for arterial oxygen saturation (SaO_2). In contrast, regional tissue oxygenation (rSO_2) as measured by NIRS is a combination of arterial, venous and capillary blood and reflects the balance between oxygen supply to the tissue and consumption. Interpretation requires careful consideration of all confounding and influencing factors.

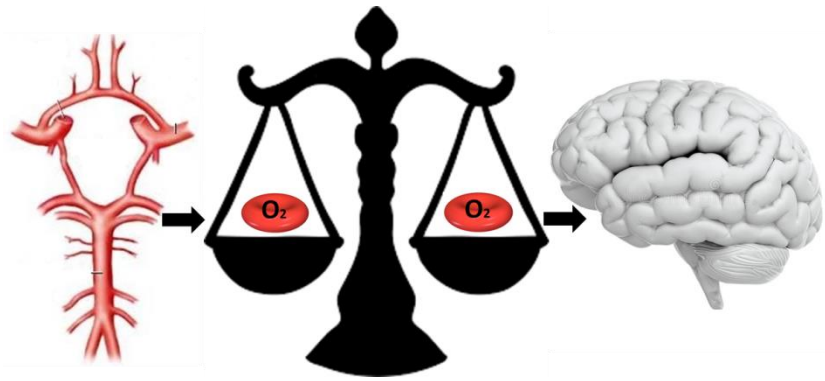


Figure 1.17: Reflecting the balance between oxygen supply and oxygen consumption.
Original diagram from Garvey AA et al. 2018 (246)

Somatic NIRS (abdominal and renal) has been investigated for its ability to detect early changes in peripheral perfusion particularly in preterm infants at risk of necrotising enterocolitis (NEC). (247, 248) Most NIRS studies in neonates to date however have described cerebral NIRS (cNIRS) to provide an assessment of cerebral oxygenation (cSO_2).

cNIRS provides an important window into the cerebral haemodynamic status of the neonate by providing an assessment of end organ perfusion and performance. For cNIRS, sensors are generally applied on the right or left frontoparietal or temporoparietal areas to avoid the central venous sinus. Lemmers et al. compared NIRS values from probes recording simultaneously from right and left frontoparietal areas in 36 preterm infants and found good correlation between the sides ($r=0.89$, $p<0.01$). (249) In addition, when different regions of the brain were measured simultaneously (left and right, frontoparietal and temporo-occipital) in a group of term and preterm infants, NIRS values did not differ between brain regions in the early neonatal period. (250)

Different forms of NIRS currently exist, the most prevalent of which is continuous wave NIRS. A constant frequency and amplitude of light is utilised, therefore any changes in absorption of light is relative to changes in absorption of the light by haemoglobin (Hb). A limitation of this modality is that it does not differentiate

between absorption and scattering of the light. Nonetheless, it is a useful trend monitor and cost effective.

Frequency domain NIRS uses varying amplitudes at high frequencies. This allows for more accurate quantification of both the scatter and absorption of light and thus a quantitative measure of absolute concentrations of oxygenated Hb. Time domain NIRS utilises short pulses of NIRS. Scattering of light and absorption will result in protons reaching the receptor at varying times. From this information, absolute measures of absorption of oxygenated haemoglobin can be obtained. Other types of NIRS includes broadband NIRS which uses white light and measures the oxidation state of cytochrome-c-oxidase and diffuse correlation spectroscopy which uses speckle patterns to quantify blood flow. Although each of these modalities provide more accurate measures of cerebral oxygenation and blood flow, they require specialised equipment which to date, are costly and bulky. (251) All commercially available NIRS monitoring devices use continuous wave NIRS and for this reason, this thesis will focus on the use of continuous wave NIRS.

Many different devices are available and approved for use in neonates, the most common of which are the INVOS (Covidien, USA), NIRO (Hamamatsu, Japan) and Foresight (CAS Medical, USA). Each device uses a slightly different algorithm and terminology to report cSO_2 values; INVOS measures cSO_2 whereas the NIRO reports tissue oxygen index (TOI) and the Foresight displays cerebral tissue oxygenation ($\%Sct0_2$), but studies have shown good correlation between devices. (252, 253, 254, 255) To further complicate interpretation, the INVOS system has three different probes available (adult, paediatric and neonatal) which can result in differences of up to 10% depending on which probe is used. (256)

Despite the variability between devices and probes, groups have suggested normative ranges. One of the largest cohorts of term infants in the neonatal unit was reported by Weiss et al. This group used the NIRO 300 to measure TOI in 155 infants from 0-365 days of age and described expected or “normal” readings for this cohort as 61% +/-12%. (257) Bernal et al. used the INVOS oximeter with a neonatal probe to

measure cSO_2 in 26 term infants over the first 5 days of life. (258) The overall average cSO_2 was 77.9% +/- 8.5% and cSO_2 decreased over the first 120 hours.

This variation in values between device and probe configurations is certainly a limitation of NIRS monitoring. Furthermore, removing and reapplying the sensors in the same region can result in a variability of up to 6%. (259) Devices report absolute values which can vary depending on interventions that influence the cerebral metabolic rate such as TH or medications. These values are also averaged over time and thus may not take into account variations in values over the recording time. Alternative signal processing methods of cNIRS signals may provide more important information. (260)

cNIRS is therefore most helpful as a trend monitor which has the potential to provide essential information on end organ oxygenation and perfusion and may provide guidance as to the need and effect of interventions.(Figure 1.18 and Figure 1.19)

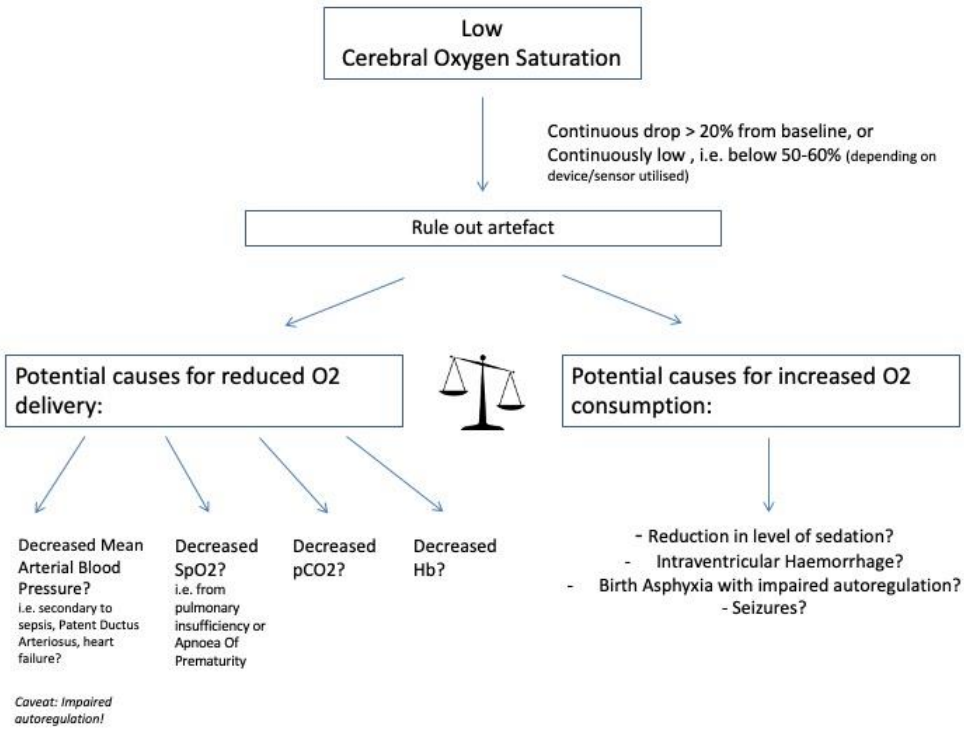


Figure 1.18: Interpretation of low cerebral NIRS values.
Original diagram from Garvey AA et al. 2018 (246)

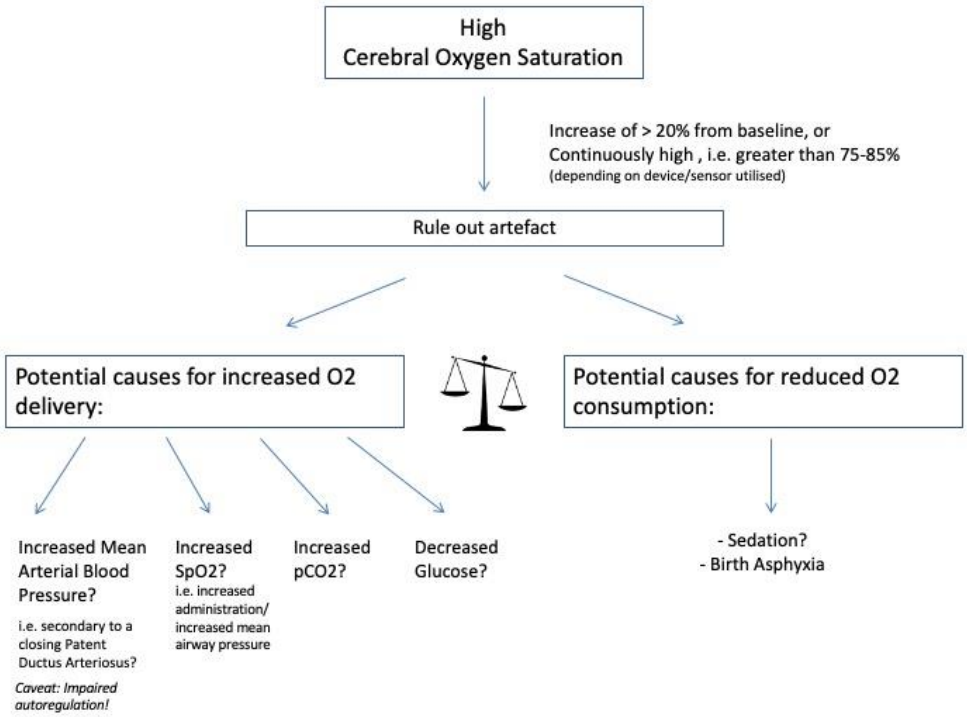


Figure 1.19: Interpretation of high cerebral NIRS values.
Original diagram from Garvey AA et al. 2018 (246)

Only one study has attempted to validate the use of NIRS in neonates. Alderliesten et al. found a strong correlation between MRI-derived measures of frontal and whole brain cerebral blood flow and venous oxygen saturation in the sagittal sinus and NIRS derived measures of cerebral oxygenation (R^2=0.71, 0.50, 0.65, p<0.01). (261) Studies examining its use in infants and young children during cardiac surgery also show good correlation across the devices. (262, 263, 264) As the cSO_2 value is a mixture of venous, arterial and capillary blood, even in adults and older children, there is no gold standard against which it can be compared. As cSO_2 is predominantly venous (75%), (265) validation studies have focused on comparing cSO_2 values to measures of venous saturation. Nagdyman et al. showed significant correlation between TOI values as measured by the NIRO oximeter and venous oxygen saturation in the jugular bulb in children with congenital heart defects during cardiac catheterisation. (262) Knirsch et al. found a close correlation between cSO_2 and central venous oxygen saturation when using the INVOS oximeter in a similar cohort (r=0.728, p<0.0001). (263) In a neonatal population, Wintermark et al. compared MRI

measurements of perfusion with cSO_2 values taken just before and after the MRI. There was a strong correlation between cerebral blood flow and cSO_2 (r=0.88; p value = 0.0085). (266)

Preclinical studies have examined the relationship between cNIRS and brain injury. Hou et al. found that in newborn piglets, cSO_2 values <40% were associated with injury to the functional zones of the mitochondria in the neurons of the hippocampus. (267) Interestingly, when cSO_2 values were between 30-40%, brain injury still occurred despite a stable mean arterial blood pressure and EEG recording. (267) Similarly Kurth et al. found evidence of functional brain impairment such as raised lactate, reduced ATP and EEG changes when cSO_2 values fell below 33-44% in their cohort of piglets. (268)

Clinical information comes mainly from studies involving infants undergoing cardiac surgery. Dent et al. found that in a cohort of infants undergoing the Norwood procedure for hypoplastic left heart syndrome, prolonged (>180minutes) postoperative cSO_2 values of <45% was associated with new or worsening ischaemia on MRI (p=0.029). (269)

Another measurement which can be helpful is fractional tissue oxygen extraction (FTOE). This is calculated by combining the cNIRS value with the peripheral oxygen saturation to give an estimate of oxygen consumption of the brain and thus is a surrogate for cerebral metabolism. It is calculated as follows and is inversely related to cerebral blood flow.

$$\frac{(SpO_2 - cSO_2)}{SpO_2}$$

This parameter correlates well with fractional oxygen extraction (FOE) measured in newborn piglets. (270) It provides further information to oxygen delivery alone and thus a better understanding of end organ performance as it can help differentiate, for example, between hypoxia and ischaemia. An increased cSO_2 could be as a result

of any of the conditions outlined above in Figure 1.19 but when combined with a decreased FTOE value, suggests less oxygen uptake by the tissue relative to oxygen delivery as may be seen during the secondary energy failure in HIE. (271) It also has the advantage of compensating for low arterial oxygen levels as can be seen in infants with congenital heart defects or preterm infants with lung disease, (272) and therefore may be a better measurement of cerebral perfusion.

cNIRS values can also be used as a surrogate for cerebral blood flow and perfusion (273) and when combined with blood pressure (BP), can provide important information on the autoregulation capacity of the brain. Cerebral autoregulation is the ability of the brain to maintain a constant cerebral blood flow in spite of alterations in cerebral perfusion pressure (CPP). (274) As CPP cannot be directly measured, many groups have looked at mean arterial BP or heart rate as surrogates for CPP. Figure 1.20 and Figure 1.21 show fluctuations in mean arterial BP and the subsequent effect on cNIRS values in times of intact and absent autoregulation.

Autoregulation can be measured in both time (correlation i.e. the linear relationship between 2 variables) and frequency (coherence) domains (275) and both have been studied in the context of HIE (276, 277, 278, 279, 280) however debate surrounds which is the best measure of autoregulation. Animal studies have found that coherence alone is a poor measure of autoregulation. (281) Govindan et al. verified this by using a piglet model of HIE to investigate and compare the different methods of assessing cerebral autoregulation with invasive intracranial pressure monitoring during both hypothermia and normothermia. Correlation was a better measure of autoregulation and was not affected by temperature. (282) Preterm studies have suggested similar findings although clinical studies lack a gold standard measurement for comparison as invasive measures of CPP are not possible in neonates. (283) Transcranial Doppler has been shown to be sensitive to fluctuations in cerebral blood flow and new ultrasound systems are being developed to allow continuous measurements. (284, 285)

Autoregulation has been studied extensively in preterm infants in whom autoregulation may be absent or impaired. (286, 287) Autoregulation seems to also be affected in infants with HIE. (288) Failure to maintain a steady cerebral blood flow may further compound HI injury. Studies have utilised cNIRS values to determine a BP range at which optimal cerebral blood flow was maintained and found that longer time spent outside these values resulted in increased risk of cerebral injury and subsequent poor neurodevelopmental outcome. (278, 279, 289) Monitoring an infant's autoregulatory capacity may be an important consideration in optimising neuroprotection and, ultimately, outcome in these infants.

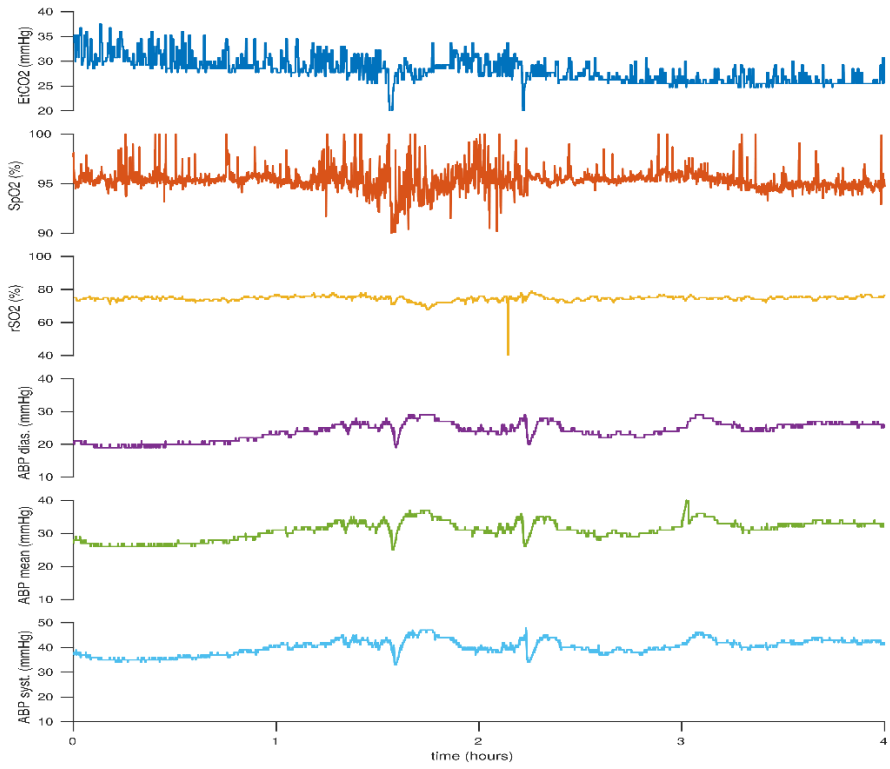


Figure 1.20: Example of intact autoregulation-rcSO₂ remains stable despite changes in blood pressure.
Dark blue line represents end-tidal CO₂. Orange line represents peripheral oxygen saturation. Yellow line represents cerebral oxygenation measured by NIRS. Purple, green and light blue lines represent diastolic, mean and systolic arterial blood pressure values respectively. Original diagram from Garvey AA et al. 2018 (246)

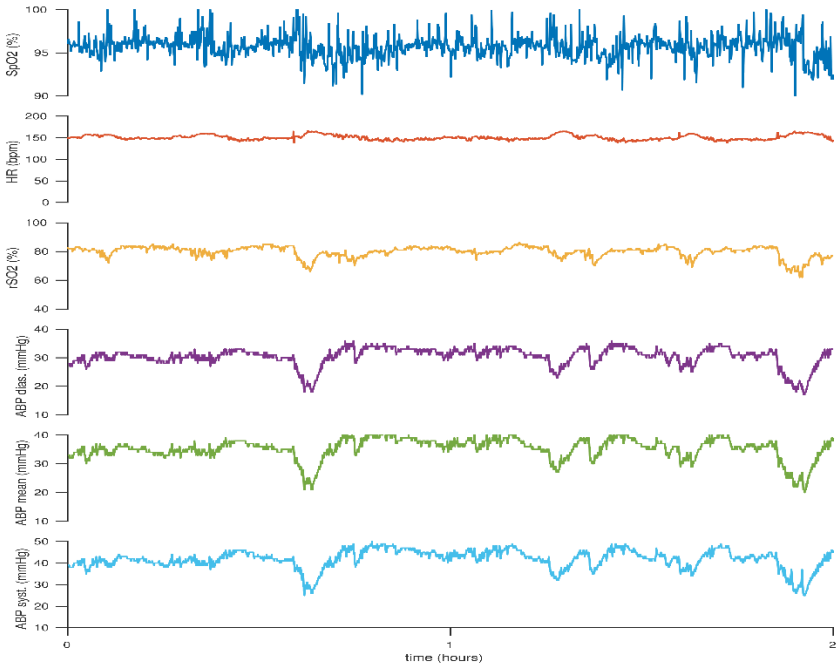


Figure 1.21: Example of impaired autoregulation-decreases in rcSO₂ in association with decreases in blood pressure.
Dark blue line represents peripheral oxygen saturation. Orange line represents heart rate. Yellow line represents cerebral oxygenation measured by NIRS. Purple, green and light blue lines represent diastolic, mean and systolic arterial blood pressure values respectively. Original diagram from Garvey AA et al. 2018 (246)

1.4.1.2.1. NIRS and HIE

HI injury results in a period of hypoperfusion at the time of initial insult followed by circulatory restoration. (290) Cerebral blood flow has been shown to increase in the first 24 hours following HI injury likely due to a disruption in haemodynamic control. (291, 292) This early hyperperfusion is associated with an increased risk of brain injury. (293) cSO_2 values have been shown to correlate with MRI findings of brain perfusion. (266) Therefore, early cerebral NIRS may contain important information that could aid in the prediction of outcome.

Higher cSO_2 and lower FTOE values, particularly beyond 24 hours of life have been associated with MRI abnormality. (294, 295) As there is a correlation between

specific patterns of changes in oxygenation and severity of brain injury, (296, 297, 298) cNIRS has the potential to be utilised as a prognostic tool.

cNIRS may also predict neurodevelopmental outcome. Toet et al. found that in the pre-TH era, infants with a higher cSO_2 value and lower FTOE beyond 24 hours had a greater risk of poor neurodevelopmental outcome at 5 years (271) and these findings have been replicated in the TH era. (271, 294, 299) Ancora et al. again showed that higher cSO_2 values were associated with poor neurodevelopmental outcomes at 18 months. (300) Although this trend was evident as early as 6 hours of age, it was not significant until 24 hours of age. These findings are likely due to a combination of increased cerebral perfusion and decreased utilisation of oxygen which may correlate with the secondary energy failure in HIE as neuronal death may also contribute to a decreased uptake in these cells. (301, 302, 303)

cNIRS can be used in combination with other monitoring tools such as BP and EEG. When combined with BP, cNIRS can provide an estimation of the autoregulatory capacity of the brain in HIE. Many groups have used cNIRS to identify the optimal mean arterial pressure (MAPopt) in which cerebral perfusion pressure was best maintained. (277, 278, 279, 302, 304, 305) All have shown that greater time spent outside the MAPopt range was associated with an increased risk of brain injury and this appears to be independent of the original degree of birth asphyxia. (279) Lemmers et al. found that at 12-18 hours, cSO_2 alone had a positive predictive value of 67% and a negative predictive value of 75% for abnormal MRI and/or adverse neurodevelopmental outcome at 18 months of age. However when cSO_2 and aEEG were used in combination, these values increased to 91% and 100% respectively. (295) Goeral et al. also found that combining NIRS with aEEG and BP improved the prediction of MRI injury. (295) These studies highlight the potential for combining data from different modalities to improve prediction. Each provides information relating to a different aspect of the underlying neurophysiology and when used in combination may allow the clinician to generate a better overall impression of the neurological status of the infant. Multi-modal monitoring may be more helpful than assessing each of these monitoring modalities in isolation.

NIRS use in infants with HIE is still in its infancy with many questions yet to be answered. Small studies using variations in devices and probes dilutes the currently available evidence and poses significant challenges in developing normative data. No centile charts currently exist for normal cSO_2 values in term infants in the first week of life. (306) Only two studies have described cSO_2 values in term infants over the first 3 days of life thus making it difficult to identify deviations from the norm. (306) Information on the use of early NIRS in HIE is limited and its early use in infants with mild HIE is almost non-existent. (307) The underlying physiology of HI injury and mechanism of NIRS monitoring places it in a prime position as a useful biomarker however its usefulness as an early indicator of severity or its use in mild HIE have yet to be studied. Studies have suggested a correlation between the severity of the brain injury, neurodevelopmental outcome and changes in the cerebral oxygenation of infants with HIE. Certainly, beyond 24 hours, its ability to predict both short and long-term adverse outcome is clearer. However, the question of whether early NIRS can predict outcome has yet to be answered.

1.4.2. Haemodynamics

1.4.2.1. Cardiac Output

In adults and older children, different invasive methods of measuring cardiac output (CO) have been reported including thermodilution, pulse contour analysis, oesophageal doppler and CO_2 rebreathing techniques. Thermodilution is considered gold standard for the continuous measurement of CO. This technique involves giving a bolus of a specified volume of cold or room temperature saline into the right atrium where it is mixed with blood. A catheter placed in the pulmonary artery detects the resultant drop in blood temperature and from this information, cardiac output can be calculated. This method is not feasible in neonates however due to the invasive nature of the catheter placement and the smaller anatomy of the infants in question.

Therefore, echocardiography is predominantly used to calculate cardiac output in neonates through measurements of blood velocity and diameter of the left or right ventricular outflow tract. Care is needed in interpretation of these measurements

however. Although adult studies have shown good correlation between measurements obtained from echocardiography and thermodilution, (308) echocardiography measurements of aortic diameter may overestimate left ventricular outflow (LVO), (309) an important consideration in neonates where precision of measurements can involve a fraction of a millimetre. Ventricular outflow values can also be affected by significant shunting due to a patent ductus arteriosus (PDA) or a patent foramen ovale (PFO). Although no studies have directly compared echocardiography measurements of CO with invasive measures such as thermodilution in neonates, Alverson et al. found that echocardiogram-derived measurements of CO were similar to values previously reported during cardiac catheterisation. (310) For this reason, point-of-care echocardiography is currently the primary method of measuring cardiac output in neonates. However not all neonatal units have access to trained personnel or indeed an echocardiography machine. In addition, it only provides a snapshot in time of cardiac function. Continuous, reliable, non-invasive measurements are required.

Non-invasive monitoring in the neonatal unit allows for continuous, bedside monitoring of the sickest and smallest of infants with minimal handling. Non-invasive monitoring of cardiac output has grown in popularity in neonatology over the past 15 years. Still used predominately in a research capacity, it has the potential to provide valuable information on the haemodynamic state of the infant. Non-invasive measures of cardiac output involve passing a low amplitude, high frequency electrical current through the thorax. The change in electrical current between emitting and receiving electrodes is due to the resistance of different tissues to current flow. This change over time represents changes in stroke volume (SV), which, when combined with HR can provide an estimation of CO. Non-invasive cardiac output monitoring generally uses one of two methods: bioimpedance and bioreactance.

Bioimpedance passes an electrical signal through the thorax, which is received by recording electrodes. By knowing the frequency and amplitude at both the emitting and receiving electrodes, it can calculate the resistance to conductivity. In traditional bioimpedance devices, resistance was due to different volumes of blood in the aorta

at different points in the cardiac cycle. The aorta expands during systole due to increased volume which results in increased resistance and is conversely decreased during diastole. CO can then be calculated from the change in resistance during different points in the cardiac cycle. A disadvantage of bioimpedance is that it is dependent on the distance between the electrodes so exact placement is crucial. Furthermore, it is susceptible to electrical "noise" or artefact and questions have been raised about its accuracy. (311) To overcome these issues, a technique known as electrical cardiometry (EC) has been developed. Using a similar theory, EC uses the resistance caused by the orientation of red blood cells (rbc) in the aorta. During systole, blood flow through the aorta results in alignment of the rbc and thus less resistance to conduction of the electrical signal. During diastole, rbc assume a random pattern resulting in increased resistance. SV is estimated using the following equation:

$$SV = CP \times v_{ft} \times FT$$

SV, stroke volume, CP, patient constant (mls); v_{ft}, mean blood velocity index (s^{-1}); FT, flow time (s)

EC values appear to be significantly affected by the presence of a PDA or a PFO and presence of both results in a cumulative effect and overestimation of CO. (312, 313) Although EC seems to overcome the accuracy issues associated with traditional bioimpedance methods, (314, 315, 316, 317) validation studies are lacking. Another important consideration of EC is that the bodyweight and length of the infant must be inputted into the device prior to recording. The algorithm uses these measurements to account for the effects of distance between the sensors. (318) This information may not be readily available particularly in an unstable baby or immediately after delivery.

Bioreactance (BR) assumes that changes in electrical current over time are not solely related to resistance of tissues but also to the ability of tissues to store an electrical current (capacitance) and to store energy in a non-electrical form (inductance). This

technology uses phase shifts, i.e. the displacement of a signal from its usual or starting position, that occurs when an alternating current is passed through the thorax to measure LVO.(Figure 1.22) Four pairs of sensors are applied to the thorax (upper/lower, left/right) in a manner that boxes the heart.(Figure 1.23) Each pair of sensors includes a transmitting and receiving electrode. SV is estimated from both sides of the thorax using the following equation and measurements are averaged to provide a final measure of CO.

$$SV = C \times VET \times \frac{d\varphi}{dt_{max}}$$

SV, stroke volume; C, constant of proportionality; VET, ventricular ejection time; $d\phi/dt_{max}$, peak rate of change of phase shift.

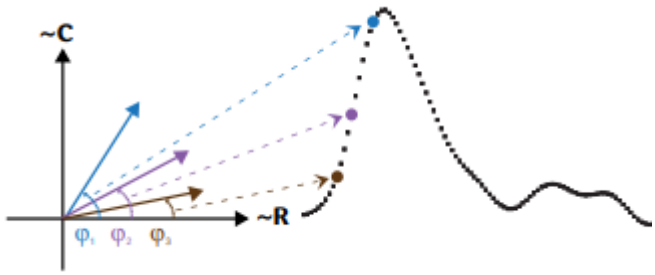


Figure 1.22: Computation of stroke volume using bioreactance.
Current is transmitted into the thorax via four transmitting sensors. Four receiving sensors detect the associated phase shift. This information is computed to generate an estimation of stroke volume. Reproduced from Cheetah Medical (USA) 2021. (319)

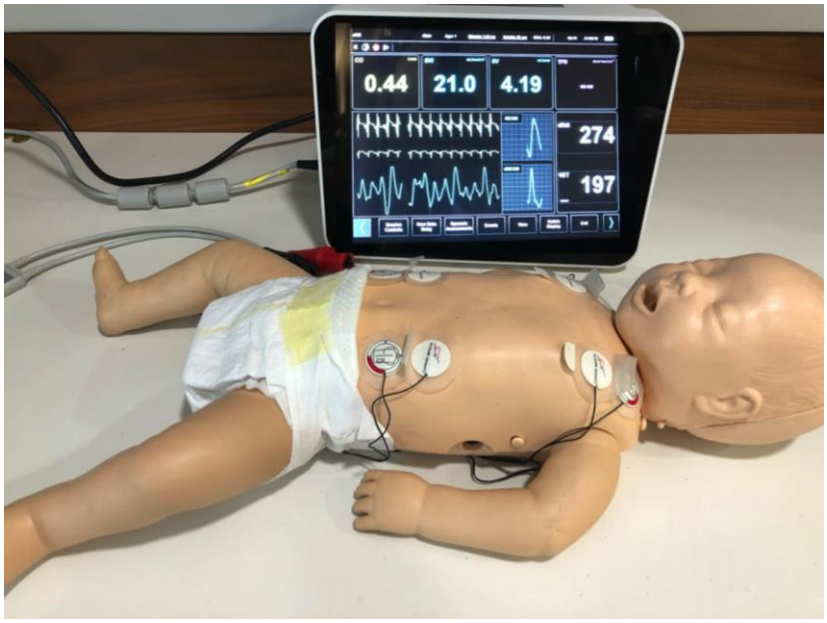


Figure 1.23: NICOM monitoring using Cheetah NICOM device©. Original diagram from McCarthy et al. 2021. (320)

Less influenced by electrical noise, its use has been validated in the adult population and correlates well with other measures of CO during mechanical ventilation, positive end-expiratory pressure (PEEP) and in adults who are spontaneously breathing. (321, 322, 323, 324, 325, 326, 327) Pre-clinical studies on small animals show good correlation between invasive CO measures and values obtained from non-invasive, bioreactance measures. (321) In the neonatal population, studies have examined its correlation with echocardiography measures of LVO. BR derived measures of CO are lower than measures obtained by echocardiogram but this difference is consistent across a range of measurements. (328, 329) This may be explained by two theories. Firstly, the algorithm used in the bioreactance system was initially based on adult data and aortic size and was subsequently extrapolated to newborns. This may account for the "under-reading" of CO. (330) Secondly, as mentioned above, echocardiography measurements may overestimate CO, so perhaps CO as measured by BR is closer to the true value. (329)

1.4.2.2. Effect of HIE on Cardiac Function

As discussed, in periods of hypoxia or asphyxia, activation of the sympathetic-adrenergic system results in vasodilation in vital organs and vasoconstriction in non-

vital organs. This allows the body to maintain an oxygenated blood supply to vital organs, specifically the brain and heart. (10, 11, 12, 13, 14) However, when the hypoxia is prolonged, this protective mechanism is lost.

Acute asphyxia leads to a significant increase in pulmonary and systemic vascular resistance. (331) With this increased afterload, the ventricles must work harder and thus require an increased oxygen supply. In addition, aortic pressure may increase, further impacting on coronary blood flow. (331)

Animal studies have shown that the myocardium relies heavily on circulating free fatty acids for energy. If these cannot be oxidised, as in times of hypoxia, the myocardium must rely on anaerobic glycolysis, stores of which deplete quickly. (332, 333)

Rowe and Hoffman were the first to describe myocardial ischaemia following acute hypoxia in 1972. (334) Barberi et al. performed electrocardiographs (ECG) and echocardiograms on infants post birth asphyxia and compared them to infants with mild respiratory distress syndrome and healthy term controls. They found that after birth asphyxia, there is evidence of ischaemic changes and left ventricular function is impaired. (335)

The papillary muscles and subendocardial areas are most at risk of ischaemia. The papillary muscles lie on the edge of coronary circulation so are the first to be compromised. (336, 337, 338, 339, 340, 341) Transmural infarction is rare. (342) Although many studies report insufficiency of the tricuspid valve following asphyxia, this effect appears to be transient in 30-80% of infants with severe ischaemia. (341, 343, 344, 345, 346)

Following birth asphyxia, we also see a reduction in left ventricular function and cardiac output. (347) Left ventricular function appears to be affected by the degree of encephalopathy. Wei et al. compared infants with mild and severe grades of encephalopathy with controls and found that infants with severe encephalopathy

had significantly reduced left ventricular function when compared to the other two groups at 24 hours. (348)

TH itself has been noted to cause hypotension and sinus bradycardia (5) and can also further decrease CO and myocardial function. (349, 350) Studies have shown that CO increases during the rewarming phase but this increase appears to be related to increased contractility as well as an increase in heart rate. (351, 352) Interestingly, in these studies, systemic BP was similar during hypothermia and normothermia.

Rakesh et al. conducted an RCT to examine the effect of hypothermia on cardiac function. Infants with encephalopathy were randomised to receive hypothermia or normothermia. Measurements of cardiac function included cardiac enzymes, ECG and echocardiography. They found increased levels of cardiac enzymes in both groups suggesting myocardial injury and these decreased over time. In the hypothermia group, however, these decreased quicker. Although they do not define what specific echocardiography measures of cardiac function they measured, they conclude that ECG and echocardiography findings were similar in both groups at randomisation but myocardial function was better in the hypothermia group by 72 hours of age suggesting hypothermia has a protective effect on myocardial function. (353)

A reduction in CO may further complicate the underlying brain injury due to reduced cerebral perfusion. (354, 355) Blood pressure and cardiac output are not interchangeable; (356, 357) CO may be affected despite a consistent BP. (351, 352) For this reason, it is important to have relevant haemodynamic information available to the clinician. (358)

Non-invasive CO monitoring in infants with HIE is feasible and correlates with echocardiography findings. (352) However, only three studies to date have used non-invasive CO monitoring in infants with HIE (one using BR and two using EC) and these studies have focused on the effect of therapeutic interventions such as TH on CO rather than monitoring the effect of the encephalopathy itself.

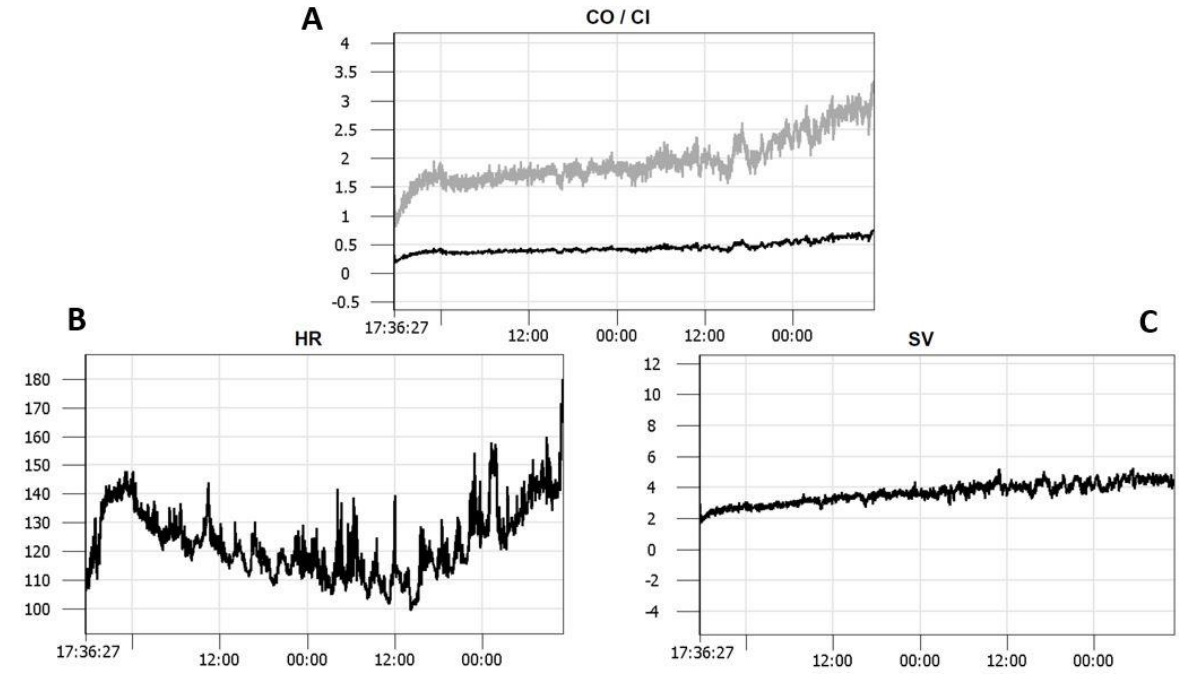


Figure 1.24: NICOM signal demonstrating an increase in CO, SV and HR over approximately 70 hours of recording.
CO is represented by the black line in image A and is displayed as L/min. HR is displayed as beats/min (B). SV is displayed as mls (C).

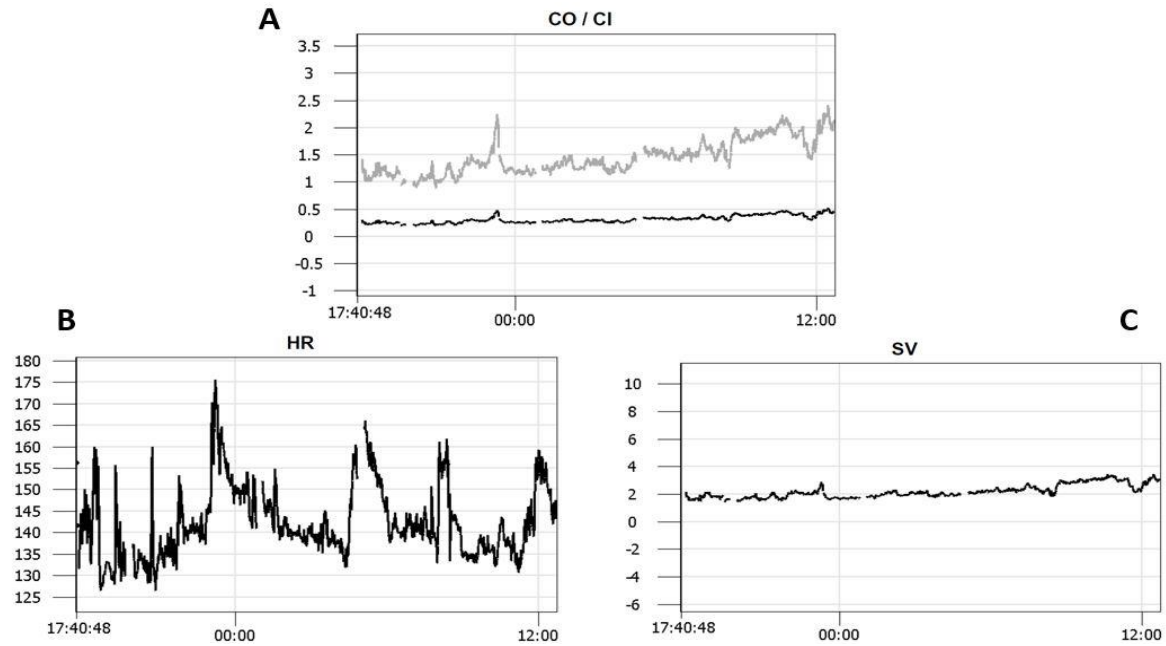


Figure 1.25: NICOM signal demonstrating a relatively stable CO despite fluctuations in HR.
The above images represent approximately 20 hours of recording.CO is represented by the black line in image A and is displayed as L/min. HR is displayed as beats/min (B). SV is displayed as mls (C).

Forman et al. measured CO non-invasively in 20 infants with moderate-severe HIE during TH and rewarming using BR. (359) CO increased during rewarming due to an increase in HR. Eight of the included infants had serial echocardiography measurements of CO performed during the recording period. Similar to previous studies, echocardiogram-derived measurements of CO were approximately 27% higher than non-invasive measures however this difference was consistent and there was a strong correlation between the two measurements.

Wu et al. used EC and echocardiography to measure CO in 20 infants with moderate to severe HIE during the rewarming period. (360) CO increased during this time predominantly due to an increase in HR. Mean arterial BP decreased during the rewarming period.

Eriksen et al. also used EC to examine the effect of TH on CO. (361) They included 15 infants with moderate and severe HIE and 10 healthy term controls. Five of the infants with HIE had CO measurements available before TH was commenced. These infants had lower CO and SV when compared to controls suggesting an effect of the encephalopathy itself. CO further decreased during TH due to a decrease in HR. In this study, they also assessed the impact of low CO on lactate clearance. Rate of lactate clearance did not correlate with CO but instead correlated with highest Thompson score.

Birth asphyxia results in transient myocardial ischaemia and myocardial dysfunction which may further complicate cerebral perfusion. (335) Monitoring blood pressure alone is not sufficient to detect infants who may benefit from haemodynamic support. (356, 357) Continuous CO monitoring in infants with HIE is relatively novel predominantly due to limitations associated with the accessibility and availability of echocardiography. Non-invasive CO monitoring may provide an accessible, easy to interpret solution however further research is warranted. There is an obvious paucity of data on non-invasive CO monitoring in infants with HIE and no study to date has examined its use in infants with mild HIE.

1.5. Magnetic Resonance Imaging

MRI is the radiological imaging method of choice for brain imaging in infants with HIE. MRI outcome is highly correlated with long-term neurodevelopmental outcome and is therefore, in the short-term, a good surrogate marker of outcome. (362, 363) It is superior to ultrasound at detecting injury in the deep grey matter (364) and is free from ionizing radiation. It utilises magnetic field and radio waves to produce an image. MRI provides a detailed assessment of the infant's brain structure and more recently, its function.

MRIs generate a strong magnetic field around the organ in question to excite predominantly hydrogen atoms in the body and cells. Excited atoms emit a radio frequency, which varies depending on the magnetic field and is detected by a receiving coil. The rate at which the atoms switch from the excited to the equilibrium phase is measured and this creates the contrast between tissues. Different pulse sequences vary in repetition and echo times and result in different imaging sequences to be generated.

The strength of the magnetic field is measured in tesla (T). Higher strength magnets allow for higher quality images to be obtained in shorter periods of time. (365) 1.5T scanners are the most widely available MRI scanners in clinical practice although availability of 3T scanners is increasing. MRI studies in neonates to date have for the most part reported 1.5T MRI outcome and current recommendations provide guidance on the optimal sequences and timing of the scan rather than the strength of scanner itself. (366) The main disadvantage of MRI in neonates is the necessity to transport the infant to the radiology department. For this reason, lower strength scanners that can be installed directly in the unit are currently under investigation. (367)

The most common sequences reported in neonatal imaging are T1 and T2 weighted images, diffusion weighted imaging (DWI) and MRS. T1 and T2 images are useful for assessing grey and white matter abnormalities and anatomical structure. DWI measures the diffusion of water molecules in a tissue and is the best modality to

detect acute infarction. MRS is used to measure different levels of metabolites in a particular tissue.

A combination of T1, T2 and DWI sequences of the brain are best for identifying HI injury (368) and MRI is considered gold standard for assessing brain injury in infants with HIE. (146, 147, 369)

Many scoring tools are available for assessing MRI injury, the most well-known include the Barkovich, NICHD, Rutherford and, more recently, the Weekes scoring systems. (147, 362, 363, 370, 371) The individual scoring systems are outlined below. They differ in the areas and sequences assessed, for example the Barkovich score does not specifically assess the posterior limb of the internal capsule (PLIC). The NICHD and Rutherford scoring systems do not assess DWI and include scans after 2 weeks of age. The Weekes score is very comprehensive and more labour-intensive than the others but all have all been validated and strongly correlate with long-term outcome. (147, 363, 370, 372) We chose the Barkovich score to assess short-term outcome in our cohort as it is concise, user friendly and has demonstrated good inter-observer agreement. (373) Furthermore, it has been utilized in many studies of infants with HIE allowing for a more direct comparison of outcome.

Table 1.5: NICHD MRI Scoring System.

0	Normal
1A	Minimal cerebral lesions only with no involvement of BG, T, ALIC, PLIC and no area of watershed infarction
1B	More extensive cerebral lesions without BG, T, ALIC or PLIC involvement or infarction
2A	Any BG, T, ALIC or PLIC involvement or watershed infarction noted without any other cerebral lesions
2B	Involvement of either BG, T, ALIC or PLIC or area of infarction and additional cerebral lesions
3	Cerebral hemispheric devastation

BG, basal ganglia; T, thalamus; ALIC, anterior limb of the internal capsule; PLIC, posterior limb of the internal capsule

Table 1.6: Barkovich MRI Scoring System.

Basal Ganglia (BG)
0 Normal or isolated focal cortical infarct
1 Abnormal signal in thalamus
2 Abnormal signal in thalamus and lentiform nucleus
3 Abnormal signal in thalamus, lentiform nucleus and perirolandic cortex
4 More extensive involvement
Watershed (W)
0 Normal
1 Single focal infarction
2 Abnormal signal in anterior or posterior watershed white matter
3 Abnormal signal in anterior or posterior watershed cortex and white matter
4 Abnormal signal in both anterior and posterior watershed zones
5 More extensive cortical involvement
Basal ganglia/watershed (BG/W)
0 Normal
1 Abnormal signal in basal ganglia or thalamus
2 Abnormal signal in cortex
3 Abnormal signal in cortex and basal nuclei (basal ganglia or thalami)
4 Abnormal signal in entire cortex and basal nuclei
Summation (S)
Arithmetic sum of BG and W
Enhancement (E)
0 No enhancement
1 Enhancement in white matter only
2 Enhancement in deep grey matter nuclei
3 Enhancement in cerebral cortex
4 Enhancement in cortex and deep grey matter or white matter

Table 1.7: Rutherford MRI Scoring System.

Posterior limb of the internal capsule score
0 Normal
1 Equivocal (reduced or asymmetric signal intensity)
2 Loss (reversed or abnormal signal intensity bilaterally on T1 and/or T2 weighted sequences)
Basal ganglia and thalamic score
0 Normal
1 Mild (focal abnormality signal intensity)
2 Moderate (multifocal abnormal signal intensity)
3 Severe (widespread abnormal signal intensity)
White matter score
0 Normal
1 Mild (exaggerated long T1 and long T2 in periventricular white matter only)
2 Moderate (long T1 and long T2 extending out to subcortical white matter and/or focal punctate lesions or focal area of infarction)
3 Severe (widespread abnormalities including overt infarction, haemorrhage and long T1 and long T2)
Cortical involvement*
0 Normal
1 Mild (1-2 sites involved)
2 Moderate (3 sites involved)
3 Severe (more than 3 sites involved)

**Scored as the presence of abnormal signal intensity, usually decreased T1 or cortical highlighting. The sites documented included specifically the central sulcus, interhemispheric fissure and the insula*

Table 1.8: Weekes Scoring System.

Items	Sequence used to assess injury	Degree		
		0	**1**	**2**
Grey matter				
1 Thalamus abnormal SI or diffusion restriction Specify location	T1/T2 DWI	No	Focal (<50%) Unilateral	Extensive (≥50%) Bilateral
2 Basal ganglia abnormal SI or diffusion restriction Specify location	T1/T2 DWI	No	Focal (<50%) Unilateral	Extensive (≥50%) Bilateral
3 PLIC myelination or diffusion restriction Specify location	T1/T2 DWI	Normal or no diffusion restriction	Equivocal/partly myelinated or partial (<50%) diffusion restriction Unilateral	Absent myelination or extensive (≥50%) diffusion restriction Bilateral
4 Brainstem abnormal SI or diffusion restriction Specify location	T1/T2 DWI	No	Focal (<50%) Unilateral	Extensive (≥50%) Bilateral
5 Peri-rolandic cortex diffusion restriction Specify location	DWI	No	Mild Unilateral	Clear Bilateral
6 Hippocampus diffusion restriction Specify location	DWI	No	Yes Unilateral	Yes Bilateral
Grey matter subscore				
Basal ganglia NAA	1H-MRS	Normal	Reduced	
Basal ganglia lactate	1H-MRS	Absent	Increased	
Grey matter subscore (including 1H-MRS)				

Items	Sequence used to assess injury	Degree		
		0	1	2
Items	**Sequence used to assess injury**	**Degree**		
		0	**1**	**2**
White matter/cortex				
1 Cortex abnormal SI or diffusion restriction not being peri-rolandic cortex Specify location	T1/T2 DWI	No	Focal (1 lobe) Unilateral	Extensive (>1 lobe) Bilateral
2 White matter increased SI or diffusion restriction not being PWML Specify location	T1/T2 DWI	No	Focal (1 lobe) Unilateral	Extensive (>1 lobe) Bilateral
3 PWML Specify location	T1/T2 DWI, SWI	No	<6 Unilateral	≥6 Bilateral
4 Haemorrhage not being PWML Specify location	T1/T2 SWI	No	Single haemorrhage <1.5cm Unilateral	≥1.5cm or multiple haemorrhages Bilateral
5 Optic radiation diffusion restriction Specify location	DWI	No	Mild Unilateral	Clear Bilateral
6 Corpus callosum diffusion restriction	DWI	No	Yes	N/A
White matter/cortex subscore				

Items	Sequence used to assess injury	Degree		
		0	1	2
Cerebellum				
1 Cerebellum abnormal SI or diffusion restriction	T1/T2 DWI	No	Focal (<0.5 cm)	Extensive (≥0.5 cm or multiple lesions)
Specify location			Unilateral	Bilateral
2 Cerebellar haemorrhage	T1/T2 SWI	No	Single haemorrhage <0.5cm	≥0.5 cm or multiple haemorrhages
Specify location			Unilateral	Bilateral
Cerebellum subscore				
Additional				
1 IVH	T1/T2, SWI	No	Yes	
2 SDH	T1/T2	No	Yes	
3 CVST	T1/T2, MRV	No	Yes	
Additional subscore				
Total score (grey matter + white matter + cerebellum + additional score)				

SI, signal intensity; PLIC, posterior limb of the internal capsule; NAA, N-acetyl aspartate; PWML, punctate white matter lesions; IVH, intraventricular haemorrhage; SDH, subdural haemorrhage; CVST, cerebral sinovenous thrombosis

The timing of when the MRI scan is performed is a very important consideration when interpreting the results. Injury may be evident on DWI sequences from the first day of life and is most obvious on days 2-3. Following this, there is a period of "pseudo-normalisation" which occurs around day 6-8, although this can be delayed to day 11-12 in infants receiving TH. (362, 374, 375) Hypoxic injury is not evident on conventional T1 and T2 weighted images until the first to second week of life (376) and therefore is not helpful in detecting or grading encephalopathy in the early postnatal period. (377)

Early MRI scans can be helpful in ascertaining the timing of the initial injury (378, 379, 380, 381) and although there were concerns that early DWI scans may underestimate the extent of the injury, (380, 382) studies have shown similar grades of injury between early and late scans. (383, 384)

Although DWI has been shown to be predictive of outcome, more robust evidence lies in a later scan and for this reason many centres still recommend performing MRI scans between days 10-14 of age.

Literature historically described two predominant patterns of injury in infants with HIE; deep nuclear grey matter injury and white matter injury (370, 376, 385, 386, 387) although other patterns of injury are increasingly being recognized. (366) Identifying certain patterns of injury can suggest timing of injury and is strongly correlated with long-term neurodevelopmental outcome. (362, 374, 385, 388) However, it must be noted that infants may demonstrate evidence of more than one pattern of injury.

Basal ganglia involvement is generally symmetrical and may extend to the peri-rolandic area. This pattern is usually associated with shorter but more profound asphyxia or a sentinel event but may also be seen in prolonged severe insults. (389, 390, 391) This type of injury pattern is present in 40-80% of cases. (362, 389, 392) Severe, acute, total hypoxic ischaemia may also involve the posterior limb of the

internal capsule, hippocampus and brain stem. This finding, although less common, is associated with a more devastating outcome. (393)

Watershed injury refers to injury at the border-zone of the cerebral arteries and is usually associated with prolonged, subacute hypoxia, hypotension or impaired autoregulation. (362, 389) Watershed injuries account for 40-60% of injuries in infants with HIE. (392)

Even short durations of asphyxia can result in white matter injury. Punctate white matter injury may be seen in infants with hypoxia without significant acidosis. (394) This pattern of injury is commonly seen in infants with mild HIE.

Global injury involves the basal ganglia, brainstem, cortex and white matter. This pattern of injury is rare and associated with profound prolonged insults. (366)

Other patterns of injury seen in infants with HIE include cerebellar injury, haemorrhages and perinatal ischaemic stroke. (366)

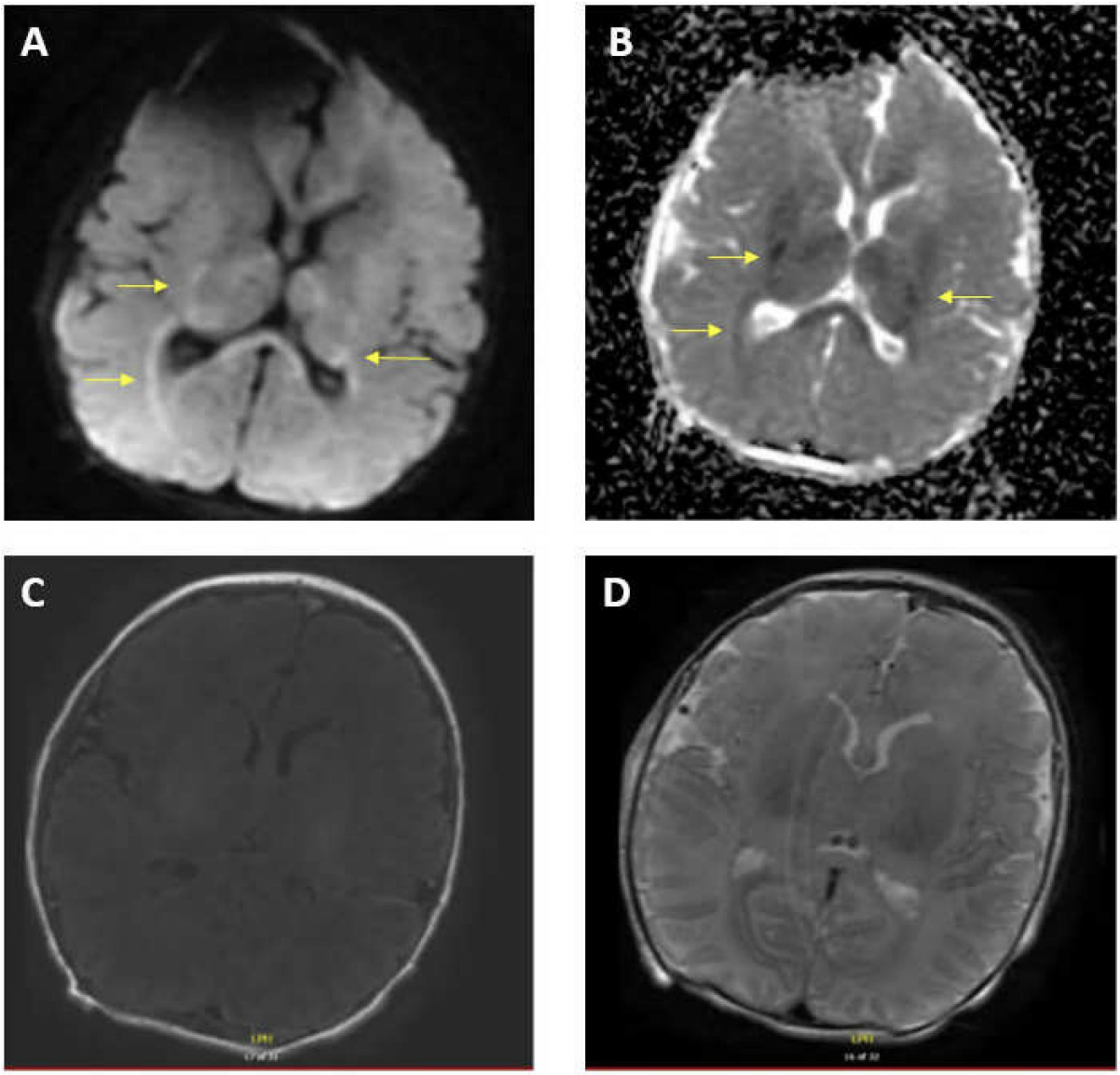


Figure 1.26: Brain MRI demonstrating acute infarction of the basal ganglia bilaterally.
Yellow arrows highlight Injury to the basal ganglia bilaterally, with some involvement of the optic tracts R>L on DWI (A) and ADC images (B). Evidence of injury evolution not yet seen on axial T1 (C) and T2 (D) weighted images suggesting acute injury.

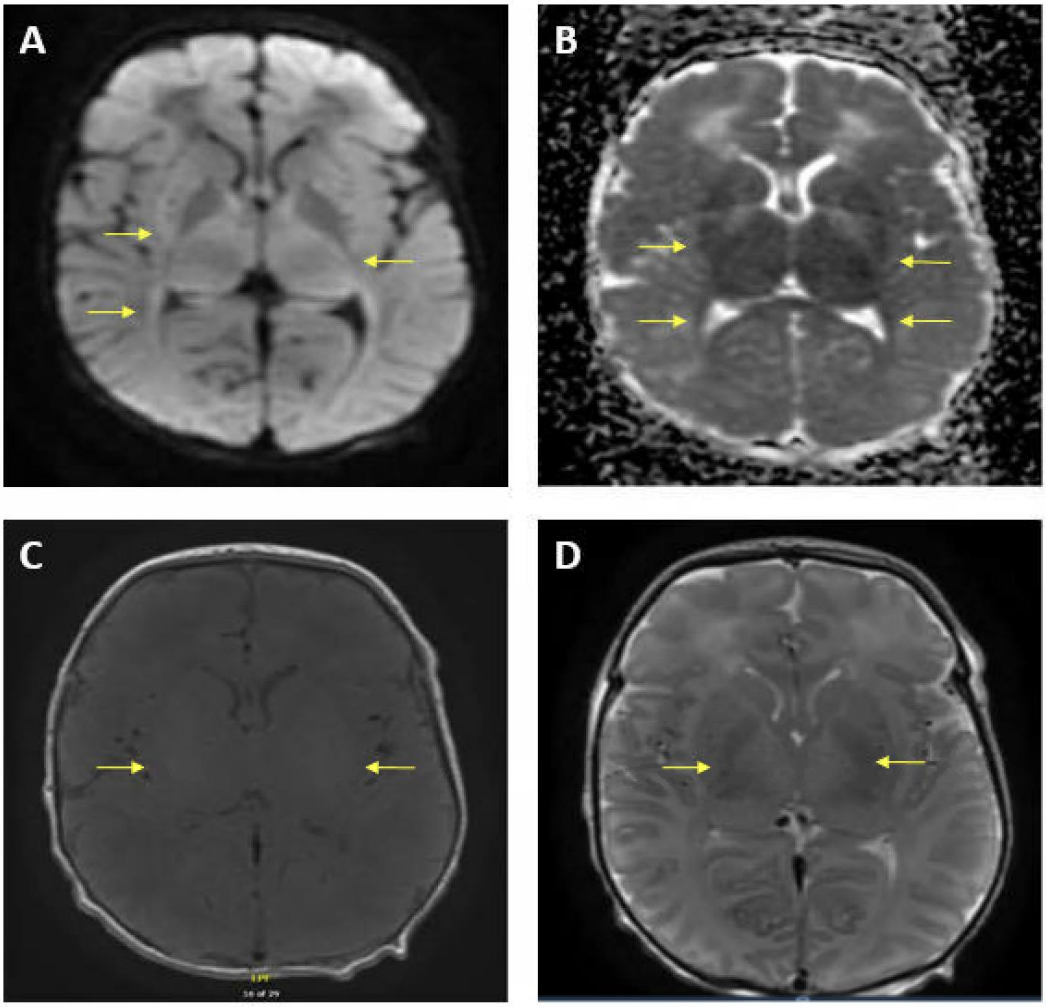


Figure 1.27: Brain MRI demonstrating extensive involvement of the deep nuclear grey matter.
Injury to the basal ganglia, posterior limb of the internal capsule and optic radiation tracts bilaterally are seen as bright areas on DWI (A) and dark on ADC (B) images. Evidence of injury evolution seen on axial T1 (C) and T2 (D) weighted images.

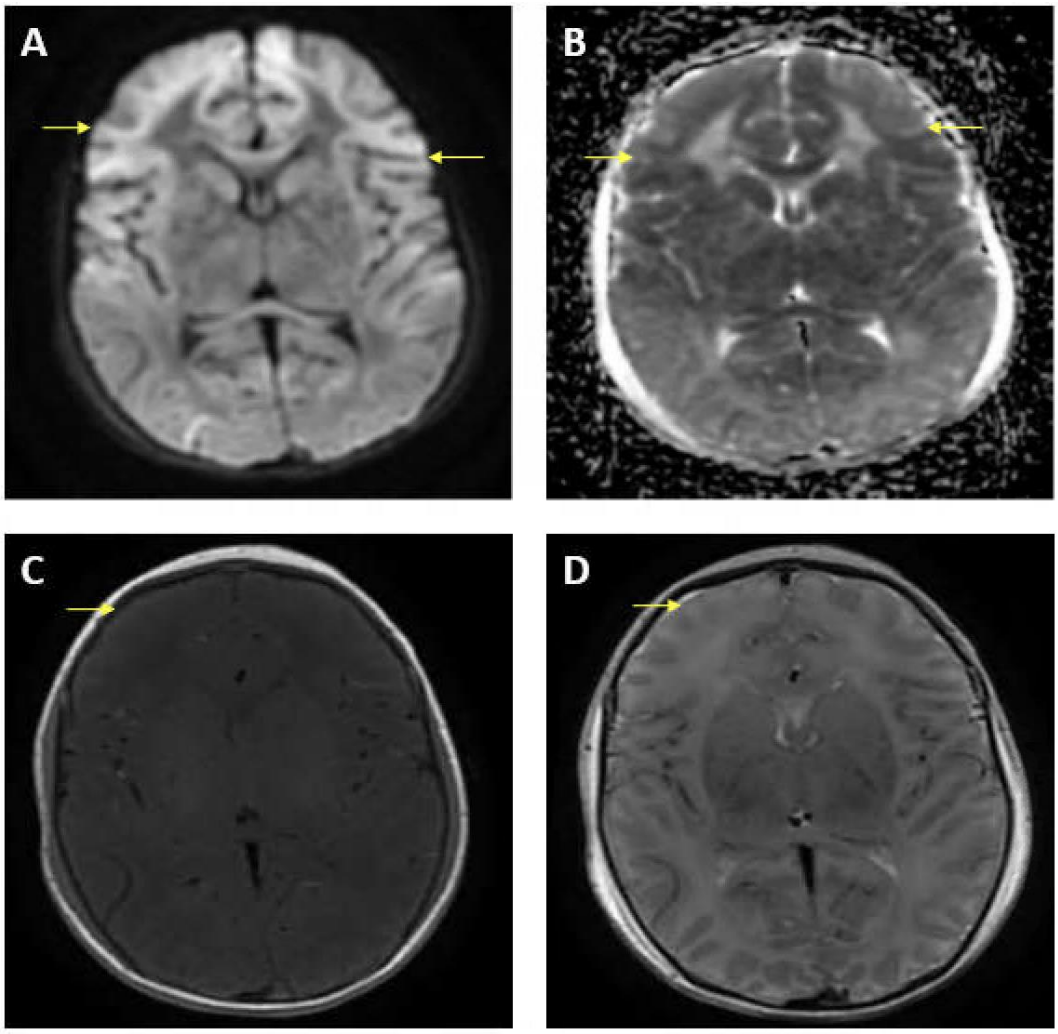


Figure 1.28: Brain MRI demonstrating diffuse white matter injury with involvement of the basal ganglia.
Diffuse white matter injury in the frontal and temporal lobes bilaterally on DWI (A), ADC (B) and axial T1 (C) and T2 weighted images (D). There is loss of cortical ribbon with some effacement of the sulci evident on axial T2 weighted image (D).

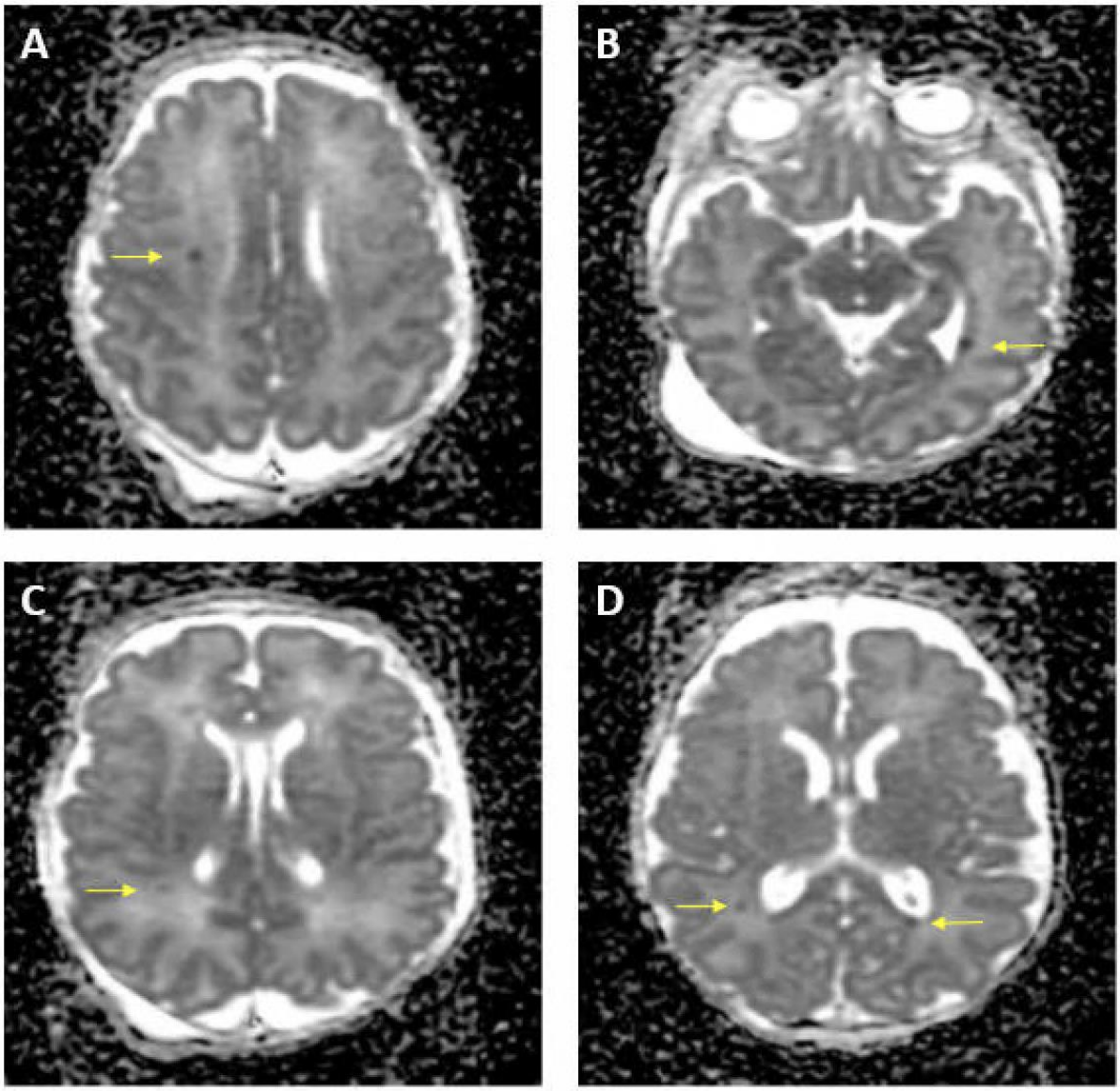


Figure 1.29: Brain MRI demonstrating punctate white matter injury.
Images A-D represent ADC images. Yellow arrows highlight punctate white matter lesions.

As mentioned, pattern of injury is important as it aids in prediction of outcome. Watershed injury is associated with suboptimal head growth and cognitive or behavioural issues at 2-4 years. (395, 396) Whereas injury to the basal ganglia or PLIC is associated with severe adverse neurodevelopmental outcome including motor delay and cerebral palsy. (374, 375, 397)

MRI is more predictive of outcome than early markers such as Apgar score. (398) A recent meta-analysis has shown that an MRI performed in the first 2 weeks after birth in infants receiving TH for HIE, can accurately predict 18 month outcome with a pooled sensitivity of 0.85 [95% CI, 0.79-0.89]. (399) DWI appears to have the best specificity (0.89 [0.62-0.98]) whereas conventional T1 and T2 weighted images had the best sensitivity (0.98 [0.80-1.00]).

MRS has been proposed as a good predictor of long-term outcome (400, 401, 402, 403, 404, 405) with one meta-analysis demonstrating that MRS was the most accurate MRI marker in the prediction of outcome. (406) In infants who received TH for HIE, thalamic N-acetyl aspartate (NAA) levels were predictive of adverse neurodevelopmental outcome at 18-24 months with an area under the curve (AUC) of 0.99 [95% CI 0.94-1.00] and a sensitivity and specificity of 100% and 97% respectively. (407) In preclinical studies, even in milder insults, there is evidence of apoptosis in the thalamus (408) and while these changes may not be evident on conventional sequences, MRS is sensitive to these changes. (126) However, previous studies have utilized different methodology to generate results and improve data quality making it difficult to generalize results. (406, 409) For this reason, this thesis focused primarily on widely available and standardized MRI sequences, specifically T1 and T2 weighted images along with DWI.

MRI injury in infants with HIE has long been described in the literature. In the pre-TH era, MRI injury was reported in up to 50% of infants with mild HIE. (410) As infants with mild HIE were considered to have a normal outcome, until more recently, studies have focused primarily on infants with moderate and severe HIE. However, just as we are gaining insight into the level of neurodevelopmental issues in infants with mild HIE so too are we realizing that a significant portion of these infants may have MRI evidence of HI injury. Studies of MRI injury in infants with mild HIE report varying rates of 10-100% although they are limited by small numbers and confounded by differing rates of TH. More recent studies show that about a half of infants with mild HIE may have an abnormal MRI to include intraventricular haemorrhage and cerebellar lesions. Specific hypoxic injury may be seen in 20-40%.

(118, 122, 411) In general, infants with mild HIE tend to have predominantly white matter injury but some do have evidence of more severe patterns of injury. (118, 385, 411)

Concern has been raised regarding the potential of newer 3T MRI scanners to identify more subtle white matter injury in infants with mild HIE as one of the main drivers for the increased incidence of white matter injury detected in infants with mild HIE. However, similar rates of injury have been reported in cohorts using 3T and 1.5T scanners. (118, 119)

Some questions have also been raised as to the significance of small punctate white matter lesions in infants with mild HIE. However, Kooij et al. demonstrated that these lesions may still be evident in childhood. Furthermore, infants with mild HIE have less motor issues and variable degrees of emotional, behavioural and cognitive impairments in childhood and beyond suggesting a possible influence of these lesions on long-term outcome.

The MARBLE study reported MRI outcomes of all infants referred to their centre for TH. Of the infants that were not cooled, 85% had mild HIE. Forty percent of these infants had injury on MRI. Twelve percent of the infants receiving TH had mild HIE, of which 31% had MRI injury suggesting a possible benefit from TH in infants with mild HIE. However, the PRIME study reported that in their multi-centre observational cohort, only 17% of infants with mild HIE receiving normothermia had an abnormal MRI. Further neuroimaging studies in this population are necessary and ultimately an RCT is required to address the impact of TH on short- and long-term outcome of infants with mild HIE.

Regardless, MRI is a good surrogate marker of long-term neurodevelopmental outcome in infants with perinatal asphyxia. Although further information is required on the incidence and pattern of MRI injury in infants with mild HIE, MRI may be helpful in identifying infants at risk for adverse neurodevelopmental outcome.

1.6. Summary

HIE is the leading cause of acquired brain injury in infants worldwide. (4) Despite recent advancements, the most significant of which has been the introduction of TH, long-term disability rates remain high. (5, 110, 412) Intervention is time sensitive and many infants may be missed during this narrow time window. (113, 143, 167) Furthermore, current available treatments are limited to infants with moderate and severe HIE, excluding the large cohort of infants categorized as having mild HIE which account for almost 40% of the HIE population. (5, 114) HIE is a spectrum and it is clinically challenging to identify infants at risk of long-term neurodevelopmental issues within the early therapeutic window. Our current markers of encephalopathy such as Apgar score, pH and lactate certainly succeed in highlighting infants who may be at risk of poor neurodevelopmental outcomes however, their sensitivity and specificity are low. (59, 75, 94) Clinical examination is a better indicator of the presence of encephalopathy but it is itself limited by subjectivity and reproducibility. (112, 113) HIE is a multi-organ pathology so it is logical that if all organs are potentially affected, all organs should be assessed for injury and potential implications for outcome. Currently available, non-invasive monitoring techniques include EEG, NIRS and non-invasive cardiac output monitoring. Data on early monitoring (before 6 hours of age) in all grades of HIE is currently lacking and infants with mild HIE are a largely understudied population. Further research is required to examine whether early monitoring of infants with HIE may provide important and clinically useful information.

In this thesis, I set out to answer these important questions and improve our knowledge of the early physiology of infants with all grades of HIE through the use of the following physiological monitoring techniques: EEG, NIRS, non-invasive CO monitoring and MRI.

The aims of my thesis were:

- To describe the early EEG features (within the first 6 hours) in infants with mild HIE.

- To systematically review currently available literature to determine whether there is evidence to support the use of early NIRS monitoring (in the first 6 hours) in the prediction of outcome in infants with all grades of HIE.

- To describe the evolution of NIRS (cSO_2 and FTOE) in infants with all grades of HIE from 3- 84 hours after birth.

- To describe the evolution of cardiac output, stroke volume and heart rate as measured by NICOM in infants with all grades of HIE from 6 – 84 hours of age.

- To determine whether early physiological monitoring (EEG, NIRS, non-invasive CO monitoring), either in isolation or combined, may be helpful in identifying infants with abnormal short-term outcomes, as defined by MRI abnormality and/or death within the first week.

2. Multichannel EEG Abnormalities during the first 6 hours in Infants with Mild Hypoxic Ischaemic Encephalopathy

2.1. Introduction

Hypoxic ischaemic encephalopathy (HIE) accounts for 1-3 per 1,000 live births per year (4) and is the leading cause of acquired brain injury in term infants. It is clinically graded as mild, moderate and severe. Adverse long-term neurodevelopmental outcome is correlated with increasing severity of encephalopathy. (2, 103, 108, 109)

Therapeutic hypothermia (TH) has become standard of care for infants with moderate to severe HIE. (5) To be effective, TH must be commenced early, within 6 hours of birth. (30, 150, 413) However, it can often be difficult to differentiate clinically between mild and moderate encephalopathy in this short timeframe. (156, 159) TH is not currently indicated for infants with mild HIE. Previously these infants were considered to have normal outcomes (2, 414, 415) and so were omitted from TH trials due to the perceived low risk of disability. However, more recent studies highlight significant levels of disability at follow-up. (116, 117, 168, 416) A systematic review by Conway et al. found that 25% of infants with mild HIE had poor neurodevelopmental outcome. (115) Their pattern of disability appears different to those with moderate to severe HIE. Infants with mild HIE have less motor difficulties but have an increased risk of learning disabilities, and emotional and behavioural issues, with 35% requiring school and/or behavioural support at 5 years. (416) This rate is similar to previous cohorts of infants with moderate HIE who were not cooled. (416)

Despite the lack of appropriate evidence, there has been a drift in practice with some centres providing TH to infants clinically categorised as mild HIE. (128, 129, 170) This is fuelled by fear of both misdiagnosis and litigation. (130) Trials evaluating TH in infants with mild HIE are required but identification of such infants can be challenging clinically. Disagreement exists regarding both the method and timing of assessment. (2, 107) Clinical and electroencephalographic (EEG) assessment tools validated for

use at 24 hours are no longer appropriate. (5) Objective parameters are required to aid in early decision making in the immediate postnatal period.

EEG plays an important role in caring for newborns with HIE, not only in seizure identification but also in prognostication. EEG findings can evolve rapidly in the first 6 hours after birth. Healthy term infants should demonstrate mixed-frequency continuous EEG with regular sleep-wake cycling (SWC) from birth and absence of SWC has been associated with poor neurodevelopmental outcome. (236, 417) Very little information exists about the EEG features in mild encephalopathy. In the pre-TH era, amplitude-integrated EEG (aEEG) within 6 hours of birth was the most useful tool for predicting outcome in infants with HIE (418); however TH has delayed the predictive ability of aEEG to 48 hours of age. (196, 419, 420) A normal continuous EEG in the first 6 hours after birth predicts a normal outcome at 2 years of age. (121)

Heart rate variability (HRV) can be assessed from simultaneous electrocardiography (ECG) recordings. It provides a measure of autonomic function by assessing the difference in time between heartbeats, denoted as either the RR (inter-beat) or NN ("normal" inter-beat) interval. HRV between 12 and 48 hours after birth has the ability to differentiate between grades of encephalopathy and correlate with neurodevelopmental outcome at 2 years (421) but no study has examined the ability of early HRV to determine grade of encephalopathy or its early use in mild HIE.

The aim of this study is to identify and describe features of early (before 6 hours of age) EEG and HRV in infants with mild HIE compared to a healthy term group.

2.2. Methods

This was a retrospective study of infants with mild HIE and healthy term infants recruited as part of previous prospective studies in Cork, Ireland between 2003 and 2019. Each study was approved by the Clinical Research Ethics Committee of the Cork

Teaching Hospitals. From these cohorts, infants who had EEG recordings before 6 hours of age were identified.

2.2.1. Cohorts

The non-HIE group was from a previous study examining brain activity in healthy term infants recruited between October 2005 and August 2008. (204) EEG was recorded on the post-natal ward. Inclusion criteria included infants >37 weeks who did not require resuscitation at birth, had normal cord pH values and had an Apgar score of >8 at 5 minutes. Sixteen infants had EEG recordings in the first 6 hours after birth.

Infants with HIE were recruited as part of four prospective cohort studies in the Unified Cork Maternity Services, Cork, Ireland between May 2003 and June 2019. From these cohorts, we identified infants with a clinical diagnosis of mild HIE who had EEG recordings commenced before 6 hours of age. Infants with evidence of perinatal asphyxia (defined as one or more of the following; cord or 1st post-natal pH <7.1; cord or first post-natal base deficit >16; Lactate >9mmol within the first hour of life; Apgar score <5 at 5 minutes of life; on-going need for resuscitation at 10 minutes of life (IPPV or intubation)) were assessed for the presence of HIE using a modified Sarnat exam by experienced clinicians in each cohort.(Table 2.1) Mild HIE was defined using the criteria set out by Chalak et al. in the PRIME Study, (116, 122) and re-affirmed in a recent expert review. (422) Specifically, if an infant had any (≥1) abnormality in any of the six domains of the modified Sarnat score, but did not meet the criteria for moderate or severe encephalopathy (i.e. ≥ 3 domains that were categorised as either moderate or severe), they were defined as mild HIE. Multichannel EEG was recorded for between 6-72 hours. Inclusion and exclusion criteria of the individual studies are outlined in Table 2.2. The EEG was recorded from frontal, central, temporal and posterior cortical regions.

Table 2.1: Modified Sarnat Exam.

Stage	Normal	Mild	Moderate	Severe
1. Level of Consciousness	Normal	Hyper-alert/ Irritable	Lethargic/ Obtunded	Stupor/ Coma
2. Spontaneous Activity	Normal	Normal	Decreased	Absent
3. Muscle Tone	Normal	Normal	Mild Hypotonia	Flaccid
4. Posture	Normal	Mild Distal Flexion	Strong Distal Flexion	Decerebrate
5. Primitive Reflexes				
Suck	Normal	Weak	Weak/Absent	Absent
Moro	Normal	Strong/ Low Threshold	Weak/Incomplete/ High Threshold	Absent
6. Autonomic Function				
Pupils	Normal	Mydriasis	Miosis	Unequal/Fixed/ Dilated/Poor Reflex
Heart Rate	Normal	Tachycardia	Bradycardia	Variable
Respirations	Normal	Normal	Periodic Breathing	Apnoea

Table 2.2: Inclusion and exclusion criteria for the four cohort studies.

	Cohort 1	**Cohort 2**	**Cohort 3**	**Cohort 4**
Period	May 2003 – Dec 2005	May 2009 – June 2011	Feb 2013 - Aug 2015	Nov 2017 - June 2019
Population	Inborn at one of 3 maternity hospitals in the Unified Cork Maternity Services, Cork , Ireland	Inborn at Cork University Maternity Hospital, Cork, Ireland	Inborn at or outborn and transferred to Cork University Maternity Hospital, Cork, Ireland	Inborn at or outborn and transferred to Cork University Maternity Hospital, Cork, Ireland.
Inclusion in primary study	GA ≥ 37+0 All grades of encephalopathy to include presence of: Capillary or arterial pH <7.1 OR Lactate >7mmol/L OR Apgar score <5 at 5mins AND Abnormal neurological examination	GA ≥ 36+0 Signs of asphyxia to include one of: 1. Cord pH <7.1, 2. Apgar score ≤6 at 5mins 3. The need for intubation or CPR at birth	GA ≥ 36+0 Signs of asphyxia to include: 1. Cord pH <7.1 2. Apgar score ≤6 at 5mins 3. The need for intubation or CPR at birth. OR Infants with requirement of EEG monitoring in the Neonatal Unit	GA ≥ 36+0 Infants with one or more of the following: 1. Apgar score <5 at 5mins 2. Postnatal resuscitation >10 minutes 3. pH <7.1 or base deficit >16 or lactate >9mmol/L on cord or first post-natal blood sample AND Presence of abnormal neurological findings on modified Sarnat score at 1 hour of age.

	Cohort 1	Cohort 2	Cohort 3	Cohort 4
Total No. HIE in primary study	65	40	59	62
Total mild HIE in primary study	29	24	36	30
No. in current study (mild HIE and EEG recording <6hours of age)	9	16	19	14
Definition of Mild HIE	≥1 abnormal finding on modified Sarnat score at 24 hours of age but not meeting criteria for moderate or severe HIE.	Worst grade of encephalopathy in 1st 24 hours using modified Sarnat score but not meeting criteria for TH.	Worst grade of encephalopathy in 1st 24 hours using modified Sarnat score but not meeting criteria for TH.	1 or more abnormal neurological finding on modified Sarnat score at 1 hour of age but not meeting criteria for TH
Exclusion from pooling		1. Infants who received TH (n=1)	1. Infants who received TH (n=4) 2. Other diagnosis than mild HIE	1. Infants who received TH (n=2)

	Cohort 1	Cohort 2	Cohort 3	Cohort 4
Main publications from cohorts to date	Murray et al. 2006[76] Murray et al. 2008[93] Murray et al. 2008[218] Murray et al. 2009[121] Murray et al. 2010[107] Murray et al. 2016[120]	Walsh et al. 2011[203] Walsh et al. 2012[547] Looney et al. 2015[545]	O'Sullivan et al. 2018[546] Rennie et al. 2018[225] O'Sullivan et al. 2019[548] Finder et al. 2019[117] Pavel et al. 2020[233]	

** Exclusion from primary studies: Infants <36+0 weeks gestation; Known genetic disorders and/or inborn errors of metabolism; Confirmed sepsis (positive blood or CSF cultures).*

TH, therapeutic hypothermia; HIE, hypoxic ischaemic encephalopathy; GA, gestational age; CPR, cardiopulmonary resuscitation; PA, perinatal asphyxia

2.2.2. EEG analysis

2.2.2.1. Qualitative

Firstly, all continuous EEG data available before 6 hours of age, for the HIE and non-HIE group were visually assessed and the background pattern was graded as normal or mildly abnormal background by two neonatal EEG reviewers independently with complete agreement for both grades. As slightly different EEG recording locations were used in the HIE and non-HIE groups, it was not possible to fully blind the reviewers to study group (in the non-HIE group, posterior electrodes were located over the right and left parietal regions rather than occipital regions). Our group has previously developed a standardised grading scheme to analyse EEG features of preterm EEG (423) and we extended this for the term EEG using seminal works from Lamblin, Andre, d'Allest and others.(Table 2.3) (424, 425) One EEG reviewer then identified specific qualitative features in both groups according to this assessment scheme.

Qualitative analysis of the EEG was divided into 3 main categories. Category 1 describes Temporal Organisation (SWC and features of continuity). Category 2 identifies if abnormal waves are present (immature or deformed waves, sharp waves or diffuse delta waves), and Category 3 describes abnormal features of a term EEG (asymmetry, asynchrony, discontinuity, seizures or low voltage activity). EEGs were reviewed and the presence or absence or the various features were noted.

Table 2.3: Qualitative EEG Assessment Scheme.

	Features	**Definition**
Group 1 (Temporal Organisation)	SWC	Clearly defined AS and QS
	Burst (3-8 seconds)	Trace alternant, 3-8 seconds
	IBI (same duration as burst)	< 6 seconds
	Continuity	Continuous activity
Group 2 (Abnormal Waves)	PRS	0.1 per minute
	PTS	400ms, >50uV, >0.1 per minute
	Sharps (diffuse/excessive/focal/negative)	> 100uV and > 0.1 per minute
	Deformed waves	Lack of smoothness, wider basis, increased peak-to-peak amplitude
	Mechanical brushes	Spindle-like, fast spikey wave bursts with maximal amplitudes higher than 40 µV and frequencies between 13 and 20 Hz
	Immature waves	Presence of waves that are usually seen in younger gestational ages
	Diffuse delta waves	

	Features	Definition
Group 3 (Abnormal Features)	Asymmetry	>50% difference in amplitude and/or frequencies between 2 hemispheres and/or if pathological waves are exceeding in one hemisphere for >50% compared to the other.
	Asynchrony	Might be present at the onset of QS
	Discontinuity	Prolonged IBI, decreased continuous activity for <50% of the recording
	Low voltage	Amplitude of 5-15 µV in wakefulness and 10-25 µV in QS
	Isoelectric	Mainly inactive tracing with activity < 5uV
	Burst Suppression	Bursts of theta and/or delta waves alternating with periods of low amplitude activity (<20 µV). No reactivity to stimuli.
	BIRDs	Paroxysms of a seizure-like rhythmic electrographic activity with a duration of <10seconds
	PLEDs	Sharp waves or spikes that repeat periodically with an almost regular interval, lateralized to one hemisphere and showing no clear evolution in terms of frequency/morphology and amplitude.
	Seizures	Sudden, repetitive, evolving and stereotyped ictal pattern with a clear beginning, middle, and ending and a minimum duration of 10 seconds.
	Status	30 minutes of seizure activity OR recurrent seizures with no recovery for at least 30 minutes OR 50% seizure burden in a 1 hour period.

SWC, sleep-wake cycling; IBI, inter-burst interval; AS, active sleep; QS, quiet sleep; PRS, positive rolandic sharp waves; PTS, positive temporal sharp waves; BIRDs, brief intermittent rhythmic discharges; PLEDs, periodic lateralised epileptiform discharges

2.2.2.2. Quantitative

All EEGs were then included in the quantitative analysis. 1 hour epochs of each EEG assessed in the qualitative analysis before 6 hours of age were selected to include a full sleep cycle if present and as little artefact as possible. Remaining artefacts were annotated and removed. EEGs were quantitatively assessed using multiple features of amplitude, spectral shape, discontinuity and inter-hemispheric connectivity using the NEURAL (**N**eonatal **E**eg feat**UR**e set in m**A**t**L**ab) software package (version 0.4.3). (237)

Measures of spectral shape included spectral power, spectral flatness (a measure of spectral entropy) and spectral difference (a measure of difference in spectral shape over time).

As different EEG or aEEG machines use different algorithms to generate an aEEG channel, we use the range-EEG (rEEG) as an alternative. This filtered and time-compressed representative of EEG is similar to the aEEG but has a unique definition which therefore allows for standard quantitative measures. (237)

2.2.3. Heart Rate Variability

HRV was computed from the same 1 hour epochs used for quantitative EEG analysis in both the HIE and non-HIE groups. R-peaks were automatically identified using a HRV software application (HRV Analysis, University College Cork, Cork, Ireland) and then visually inspected and corrected if necessary. Artefacts were annotated and removed. Quantitative HRV features were extracted from the R-R interval (426) including both time-domain and frequency-domain features. These features are consistent with previous neonatal studies. (421, 426)

2.2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 24.0, IBM Corp, U.S.A.). Continuous variables were described using the mean and standard deviation (SD) or the median and interquartile range (IQR) and categorical variables using

frequency and percentage. For comparisons between the two groups (mild HIE, non-HIE) the Mann-Whitney U test was used for continuous variables and Fisher's Exact test for categorical variables. Receiver operator characteristic (ROC) curve analysis was used to assess the predictive ability of qualitative EEG features and quantitative EEG and HRV features in identifying mild HIE. All tests were two-sided and a p-value<0.05 was considered statistically significant.

2.3. Results

2.3.1. Population

2.3.1.1. Non-HIE

Sixteen healthy term infants were included in the non-HIE group. Mean birth weight was 3497g (SD 381g) and median gestational age was 39.3 weeks (IQR 38.9 – 40.6 weeks). Fifty percent (n=8) were born by elective lower section caesarean section (LSCS), 31% (n=5) were born by spontaneous vaginal delivery, 13% (n=2) were born by emergency LSCS and 6% (n=1) required instrumental assistance.(Table 2.4)

2.3.1.2. Mild HIE

Fifty-eight infants with mild HIE were included. Mean birth weight was 3467g (SD 527g) and median gestational age at birth was 40.4 weeks (IQR 39.2 – 41.3 weeks). Forty-seven percent (n=27) were born by instrumental assisted vaginal delivery, 26% (n=15) were born by emergency LSCS, 24% (n=14) were born by spontaneous vaginal delivery and 2% (n=1) were born by elective LSCS. Mode of delivery was not documented in 1 infant.(Table 2.4)

Table 2.4: Demographics and Mode of Delivery of infants included.

	Non-HIE n = 16	**Mild HIE n = 58**
Gestational Age at birth (weeks) [median(IQR)]	39.3 (38.9-40.6)	40.4 (39.2 - 41.3)
Birthweight (g) [mean(SD)]	3497 (381)	3467 (527)
Mode of Delivery [n(%)]:		
SVD	5 (31)	14 (24)
Instrumental	1 (6)	27 (47)
Emergency LSCS	2 (13)	15 (26)
Elective LSCS	8 (50)	1 (2)
Not documented	0 (0)	1 (2)

IQR, interquartile range; SD, standard deviation; SVD, spontaneous vaginal delivery; LSCS, lower section caesarean section

2.3.2. Qualitative analysis

Qualitative analysis of the EEG was divided into 3 main categories as described above: temporal organisation, abnormal waves and abnormal features. The main results are displayed inTable 2.5.

2.3.2.1. Non-HIE Group

Infants in the non-HIE group demonstrated normal SWC with continuous mixed-frequency activity with absence of abnormal waves and features.

2.3.2.2. Mild HIE

On assessing temporal organisation, 53% demonstrated clear sleep-wake cycling. Approximately 34% had prolonged inter-burst intervals in quiet sleep. Regarding abnormal waves and features, 48% had diffuse slow waves and 29% had excessive sharp waves. Twenty-one percent were noted to have low voltage recording and 19% were excessively discontinuous with 24% of the EEGs showing periods of discontinuity (<50% of the recording).

Overall, 72% of the infants with mild HIE had at least one abnormal EEG feature in the first 6 hours after birth, including absent or abnormal SWC, intermittent

discontinuity, diffuse slow wave activity or excessive sharp waves. The most striking difference visually was the high frequency of slow and sharp waves, periods of excessive discontinuity and lower amplitude. ROC analysis revealed that absence of SWC or presence of diffuse slow waves were the features that were most predictive of mild HIE.

Table 2.5: Qualitative EEG features of infants with mild HIE and infants in the non-HIE group.

		Non-HIE n=16 n(%)	**Mild HIE n=58 n(%)**	**p-value**	**AUC (95% CI)**
Group 1 (Temporal Organisation)	Normal Sleep Wake Cycling	16 (100)	31 (53)	**<0.001**	0.73 (0.67-0.80)
	Predominant continuous activity	16 (100)	47 (81)	0.107	0.59 (0.54-0.65)
Group 2 (Abnormal Waves)	Diffuse delta waves	0	28 (48)	**<0.001**	0.74 (0.68-0.81)
	Sharps (diffuse/excessive/focal/negative)	0	17 (29)	**0.016**	0.65 (0.59-0.71)
	Deformed waves	0	6 (10)	0.329	0.55 (0.51-0.59)
	Immature waves	0	5 (9)	0.579	0.54 (0.51-0.58)
	Mechanical brushes	0	2 (3)	1	0.52 (0.49-0.54)
	Positive Temporal Sharp waves	0	2 (3)	1	0.52 (0.49-0.54)
	Positive Rolandic Sharp waves	0	0		
Group 3 (Abnormal Features)	Periods of Discontinuity	0	14 (24)	**0.031**	0.62 (0.57-0.68)
	Low voltage	0	12 (21)	0.058	0.60 (0.55-0.66)
	Brief Intermittent Rhythmic Discharges	0	2 (3)	1	0.52 (0.49-0.54)
	Asymmetry	0	2 (3)	1	0.52 (0.49-0.54)
	Asynchrony	0	1 (2)	1	0.51 (0.49-0.53)
	Burst Suppression	0	0		
	Isoelectric	0	0		
	Periodic lateralized epileptiform discharges	0	0		
	Seizures	0	0		
	Status	0	0		

p-values derived from Fisher's Exact test. Values in bold indicate a p-value <0.05

2.3.3. Quantitative analysis

Epochs were analysed at a median time of 0.6 hours (IQR 0.3 – 1.2 hours) after start of EEG recording. Quantitative analysis revealed significant differences in spectral features between infants with mild HIE and those without. (Table 2.6) Ninety-one percent of spectral power in the EEGs of infants with mild HIE was in the delta band (<4 Hz), with 95% total power <5Hz. Both spectral flatness and spectral difference were significantly lower in the delta and theta frequency bands for the mild HIE group compared with the non-HIE group. ROC analysis revealed that these features were also most predictive of mild HIE.

There were no differences in quantitative measures of amplitude including range-EEG, discontinuity and inter-hemispheric coherence between the groups.

Table 2.6: Quantitative EEG features of infants with mild HIE and infants in the non-HIE group.

	Non-HIE [med (IQR)] n=16	**Mild HIE [med (IQR)] n=58**	**p-value**	**AUC (95% CI)**
POWER				
Spectral power FB1 (μV^2)	282.1 (224.9-386.0)	332.9 (238.0-637.1)	0.309	0.59 (0.47-0.70)
Spectral power FB2 (μV^2)	17.5 (15.1-23.9)	18.5 (13.7-25.8)	0.940	0.51 (0.39-0.63)
Spectral power FB3 (μV^2)	9.1 (8.1-11.6)	8.2 (5.9-10.8)	0.162	0.62 (0.50-0.73)
Spectral power FB4 (μV^2)	4.7 (4.1-5.7)	5.0 (3.5-8.4)	0.748	0.53 (0.41-0.65)
rEEG median (μV)	52.1 (44.0-55.1)	47.4 (42.4-54.9)	0.379	0.57 (0.45-0.69)
rEEG lower margin (μV)	27.0 (22.2-31.1)	26.1 (22.1-30.9)	0.667	0.54 (0.41-0.65)
rEEG upper margin (μV)	94.6 (91.3-97.1)	90.7 (76.7-103.9)	0.350	0.58 (0.45-0.69)
rEEG asymmetry	0.322 (0.297-0.349)	0.324 (0.293-0.356)	0.995	0.50 (0.39-0.63)
SPECTRAL SHAPE				
Spectral edge frequency (Hz)	6.3 (5.3-6.7)	5.2 (3.6-5.9)	**0.008**	0.72 (0.61-0.82)
Spectral relative power FB1 (%)	89.3 (88.0-91.1)	91.0 (89.6-93.0)	**0.023**	0.69 (0.58-0.80)
Spectral relative power FB2 (%)	5.8 (5.2-6.7)	4.9 (3.7-5.9)	**0.021**	0.69 (0.58-0.80)
Spectral relative power FB3 (%)	3.0 (2.5-3.4)	2.2 (1.8-2.5)	**0.001**	0.77 (0.65-0.86)
Spectral relative power FB4 (%)	1.7 (1.4-1.9)	1.4 (1.1-1.8)	0.371	0.42 (0.31-0.55)
Spectral flatness FB1	0.47 (0.41-0.52)	0.42 (0.29-0.48)	**0.035**	0.68 (0.57-0.79)
Spectral flatness FB2	0.89 (0.88-0.90)	0.88 (0.86-0.89)	**0.007**	0.73 (0.61-0.82)
Spectral flatness FB3	0.89 (0.87-0.90)	0.89 (0.87-0.91)	0.440	0.57 (0.44-0.68)
Spectral flatness FB4	0.76 (0.71-0.77)	0.78 (0.72-0.82)	0.139	0.63 (0.51-0.74)

	Non-HIE [med (IQR)] n=16	Mild HIE [med (IQR)] n=58	p-value	AUC (95% CI)
Spectral difference FB1	0.010 (0.008-0.011)	0.007 (0.004-0.009)	**0.003**	0.75 (0.64-0.85)
Spectral difference FB2	0.026 (0.024-0.029)	0.023 (0.021-0.026)	**0.007**	0.73 (0.61-0.82)
Spectral difference FB3	0.021 (0.018-0.022)	0.020 (0.018-0.022)	0.875	0.49 (0.36-0.60)
Spectral difference FB4	0.010 (0.009-0.012)	0.011 (0.009-0.013)	0.179	0.61 (0.50-0.73)
INTER-HEMISPHERE CONNECTIVITY				
Coherence FB1	0.172 (0.122-0.195)	0.118 (0.088-0.202)	0.379	0.43 (0.31-0.55)
Coherence FB2	0.073 (0.054-0.084)	0.049 (0.035-0.084)	0.128	0.37 (0.26-0.49)
Coherence FB3	0.048 (0.044-0.068)	0.046 (0.035-0.079)	0.562	0.45 (0.34-0.57)
Coherence FB4	0.036 (0.032-0.045)	0.038 (0.030-0.074)	0.647	0.54 (0.41-0.65)

FB, frequency band; rEEG, range EEG.
FB1 = 0.5–4 Hz; FB2 = 4-7 Hz; FB3 = 7-13 Hz; FB4 = 13-30 Hz
p-values derived from Mann-Whitney U Test. Values in bold indicate a p-value <0.05.

2.3.4. Heart Rate Variability

There were no differences between infants with mild HIE and infants in the non-HIE group.(Table 2.7)

Table 2.7: Quantitative HRV features of infants with mild HIE and infants in the non-HIE group.

Feature	**Non-HIE [med (IQR)] n=16**	**Mild HIE [med (IQR)] n=47**	**p-value**	**AUC (95% CI)**
Mean NN (msec)	504.6 (477.0-537.6)	490.5 (465.4-520.9)	0.347	0.58 (0.46–0.71)
SDNN (msec)	29.0 (13.9-34.7)	20.8 (15.2-29.2)	0.294	0.59 (0.46–0.71)
VLF power ($msec^2$)	4142.5 (1017.3-6295.0)	1888.5 (950.5-3419.6)	0.076	0.65 (0.52–0.77)
LF power ($msec^2$)	487.2 (158.1-1051.8)	323.8 (146.8-615.1)	0.227	0.60 (0.47–0.72)
HF power ($msec^2$)	8.9 (3.0-32.8)	10.1 (3.5-31.9)	0.906	0.49 (0.36–0.62)
LF/HF ratio	50.2 (26.2-74.4)	34.4 (14.1-65.4)	0.102	0.64 (0.50–0.75)
TINN (msec)	82.0 (58.6-101.6)	54.7 (46.9-76.2)	0.102	0.64 (0.50–0.75)

NN, normalised RR interval; SD, standard deviation; VLF, very low frequency; LF, low frequency; HF, high frequency; TINN, triangular interpolation of the NN interval histogram.
p-values derived from Mann-Whitney U Test

2.4. Discussion

This is the first detailed study describing multichannel EEG and HRV in infants with mild HIE within 6 hours of birth. We have found significant differences between the EEG features of infants with mild HIE and healthy term infants. Our qualitative analysis identified the presence of specific abnormal EEG features in the HIE group. Sleep-wake cycling should be present from birth (427) and the absence of SWC is associated with poor neurodevelopmental outcome. (236, 417) Good quality sleep is crucial for an infant's development and studies have shown disruptions in both the presence and composition of the SWC in term infants post asphyxia injury, specifically a decrease in the proportion of active sleep and an increase in the amount of quiet and indeterminate sleep within the sleep cycle. (428, 429, 430, 431) Almost half of the infants with mild HIE had absent or poor SWC in the first 6 hours after birth, active sleep was absent in 35% and quiet sleep was abnormal, as it contained prolonged inter-burst intervals, in 34%. It is important to consider the effect that interventions in the neonatal unit may have on SWC however, it is our practice to only perform necessary procedures and cares on admission and nurse these infants in incubators with minimal handling thereafter. We have also previously shown that normal continuous SWC activity is present from birth in healthy control infants without perinatal asphyxia. (427) As well as altered SWC, we also found that 24% of the EEGs in infants with mild HIE had periods of excessive discontinuity and 19% were predominantly discontinuous. Excessive sharp waves were seen in 30%. Qualitative analysis demonstrated excessive slow waves in infants with mild HIE which was also confirmed on quantitative analysis.

Previous studies have shown altered HRV features with increasing grade of encephalopathy (421, 426) however no study to date has assessed HRV in the first 6 hours after birth. Animal studies have found an increased variability in the HRV (increased SDNN) in preterm foetal sheep between 4-6 hours post occlusion with severe hypoxia ischemia compared to those with mild hypoxia ischemia or controls. (432) In this study, we found no differences in measures of HRV in infants with mild HIE compared with healthy term infants in the first 6 hours.

There is a growing body of evidence that infants with mild HIE have significant levels of disability at follow-up, yet no current evidence or guidance exists regarding potential therapeutic interventions in this group. This, coupled with the medico-legal implications of not offering TH to infants who may have benefited, leads to unease and difficulty for clinicians in objective decision-making regarding treatment. In addition, there is therapeutic creep and many centres are now cooling infants with mild HIE. (128, 129, 170)

Although TH has a wide margin of safety, it is not without side-effects. Inappropriate TH has a number of potential adverse consequences. Animal studies have suggested that induced hypothermia in a normal brain may lead to apoptosis. (433) TH results in a prolonged NICU stay, separation from mother, delayed breastfeeding initiation, risks associated with sedative medications, risk of coagulopathy and pulmonary hypertension, and ultimately increased healthcare economic costs. (5, 434) Sedation is often required in infants undergoing TH due to the discomfort associated with a low core temperature. Concerns have been raised about commonly used drugs such as morphine as it may contribute to neuronal and microglial apoptosis. Tolerance has also been described, requiring increased doses and problems with withdrawal on discontinuation. (435, 436, 437)

Therefore it seems logical that a randomised trial of TH in infants with mild HIE is now required. (131) However, consensus must be reached on how we identify these infants. Improved identification and selection of infants who may potentially benefit from TH would limit the numbers required to power such a study.

Our current methods of identifying infants are flawed as the primary and most widely available assessment is based on clinical examination, which is highly subjective. (113) Although several studies have assessed different scoring tools, (108, 109, 438, 439) it is often difficult to clinically differentiate between mild and moderate grades of HIE. (156, 159) Furthermore, it is based on a modified Sarnat score which was initially validated to examine infants repeatedly and at 24 hours. (2) Many centres now use the Thompson score. Initially developed as a quick and easy tool to assess

infants with encephalopathy, it was also developed to examine infants on a daily basis and is most predictive of outcome on days 3-4. (108) Early EEG has been shown to be superior to clinical examination alone for the prediction of outcome. (421, 440)

aEEG is preferred in many neonatal units as it can be easily applied and interpreted through pattern recognition. In the pre-TH era, aEEG within 6 hours of birth was the best predictor of outcome and aEEG was incorporated into many of the TH RCTs as an inclusion criteria for randomisation. As mentioned, different EEG or aEEG machines use slightly different algorithms to generate an aEEG channel. For quantitative analysis, we used rEEG which has a standard definition. (237) This allowed us to assess the ability of aEEG to distinguish between infants with mild HIE and those without HIE in our study. In our cohort, rEEG features alone such as median amplitude, upper and lower margins did not distinguish infants with mild HIE from the non-HIE group. Studies have previously shown a discrepancy in HIE grade between continuous multi-channel EEG and aEEG in the same infants. (200) This is likely due to the fact that the raw EEG is uncompressed and specific features such as sharp and slow waves, short periods of discontinuity, asymmetry and asynchrony can be easily seen but these are lost in the compressed or summarised aEEG. Nonetheless, aEEG is very useful for the visual identification of SWC, a feature that was absent or poorly defined in our cohort of infants with mild HIE. Whilst continuous multichannel EEG may not be available in all neonatal units, most aEEG devices allow visualisation of at least some raw EEG channels which can provide richer information and a more enhanced aEEG interpretation.

Multichannel EEG does require expert interpretation and many units do not have 24-hour access to a neonatal neurophysiologist or neurologist. Quantitative EEG analysis of multichannel EEG provides an automated objective description of the EEG without the need for expert interpretation. It also has the potential to detect more subtle differences that may not be easily identified on visual assessment alone. In this cohort, quantitative analysis demonstrated significant differences in all measures of spectral shape at the lower frequency bands when compared to the non-HIE group. These features of the EEG would be very difficult, if not impossible, to detect visually.

Quantitative EEG features provides a scalable, continuous, and objective assessment of the EEG that may be useful in the future to improve our identification of at-risk infants. It is also easily applied to a smaller number of EEG channels.

Our study is limited by the fact that it was a retrospective analysis of data; however EEGs of infants with HIE were collected over different cohorts in both the TH and pre-TH era capturing the clinical variability in presentation. In addition, it was not possible to completely blind the neurophysiologists conducting the qualitative analysis to study group however, their findings were remarkably consistent with the objective quantitative analysis. Outcome data is currently unavailable for the entire population however, neurodevelopmental follow-up is underway. We plan to clinically follow these infants to 5 years of age to determine which, if any, of these abnormal features correlate with outcome. We do know from previous studies that a proportion of infants with mild HIE will have cognitive or behavioural disability on follow-up, however these difficulties may not be evident until 5 years of age or later. (416)

Mild HIE is not "normal" as previously thought; (2, 414, 415) these infants have an encephalopathy and may have significant learning, behavioural and emotional difficulties on follow-up, yet have largely been ignored by research to date. (115, 117, 416) Our current criteria of identifying infants at risk of long-term developmental issues is clearly inadequate as many infants who have significant disability on follow-up currently fall outside the current criteria for intervention.

In conclusion, this is the first study to describe early multichannel EEG findings (<6 hours of age) in infants with mild HIE and compare them with healthy term infants at the same time point. There are clear differences between the EEGs of infants with mild HIE and healthy term infants. Visual analysis shows that 72% of infants with mild HIE have some abnormal EEG features such as sleep cycle disruption or excessive sharp and slow waves within 6 hours of birth, which cannot all be attributed to the difficult sleep environment of the NICU. Quantitative analysis of the EEG reveals significant differences in spectral measures of the lower frequency bands. The challenge now is to correlate these features with outcome and determine the

importance of each feature. Incorporation of early quantitative EEG features could be useful for future trials of TH in infants with mild HIE to aid in the early and objective identification of cases.

3. Near-Infrared Spectroscopy in Infants with Hypoxic Ischaemic Encephalopathy

3.1. Systematic Review: Does early Cerebral Near-infrared Spectroscopy (NIRS) monitoring predict outcome in Neonates with Hypoxic Ischaemic Encephalopathy (HIE)? A Systematic Review of Diagnostic Test Accuracy.

3.1.1. Introduction

Hypoxic Ischaemic Encephalopathy (HIE) is the leading cause of acquired brain injury in term newborns and accounts for 1-3/1000 live births. (4). HIE results from an initial insult due to cerebral hypoxia and ischaemia resulting in energy depletion and lactate accumulation secondary to anaerobic metabolism. This is followed by a secondary insult, termed the reperfusion injury, following circulatory restoration. (441, 442) HIE is clinically graded as mild, moderate and severe based on neurological examination after birth and long-term neurodevelopmental outcome correlates with grade of encephalopathy. Therapeutic hypothermia (TH) is the current approved treatment for infants with moderate and severe grades of HIE and should be commenced within 6 hours of birth, before the secondary energy failure commences, to be most effective. (143, 150, 442) Historically, infants with mild grades of encephalopathy were thought to have normal outcomes (2); however, it is becoming more apparent that these infants are at increased risk of adverse neurodevelopmental outcome, specifically significant levels of learning, emotional and behavioural difficulties. (115)

This presents significant challenges as current methods of identifying such infants at risk of long-term neurodevelopmental difficulties are somewhat limited. It is clear that some infants who have difficulties later are currently falling below the threshold for therapeutic intervention. (115) The earliest available tools including Apgar score, pH and lactate levels do not correlate with outcome, rather they succeed in focusing attention on newborns at risk of HIE. (59, 60, 94)

Currently, assessment is centred around clinical evaluation, and the modified Sarnat and Thompson scores (2, 108) are most commonly used in clinical practice. Originally validated to examine infants repeatedly in the first week of life, (2, 108) when used in the first 6 hours of life, they can be influenced by factors including mode of delivery and maternal analgesia.

Electroencephalography (EEG) plays a key role in both prognostication and detection of seizures in infants with HIE. Amplitude-integrated EEG (aEEG) in the first 6 hours after birth was the most useful tool in predicting outcome in the pre-TH era, however this predictive ability has now altered if TH is commenced very early and the best predictive ability is now seen at 48 hours. (196) Continuous, multichannel EEG requires expert interpretation and most units do not have 24-hour access to a neonatal neurophysiologist or neurologist.

Hypoxic injury is not evident on conventional magnetic resonance imaging (MRI) until the first to second week of life (376) and is therefore not helpful in detecting or grading encephalopathy in the early postnatal period. (377) Early MRI scans may be helpful in ascertaining the timing of the initial injury, (378, 379, 380) although there are concerns that early diffusion weighted imaging (DWI) scans may underestimate the extent of the injury. (380, 382)

Prediction of long-term outcome remains challenging and it is important to consider other confounding factors in the neonatal unit and beyond. However, at present, we have no single test to accurately identify infants at risk of long-term neurodevelopmental outcome in the setting of HIE. Alternative methods of identification are thus required.

HI injury results in a period of hypoperfusion at the time of initial insult followed by circulatory restoration. (442) Cerebral blood flow (CBF) has been shown to increase in the first 24 hours following HI injury, likely due to a disruption in haemodynamic control. (291, 292) This early hyperperfusion has been shown to be associated with increased risk of brain injury. (293) Clinicians commonly associate time of injury with

time of birth, which is not always the case and may explain why studies on the use of early near-infrared spectroscopy (NIRS) as a prognostic tool are conflicting but beyond 24 hours of age, NIRS, specifically cerebral oxygenation (cSO_2) and metabolism, appears to be a good predictor of neurodevelopmental outcome. (299, 443) Data on NIRS in the first 6 hours of life (within the therapeutic window) may aid in prognostication and objective identification of cases for therapeutic intervention.

The aim of this systematic review is to determine whether early cerebral NIRS monitoring (<6 hours of age) can predict neonatal outcome as defined by grade of encephalopathy (defined clinically using Thompson or Sarnat scoring, or electrophysiologically using visual EEG analysis), brain MRI findings and/or neurodevelopmental outcome at 1-2 years of age.

3.1.2. Methods

As per the Preferred Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement, a systematic stepwise approach was taken to search various databases. (444)

3.1.2.1. Search strategy

Relevant articles were identified from the following databases by two authors, (A Garvey, A Pavel): PubMed, Scopus, Web of Science, Embase and The Cochrane Library. Searches were filtered to include human studies published in the English language in the last 30 years (Jan 1988 to July 2019). Three broad search terms were used (Neonate, HIE and NIRS) along with their corresponding synonyms and MeSH terms.(Table 3.1) Additional published reports identified in review articles or screened articles were subsequently included.

Table 3.1: Search terms used in database search.

Search concepts	1. Newborn	2. Hypoxic Ischaemic Encephalopathy	3. NIRS monitoring
PubMed	infant, newborn[MeSH Terms] OR infant[All Fields] OR "newborn"[All Fields] OR newborn* OR neonat* OR infan*	"hypoxia ischaemia" OR hypoxic ischemia OR "hypoxic ischaemia" OR "hypoxic ischemic" OR "hypoxic ischaemic" OR HIE OR asphyxia* OR "anoxia" [MeSH] OR encephalopathy OR "asphyxia neonatorum"[MeSH] OR "Hypoxia-Ischemia, Brain"[MeSH] OR "neonatal encephalopathy"	"spectroscopy, near-infrared"[MeSH] OR "(cerebr* or regional* or tissue* or brain*) AND (oxymet* or oximet* or oxygen*)" OR "near infrared spectroscopy" OR "near infrared reflectance spectroscopy" OR nirs* OR "near ir spectroscopy" OR invos* OR equanox* OR foresight* OR "for e sight*" OR cerox* OR "cer ox*" OR "in spectra" OR inspectra* OR niro* OR "near ir spectroscop*" OR "near* infrared* spectroscop*" OR sto2 OR rsco2 OR rso2 OR scto2 OR "brain oxygenation" OR "cerebral oximeter" OR "cerebral oxim*" OR "cerebral oxygen
Scopus	infan* OR newborn* OR neonat*	hypoxia OR hypoxemia OR hypoxi* OR h ypoxe* OR hypoxic OR anoxia OR anoxae mia OR asphyxia OR asphyxi* OR "hypoxi c ischemic encephalopathy" OR "newborn hypoxia" OR ischaemia OR ischaemi* OR ischemi* OR "neonatal encephalopathy" OR "brain hypoxia" OR "perinatal asphyxi*"	near infrared spectroscopy OR "near infrared reflectance spectroscopy" OR nirs* OR "near ir spectroscopy" OR invos* OR equanox* OR foresight* OR "for e sight*" OR cerox* OR "cer ox*" OR "in spectra" OR inspectra* OR niro* OR "near ir spectroscop*" OR "near* infrared* spectroscop*" OR sto2 OR rsco2 OR rso2 OR scto2 OR "brai n oxygenation" OR "cerebral oximeter" OR "cerebral oxim*" OR "cerebral oxygen saturation" OR "cerebral oxygenation" OR "cerebral oxygen*" OR "toe*" OR "regional oxymet*"

Search concepts	1. Newborn	2. Hypoxic Ischaemic Encephalopathy	3. NIRS monitoring
WOS	newborn* OR neonat* OR infan*	"hypoxi? isch$emi?" OR HIE OR asphyxia OR anoxia OR (hypoxi? OR ischaemi?) NEAR encephalop* OR (hypoxi? OR ischaemi?) NEAR brain OR encephalopath* OR "asphyxia neonatorum" OR "neonatal encephalopathy"	"spectroscopy, near-infrared"[MeSH] OR "(cerebr* or regional* or tissue* or brain*) AND (oxymet* or oximet* or oxygen*)" OR "near infrared spectroscopy" OR "near infrared reflectance spectroscopy" OR nirs* OR "near ir spectroscopy" OR invos* OR equanox* OR foresight* OR "for e sight*" OR cerox* OR "cer ox*" OR "in spectra" OR inspectra* OR niro* OR "near ir spectroscop*" OR "near* infrared* spectroscop*" OR sto2 OR rsco2 OR rso2 OR scto2 OR "brai n oxygenation" OR "cerebral oximeter" OR "cerebral oxim*" OR "cerebral oxygen
Embase	infant'/exp OR infant OR infan* OR newborn* OR neonat*	hypoxia'/exp OR 'hypoxemia'/exp OR hypoxi* OR hypoxe* OR hypoxic OR 'anoxia'/exp OR 'anoxaemia'/exp OR 'asphyxia'/exp OR asphyxi* OR 'hypoxic ischemic encephalopathy'/exp OR 'newborn hypoxia'/exp OR 'ischemia'/exp OR 'ischaemia'/exp OR ischaemi* OR ischemi* OR 'neonatal encephalopathy'/exp OR 'brain hypoxia'/exp	near infrared spectroscopy'/exp OR 'near infrared reflectance spectroscopy'/exp OR 'nirs*' OR 'near ir spectroscopy' OR invos* OR equanox* OR foresight* OR 'fore sight*' OR cerox* OR 'cer ox*' OR 'in spectra' OR inspectra* OR niro* OR 'near ir spectroscop*' OR 'near* infrared* spectroscop*' OR 'sto2 or rsco2 or rso2 or scto2' OR 'brain oxygenation'/exp OR 'cerebral oximeter'/exp OR 'cerebral oxim*' OR 'cerebral oxygen saturation'/exp OR 'cerebral oxygenation'/exp OR 'cerebral oxygen*' OR 'toe*' OR 'regional oxymet*'

Search concepts	**1. Newborn**	**2. Hypoxic Ischaemic Encephalopathy**	**3. NIRS monitoring**
Cochrane library	newborn* OR neonat* OR infan*	“hypoxia ischaemia” OR “hypoxia ischemia” OR “hypoxia ischaemic” OR “hypoxia ischemic” OR “hypoxic ischemia” OR “hypoxic ischaemia” OR “hypoxic ischemic” OR “hypoxic ischaemic” OR HIE OR asphyxia* OR "hypoxia" [MeSH] OR “anoxia” [MeSH] OR encephalopath* OR “asphyxia neonatorum” [MeSH] OR Hypoxia-Ischemia, Brain[MeSH] OR “neonatal encephalopathy”	“spectroscopy, near-infrared” [MeSH] OR “INVOS* or EQUANOX* or FORESIGHT* or FORE-SIGHT* or CerOX* or Cer-OX* or IN-Spectra or INSpectra* or Niro*” OR “NIRS*” OR “near IR spectroscop*” OR “near* infrared* spectroscop*” OR “(cerebr* or regional* or tissue* or brain*) AND (oxymet* or oximet* or oxygen*)” OR “StO2 or rscO2 or rSO2 or SctO2” OR *NIRS OR *TOE*

3.1.2.2. Study Selection

All articles were indexed to EndNote and duplicates were removed. Articles were initially screened based on title and then by abstract by one author (A Garvey). The remaining articles were then screened independently for inclusion criteria by both A Garvey and E Dempsey and any discrepancies were discussed and resolved.

Inclusion criteria consisted of:

- Observational cohort study or clinical randomised or quasi-randomised controlled trials
- Human study
- Newborn infants born at ≥36 weeks gestational age
- Neonatal HIE defined clinically, using Thompson or Sarnat scoring, and/or electrophysiologically, using amplitude-integrated (aEEG) or continuous EEG (cEEG)
- Cerebral NIRS monitoring commenced early, defined as before 6 hours of age

Animal studies, studies that were not in English or conference abstracts were not included.

3.1.2.3. Strategy for data analysis

We planned to include a narrative of all the studies included, detailing the early findings of NIRS monitoring.

If the studies were similar clinically and methodologically, we planned to pool the results of the studies in a meta-analysis. Data from each study would be summarised in a 2X2 table (true-positives, false-positives, false-negatives and true negatives) and the sensitivity and specificity for each study calculated. A coupled forest plot

displaying the sensitivity and specificity and corresponding 95% CIs for each study and a summary plot displaying the sensitivity-specificity point for each study would be created. Heterogeneity among studies would be investigated by visual inspection of these plots. If no substantial heterogeneity was observed, pooled sensitivity and specificity estimates and corresponding 95% CIs would be calculated for studies with a common threshold using the bivariate random effects regression model or the univariate random effects model (if the number of studies is small). If different thresholds were reported in the individual studies, a hierarchical summary receiver operating characteristic (HSROC) curve would be created and a global summary of test accuracy calculated. The analysis would be performed using Stata (version 13.0, StataCorp LP, USA).

3.1.2.4. Risk of bias (quality) assessment

Studies were critically appraised using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool to assess their validity and applicability. Each article was scored by both reviewers independently.

The review protocol was published on the International Prospective Register of Systematic Reviews (PROSPERO) prior to conducting database searches (Registration number PROSPERO 2019 CRD42019127907).(Appendix G)

3.1.3. Results

Our initial search identified 3826 articles. Figure 3.1 provides a flow diagram of the search results.

From these articles, 1116 duplicates were removed and 2590 articles were excluded based on title or abstract. One hundred and twenty full-text articles were assessed for eligibility criteria and from these we identified 7 original articles that described the use of NIRS in infants with HIE within the first 6 hours after birth. The details of included articles are summarised in Table 3.2. The principal reasons for non-inclusion of articles are outlined in Table 3.3, but predominantly were related to a NIRS device not being used (e.g. MRI to measure cerebral metabolism and blood flow), start time of NIRS recording not documented or NIRS monitoring commenced after 6 hours of age.

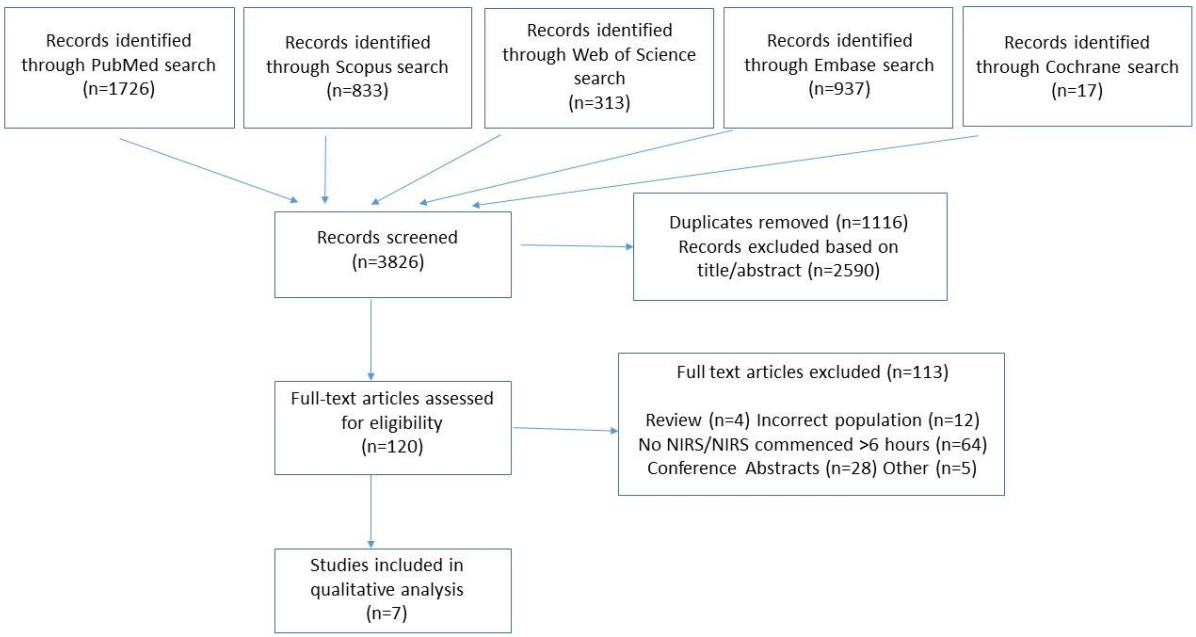


Figure 3.1: PRISMA diagram of literature search.

3.1.3.1. Included Studies

Details of included studies are outlined in Table 3.2 and a brief narrative is included below. Most studies used mean values of NIRS measures calculated over a 30–60 minute time window. Goeral et al. calculated mean values over 6-hour timeframes and Nakamura et al. did not specify the timeframe encephalopathy. (295, 307).

3.1.3.2. Early NIRS to determine grade of encephalopathy

In all of the included studies, grade of encephalopathy was determined at or before time of enrolment using standardised assessment tools such as the Sarnat or Thompson score. Six of the seven trials focused on moderate to severe encephalopathy only. Early NIRS was not assessed for its ability to predict grade of encephalopathy nor were they correlated with the individual grades. Only one study included infants with mild encephalopathy and no study included a control group.

3.1.3.3. Early NIRS monitoring to predict outcomes

(a) MR injury only: Only 2 studies addressed

Peng et al. found consistently higher cSO_2 values in moderately or severely encephalopathic infants receiving TH with later evidence of injury on MRI. (294) In a cohort of 32 cooled infants with moderate encephalopathy, Goeral et al. found no difference in TOI or FTOE values at 6 hours in those with favourable or unfavourable MRI outcomes. (295)

(b) Long-term outcome only: Only 1 study addressed

Examining the ability of NIRS to predict long-term outcome, Ancora et al. reported a trend towards a higher TOI at 6 hours of age in infants with an adverse outcome at 18 months (80.0% +/- 10.5 vs 66.9% =/- 7.0, p=0.057) and this difference was statistically significant by 24 hours of age (79.7% +/- 9.4 vs 67.1% +/- 7.9, p=0.034). (300) These infants all received TH for moderate or severe grades of encephalopathy.

Table 3.2: Studies included in systematic review, NIRS measurement commenced <6hours, outcome method used and synopsis of main findings.

Author	**n**	**Grade of HIE (n)**	**NIRS Device**	**Location of Sensor**	**NIRS Measure Reported**	**MRI Brain**	**Long-term Outcome**	**Summary of Findings**
Ancora et al. 2013 [300]	12	Moderate (8), Severe (4)	NIRO 200	Bifrontal	TOI	n/a	Griffiths at 18mths	No difference in TOI at 6 hours. Significantly higher TOI values at 24 hours in adverse outcome group.
Goeral et al. 2017 [295]	32	Moderate (32)	INVOS 5100C neonatal sensor	Frontoparietal	cSO_2, FTOE	First 2 weeks	not done	No difference in cSO_2/FTOE values at 6 hours in those with favourable/unfavourable MRI outcomes
Gucuyener et al. 2012 [500]	10	Moderate (6), Severe (4)	NIRO 200	Parietal	TOI, FTOE	Day 7	Bayley's at 24 months	Early NIRS (TOI, FTOE) did not predict long-term outcome.
Lemmers et al. 2013 [249]	39	Moderate, Severe* (39)	INVOS 4100-5100 adult sensor	Frontoparietal	cSO_2, FTOE	Day 4-6	Griffiths at 18mths	No difference in early cSO_2 or FTOE. Significantly higher cSO_2 beyond 24-72 hours in adverse outcome group.

Author	n	Grade of HIE (n)	NIRS Device	Location of Sensor	NIRS Measure Reported	MRI Brain	Long-term Outcome	Summary of Findings
Nakamura et al. 2015 [307]	11	Mild (4), Moderate (6), Severe (1)	TRS 10	Forehead	cSO_2, CBV	Day 7 - 14	Kyoto Scale of Psychological Dev & exam at 12-18mths	No difference in early cSO_2. Significantly higher CBV at 6 hours in adverse outcome group. Significantly higher cSO_2 and CBV at 24 hours in adverse outcome group.
Niezen et al. 2017 [197]	39	Moderate, Severe* (39)	INVOS 5100 paediatric sensor	Left or right frontoparietal	cSO_2	Day 4 - 13	GMFCS and Bayley's at 30 months	No difference in early cSO_2. Significantly higher cSO_2 beyond 24-72hours in adverse outcome group.
Peng et al. 2015 [294]	18	Moderate (8), Severe (10)	Fore-sight	Bifrontal	cSO_2	Day 2-3 and 10	not done	Higher cSO_2 values at all time points in infants with evidence of MR injury.

TOI, tissue oxygen extraction; cSO_2, cerebral oxygen saturation; FTOE, fractional tissue oxygen extraction; CBV, cerebral blood volume
**Numbers of each grade not specified*

Table 3.3: Excluded NIRS studies and reasons for exclusion.

NIRS not reported (n=14)	**Start time not documented (n=15)**	**NIRS commenced after 6 hours of life (n=25)**
Cunningham et al. 1993 (445)	Burton et al. 2015 (458)	Alderliesten et al. 2017 (470)
De Vis et al. 2014 (446)	Carrasco et al. 2018 (459)	Bale G et al. 2016 (471)
Edwards-Bailey et al. 2016 (447)	Chalak et al. 2016 (460)	Campbell et al. 2018 (472)
Frewen et al. 1991 (448)	Chavez-Valdez et al. 2017 (461)	Chalak et al. 2017 (473)
Hanrahan et al. 1996 (449)	Govindan et al. 2016 (462)	Costa et al. 2012 (474)
Hebden et al. 2004 (450)	Howlett et al. 2013 (289)	Dehaes et al. 2014 (475)
Kapadia et al. 2013 (451)	Lee et al. 2017 (463)	Edwards et al. 1988 (476)
Kelly et al. 2018 (74)	Lee et al. 2017 (279)	Estrin et al. 2011 (477)
Klinger et al. 2005 (452)	Meek et al. 1999 (292)	Forman et al. 2017 (352)
Kusaka et al. 2001 (453)	Shellhaas et al. 2013 (464)	Govindan et al. 2014 (478)
Lingappan et al. 2016 (454)	Shellhaas et al. 2015 (465)	Govindan et al. 2016 (479)
Tokuhisa et al. 2015 (455)	Tekes et al. 2015 (466)	Govindan et al. 2018 (480)
Singh et al. 2014 (456)	Tian et al. 2016 (467)	Holper et al. 2017 (481)
Singh et al. 2016 (457)	Wintermark et al. 2014 (468)	Jain et al. 2017 (443)
	Zaramella et al. 2007 (469)	Kaya et al. 2014 (482)
		Kondo et al. 2014 (483)
		Massaro et al. 2013 (303)
		Massaro et al. 2015 (276)
		Mitra et al. 2016 (484)
		Mitra et al. 2019 (485)
		Shellhaas et al. 2014 (486)
		Shellhaas et al. 2017 (487)
		Toet et al. 2006 (488)
		Wu et al. 2018 (360)
		Wyatt et al. 1986 (489)

Others (n=10)	
Bale et al. 2014 (490)	NIRS during desaturation episodes only
Bale et al. 2019 (491)	NIRS during desaturation episodes only
Chen et al. 2002 (492)	Effect of auditory stimuli on NIRS
Gagnon et al. 2016 (493)	Effect of PPHN and HIE
Kovacsova et al. 2018 (494)	Effect of confounders on different spatially resolved spectroscopy devices
Skov et al. 1993 (495)	Effect of head tilting in preterm and asphyxiated infants
Sokoloff et al. 2015 (496)	Effect of seizures on NIRS
Van Bel et al. 1993 (497)	No standardised assessment of encephalopathy grade used
van Bel et al. 1998 (498)	Effect of allopurinol on NIRS
Vesoulis et al. 2018 (499)	NIRS to determine autoregulation threshold

(c) Composite outcome of MR or Long-term outcome: 4 studies addressed

Both Lemmers et al. and Niezen et al. found that early cSO_2 and FTOE values did not differentiate between infants with a favourable or adverse outcome. (197, 299) They included infants with both moderate and severe grades of HIE undergoing TH. However, in both studies, the predictive ability of these NIRS measurements improved beyond the first 24-72 hours of age at which time cSO_2 was significantly higher in the adverse outcome group.

Nakamura et al. also found no difference in cSO_2 values at 6 hours between those with a favourable and unfavourable outcome across all grades of encephalopathy. (307) Mean CBV at 6 hours, however, was significantly higher in the infants who had an adverse outcome (3.1 +/- 0.4 vs 2.4 +/- 0.5mls/100g brain, p=0.03). At 24 hours after birth both cSO_2 and CBV were higher in the infants with an adverse outcome and they found that combining CBV and cSO_2 at 24 hours after birth had a 100% sensitivity, specificity, positive and negative predictive values for neurological outcome.

Gucuyener et al. noted that three infants in their cohort, all of whom were cooled for moderate grade of encephalopathy, had a low TOI and high FTOE before the initiation of TH. (500) These same infants had repetitive seizures as confirmed by aEEG within the first few hours of life, suggesting increased oxygen consumption during seizures and by the end of TH, their values were within normal ranges. Although two of these infants had an abnormal MRI on day 7, all three had normal Bayley's scores at 2 years of age, highlighting the importance of long-term follow-up as an abnormal MRI is not synonymous with adverse neurodevelopmental outcome.

3.1.3.4. Quality Assessment

Quality of the included studies were assessed using the QUADAS-2 tool.(Table 3.4) Regarding patient selection, it was unclear in some studies as to whether patients were consecutively enrolled or selected randomly, thus avoiding selection bias. In all studies, it was unclear as to whether the index test was interpreted without

knowledge of the reference standard and similar issues with blinding were present in some studies regarding the interpretation of the outcome data. Generally, concerns regarding applicability were low in the majority of studies.

Table 3.4: Assessment of risk of bias of included studies using Quality Assessment of Diagnostic Accuracy Tests – 2.

	Risk of Bias				Applicability Concerns		
	Patient Selection	**Index Test**	**Reference Standard**	**Flow and Timing**	**Patient Selection**	**Index Test**	**Reference Standard**
Ancora et al. [300]	Low	Unclear	Low	Low	Low	Low	Low
Goeral et al. [295]	Low	Unclear	Low	Low	Low	Low	Low
Gucuyener et al. [500]	Unclear	Unclear	Unclear	High	Low	Low	Low
Lemmers et al. [249]	Low	Unclear	Low	Low	Low	Low	Low
Nakamura et al. [307]	High	Unclear	High	Low	Low	Low	Low
Niezen et al. [197]	Low	Unclear	Unclear	Low	Low	Low	Low
Peng et al. [294]	Unclear	Unclear	Low	Low	Unclear	Low	Low

3.1.4. Discussion

HIE is one of the top ten contributors to the global burden of disease and has life-long effects for survivors and their families. (6) The use of TH in infants with moderate and severe encephalopathy has improved neurological outcomes. However, with significant disability still reported in over 1 million survivors, (114) further improvement in therapy is required. Early NIRS monitoring may have an important role to play, not only in the prediction of poor neurodevelopmental outcome, but also in the objective selection of infants for therapeutic intervention.

One of the main findings from this systematic review was the variation in methodology seen, specifically the device and sensors used, the measurements reported and the outcome milestones assessed. Due to this heterogeneity, we were unable to perform a quantitative meta-analysis. In the 7 included studies, 6 different devices or sensors were used, making it very difficult to draw firm conclusions. Although normative ranges for term infants have been suggested, these values have a narrow range and vary greatly between configurations of devices and sensors used. (501, 502) Using the INVOS oximeter, for example, cSO_2 values vary up to 10% depending on whether the neonatal or adult probes are used. In addition, the INVOS oximeter truncates values at 95%, the range above which an adverse outcome may be expected, and is an important point to consider when neonatal probes are used. Reapplication of a sensor in the same area may also result in variability of values of up to 6%. (503) Furthermore, there is significant variation in the probe location used in the above studies e.g. frontal, parietal, frontoparietal. Some studies obtained measurements bilaterally and some did not specify whether it was right or left. This is important, as different NIRS systems have different penetration depths so values may vary depending on the site used. (504)

There was also variation in the short and long-term outcome assessment methods used. Overall, 6 of the studies examined the ability of NIRS to predict brain injury on MRI but the timing of these scans differed with some performing MRI in the first week of life and others into the second week of life. Long-term neurodevelopmental assessment also varied from 12 to 30 months of age. Furthermore, it is also unclear

in many of the studies whether the authors were blinded to the NIRS values when assessing the outcome data and vice-versa, raising concerns for the potential for bias.

It is important, however, to also consider the timing of injury. As previously mentioned, timing of injury is frequently tacked to time of birth when in fact the timing of the sentinel event may vary and the resultant asphyxia may be acute, chronic or acute-on-chronic placing the infant at different stages along the injury pathway at time of birth. (70) Nonetheless, NIRS provides an indirect measure of cerebral perfusion and oxygen consumption and has been shown to be very sensitive to acute changes in cerebral oxygenation. (505) Alterations in cerebral oxygenation and metabolism are pathognomonic of HIE, supporting the theoretical benefit of NIRS as a potential bedside biomarker. CBF has been shown to increase in the first 24 hours following HI injury. (291, 292) In the pre-TH era, a low resistance index as measured by Doppler ultrasound was a good predictor of outcome in infants with HIE indicating impaired cerebral vasodilation and blood flow. This is no longer the case in the advent of TH. (506) However in infants receiving TH, Wintermark et al. found decreased CBF on day of life 1 in infants who later demonstrated hypoxic-ischaemic injury on MRI. (293) Therefore, early cerebral NIRS measurements of cerebral oxygenation, metabolism and blood flow may contain important information that could aid in the prediction of outcome.

It is also necessary to address any potential confounding influencers on cSO_2. cSO_2 values are comprised of arterial, venous and capillary saturations with a largely venous component (75%). For this reason, care is needed in its interpretation as it portrays the fine balance between oxygen delivery and consumption and can thus be influenced by many variables including blood pressure, cardiac output, ventilator status and concomitant medication. Nonetheless, trends in NIRS values can give important clues as to the haemodynamic status of the individual organs being monitored. (246, 507, 508) Sedation was described in 4 of the studies in which all infants received sedation as routine. (197, 300, 307, 500) Three studies described the use of inotropes but found no difference in the use of inotropes between infants with a favourable or unfavourable outcome. (294, 295, 299) The impact of TH on NIRS is

unclear. TH is thought to reduce metabolism and thus may decrease oxygen extraction. However, TH is also known to cause bradycardia, which may influence cardiac output and thus oxygen delivery. Only Lemmers et al. and Nakamura et al. provide cSO_2 values during both hypothermia and normothermia however, they do not address the impact of TH on cerebral oxygenation. (299, 307)

Previous studies have shown that cSO_2 levels beyond 24 hours of age correlate with MRI findings and long-term neurodevelopmental outcome. (299, 443) Persistently higher cSO_2 values are seen in infants with subsequent MRI injury and poor neurological outcomes. (271, 443) We set out to determine if early cerebral NIRS monitoring before 6 hours of age could predict both short- and long-term outcomes, and thereby aid in the identification of infants who would benefit most from therapeutic hypothermia. Although two studies demonstrated higher cSO_2 and CBF values before 6 hours in infants with adverse outcomes, in the majority, this difference was not significant until beyond 24 hours of age. (197, 294, 295, 300, 307, 500)

Cerebral NIRS may be used in combination with blood pressure to assess autoregulation in infants with HIE. (274) Studies have used NIRS to determine the optimal mean arterial blood pressure at which autoregulation is maintained. Studies to date have found that infants who spent longer outside this optimal pressure range had a greater risk of adverse outcome. (279) This approach did not form part of our review but information pertaining to early cerebral autoregulation may be beneficial in enabling early identification of infants at risk.

NIRS can also be used in combination with other modalities such as EEG or aEEG. As discussed, aEEG in the first 6 hours of life was the most useful tool in predicting outcome in the pre-TH era. Although its predictive ability is now best seen at 48 hours following the introduction of TH, (196) the combination of NIRS and aEEG in the first 6 hours after birth may be useful in predicting outcome and thus the need for TH. Lemmers et al. found that when aEEG and cSO_2 scores were combined, both positive and negative predictive values improved to 70-91% and 90-100% respectively. (299)

Goeral et al. found that a combination of both cSO_2 and aEEG predicted MRI outcome as early as 18 hours. (295)

Cerebral NIRS is both a feasible and acceptable monitoring tool in the neonatal unit. However, whether it results in a meaningful benefit to the neonatal HIE population remains to be seen. Based on limited available data, methodological heterogeneity in design, execution and available findings, early cerebral NIRS has not been evaluated for prediction of grades of HIE, and is not associated with prediction of MRI evidence of brain injury or neurodevelopmental outcome.

The included papers in this review focused primarily on continuous wave NIRS to measure cerebral oxygenation. Other available methods include broadband NIRS or diffuse correlation spectroscopy, which measure changes in cytochrome c oxidase (CCO) and the cerebral metabolic rate of oxygen ($CMRO_2$). These devices however are not commercially available and are therefore predominantly used in a research setting. (509)

Whilst research to date has primarily focused on the value of NIRS monitoring as a trend monitor, signal-processing techniques have shown that the NIRS signal itself may be rich in information. (508) Perhaps it is not the absolute values, rather the variation in values over time or quantitative analysis of the signal itself that is useful in outcome prediction. Quantitative analysis has the potential of providing an objective, continuous and scalable analysis of the NIRS signal but further research in this area is required. Future research should also focus on obtaining early measurements and including all grades of HIE. Combining NIRS with other monitoring modalities such as EEG and BP may enhance its predictive ability however, it is also important that saturation values are measured using the same device, sensors and methods if NIRS is to be used as a predictive tool.

This review is limited by the fact that it only includes articles written in English and was filtered to include human studies only as we wished to assess the usefulness of NIRS in clinical practice. As discussed, a small number of studies with varying

methodologies were identified so a meta-analysis was not feasible; however, this review was systematic and transparent. All full text articles were independently reviewed and we have clearly outlined reasons for inclusion and exclusion of studies in a PRISMA flowchart.

In conclusion, cerebral NIRS has the potential to be an important monitoring tool in infants with HIE. It can be used early after birth as it is quick and easy to apply and can provide continuous, non-invasive measurements. Unlike other monitoring modalities such as EEG, NIRS interpretation requires little training and values are available instantaneously with a high sampling frequency. However, there are many questions to be answered before NIRS can be incorporated as an objective tool for identifying at-risk infants. Only 161 infants in total were included in the studies reviewed and individually the studies had insufficient numbers to address their primary objectives. Only 4 infants with mild HIE were included in the studies and therefore we are unable to determine whether there is a benefit to NIRS monitoring in this group. Furthermore, differing methodologies and conflicting results make it very difficult to draw any firm conclusions. There may be a trend towards higher cSO_2 values at 6 hours of age in infants with HIE who subsequently have adverse outcomes but larger studies using a standardised approach and including all grades of encephalopathy are required. Furthermore, more precise measures of oxygen metabolism are likely required to identify the impairment in cerebral metabolism which occurs in the early stages of HIE prior to its incorporation into the neonatal unit as a predictive or decision making tool.

3.2. Evolution of Early Cerebral NIRS in Hypoxic Ischaemic Encephalopathy.

3.2.1. Introduction

Hypoxic ischaemic encephalopathy (HIE) is one of the leading causes of acquired brain injury in term infants. (4) Although therapeutic hypothermia (TH) has significantly improved outcomes in infants with moderate and severe grades of HIE, approximately 40% of infants continue to have adverse neurodevelopmental outcomes. (5) Growing evidence suggests that infants with mild HIE are also at risk of disability. (115) However as evidence for the use of TH in mild HIE is currently lacking, these infants generally receive supportive management in the neonatal unit.

Alterations in cerebral blood flow (CBF) and metabolism are pathognomonic of HIE. Initially low following the acute HI injury, CBF increases over the first 24 hours. Near-infrared spectroscopy (NIRS) monitoring in HIE has many theoretical benefits as it provides continuous, non-invasive monitoring of tissue oxygenation. Regional cerebral oxygen saturation (cSO_2) may provide useful information on cerebral haemodynamics and can be used as a surrogate of cerebral perfusion. (273) Fractional Tissue Oxygen Extraction (FTOE) is a measure of oxygen extraction and is calculated using the formula:

$$\frac{(SpO_2 - cSO_2)}{SpO_2}$$

By accounting for peripheral arterial oxygen saturation (SpO_2), it therefore allows for further interrogation of the cSO_2 trends by providing information, not only on oxygen delivery, but regional oxygen uptake and utilisation. Thus, FTOE provides further insight into cerebral perfusion. (272)

Many studies have assessed the utility of NIRS in the care of infants with HIE, specifically infants undergoing TH. (271, 294, 299, 510) Increased cSO_2 and lower FTOE beyond 24 hours have been associated with adverse outcome. The evolution

of values over time is also important as Jain et al. identified greater magnetic resonance imaging (MRI) injury in infants with more rapidly increasing cSO_2. (443) However, very few studies have examined the use of NIRS at earlier time points and its evolution over time in the setting of HIE. (511)

Clinically it can be difficult to differentiate between grades of HIE, particularly between infants with mild and moderate encephalopathy in the first hours after birth, and infants who may potentially benefit from TH may be missed. (113) In addition, earlier initiation of TH is associated with improved outcome and TH commenced later, after 6 hours, has been shown to be less effective. (30, 143, 150, 167) Early, objective identification of infants who would benefit from TH within the therapeutic window is crucial.

To date, studies examining the use of early NIRS in HIE include small patient numbers and vary greatly in their methodology. (511) Furthermore, no study has examined the evolution of cSO_2 in infants with mild HIE. The aim of this study was to describe early cSO_2 and FTOE in infants with all grades of HIE (uncooled infants with mild HIE and infants with moderate and severe HIE receiving TH) and to examine their evolution over time.

3.2.2. Methods

This study was part of a larger prospective observational study conducted in Cork University Maternity Hospital, Cork, Ireland between November 2017 and March 2020. Infants with all grades of HIE had multi-modal monitoring including NIRS, electroencephalography (EEG), non-invasive cardiac output monitoring, echocardiography, MRI and blood biomarkers. Ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals (ECM 5(5) 04/07/17). Parents of infants meeting inclusion criteria were approached after delivery and written informed parental consent was obtained in each case.

Infants > 36 weeks gestation at birth admitted to the neonatal unit with evidence of HIE were eligible for inclusion. Specifically, infants had one or more of the following: an Apgar <5 at 5 minutes, postnatal resuscitation >10 minutes, pH <7.1 or base deficit >16 or lactate >9mmol on cord or first post-natal blood sample, AND clinically evolving encephalopathy defined as the presence of abnormal neurological findings on the modified Sarnat score performed at approximately 1 hour of age. (71, 512, 513)

Exclusion criteria included congenital abnormalities, inborn errors of metabolism, congenital infections or confirmed sepsis.

Infants were clinically categorised into grade of encephalopathy (mild, moderate, severe) based on assessment using the modified Sarnat score at one hour of age. (110, 122) Therapeutic hypothermia was provided to infants with moderate and severe grades of encephalopathy. A clinical decision was made by the attending physician to provide TH to two of the infants graded as having mild HIE. As there were only 2 infants in this group, they were excluded from the analysis. It is practice in our unit that infants undergoing TH receive a low dose morphine infusion (10-20mcg/kg/hour) which is titrated to clinical response.

3.2.2.1. NIRS

Following enrolment, cerebral NIRS monitoring commenced as soon as possible after delivery using the INVOS 5100 and the neonatal OxyAlert^{TM} NIRSensor (Covidien, USA) on the right frontal area. Continuous measurements were recorded during the inpatient stay for up to 4 days where feasible.

SpO_2 was measured with the Nellcor SpO_2 Neonatal Sensor (Covidien, USA) and the IntelliVue MP70 (Philips Healthcare, The Netherlands) and stored with the EEG signals using the NicoletOne EEG System (Natus, USA) or Nihon Kohden (Nihon Kohden, Japan). All machines were time-synchronised at the beginning of each recording and monitored during the recording period.

One-hour epochs of cSO_2 and corresponding SpO_2 were selected at 3, 6, 9, 12, 18, 24, 36, 48, 72 and 84 hours of age for each individual infant. The INVOS 5100 NIRS device records cSO_2 at a non-uniform sampling rate of approximately 1/5 to 1/6 Hz. (508) We up-sampled to a uniform 100 Hz by interpolation using a piece-wise cubic Hermite polynomial and then down-sampled to 1/5 Hz. We used the default anti-aliasing filter in our software package before down-sampling: a finite-impulse response filter with zero-phase and a Kaiser window with parameter β=5.

The Philips IntelliVue device has a sampling rate of 1/12 Hz. SpO_2 values were stored with the EEG signals and subsequently extracted from the EEG files. The Nicolet device records SpO_2 at a sampling rate of 1 Hz. The Nihon Kohden machine records the SpO_2 at a sampling rate of approximately 200 Hz. SpO_2 for the Nihon Kohden data was down-sampled to 1 Hz, after applying an anti-aliasing filter as described above.

The INVOS 5100 NIRS device measures cSO_2 values between 15-95%. Values less than 15% suggest loss of sensor contact and were therefore removed from analysis. SpO_2 recording may be affected by movement artefact. All values less than 40% were reviewed using the synchronised video EEG and removed if deemed to be due to movement artefact or loss of probe contact with skin.

To calculate FTOE, an average of SpO_2 and cSO_2 was calculated over 30 second periods. FTOE was then calculated using the following standard formula for each infant.

$$\frac{(SpO_2 - cSO_2)}{SpO_2}$$

For each infant, median cSO_2 and FTOE values across each hour (3, 6, 9, 12, 18, 24, 36, 48, 72 and 84 hours) were calculated.

3.2.2.2. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 26.0, IBM Corp, U.S.A.) and Stata (Version 13.0, StataCorp LP, USA). Continuous variables were described using the mean and standard deviation (SD) or median and interquartile range (IQR) and categorical variables using frequency and percentage.

Longitudinal mixed models (514) were used to investigate changes in cSO_2 and FTOE over time (from 6 to 24 hours after birth) in infants with moderate HIE compared to infants with mild HIE. For the unconditional model of time, the best fitting polynomial (a straight line, a quadratic curve or a cubic curve) was selected. The sequence of testing fixed and random polynomial effects of time was as follows: the first model was an empty random intercepts model and then each fixed effect (linear, quadratic, cubic) followed by its corresponding random time effect (linear, quadratic, cubic) was fitted, in turn. The fixed effect was added to the model if its Wald test was statistically significant and its corresponding random effect was added to the model if the likelihood ratio statistic was statistically significant. To examine the effect of HIE group on changes in cSO_2 and FTOE between 6 and 24 hours after birth, the fixed effects of HIE group and the interactions of HIE group by time (linear, quadratic and cubic, as appropriate) were added to the best fitting unconditional model of time. These fixed effects were included in the final model if their Wald tests were statistically significant. All tests were two-sided and p-values <0.05 were considered statistically significant.

Five infants in our cohort had a single seizure during one of the included time points. Three of these had severe HIE and therefore were not included in the mixed model analysis. The 2 included seizures were brief (seizure burden of <5minutes/hour). Analysis was performed both with and without the infants. As their included values were similar to other infants in their group at the time and as there was no difference in the overall results, they were included in the final analysis.

3.2.3. Results

3.2.3.1. Study participants

Sixty-one infants were enrolled in the study. Two infants with mild HIE received TH and one infant with moderate HIE had culture negative sepsis (elevated CRP 137, Day 2) and were subsequently excluded. Fifty-eight infants were included in the analysis. Twenty-eight infants had a mild grade of HIE, 24 were moderate and 6 were severe. Eleven infants (7 moderate, 4 severe) were outborn in another hospital and transferred to our centre for TH. The median gestational age (GA) across the entire group was 39.9 weeks (IQR 38.1-40.7) and median birth weight (BW) was 3.35 kgs (IQR 2.97 – 3.71). Demographic and perinatal data according to grade of encephalopathy are illustrated in Table 3.5. Approximately one third of infants in the mild and moderate groups were born by caesarean section. By 5 minutes of age, 11% of infants in the mild group had an Apgar score <5 compared with 50% of infants in the moderate group and 100% of infants in the severe group.

All infants with moderate and severe grades of encephalopathy received TH and NIRS values reported are during hypothermia treatment. None of the included infants with mild HIE received TH.

Table 3.5: Demographic and perinatal data of infants included in the study.

	Mild n=28	**Moderate n=24**	**Severe n=6**
median (IQR)			
Gestational Age (*weeks*)	40.1 (38.2-40.8)	39.3 (37.8-40.4)	40.4 (39-40.8)
Birthweight (*kg*)	3.36 (3.04-3.77)	3.29 (2.85-3.63)	3.51 (3.37-3.84)
Lowest pH	7.01 (6.92-7.08)	6.88 (6.81-7.09)	7.05 (6.78-7.12)
n (%)			
Mode of delivery			
SVD	7 (25)	7 (29)	3 (50)
Instrumental	11 (39)	8 (33)	0
EmLSCS labour	8 (29)	6 (25)	3 (50)
EmLSCS not in labour	2 (7)	3 (13)	0
Apgar score at 5 minutes			
0-4	3 (11)	12 (50)	6 (100)
5-7	8 (29)	11 (46)	0
8-10	17 (61)	1 (4)	0
Resuscitation			
Facial O_2	1 (4)	0	0
Positive Pressure Ventilation	14 (50)	8 (33)	0
CPAP	2 (7)	5 (21)	0
Intubation	1 (4)	5 (21)	2 (33)
CPR	2 (7)	5 (21)	1 (17)
Adrenaline	0	1 (4)	3 (50)
Seizures (EEG)	1	2 (8)	4 (67)
Medication			
Anti-seizure medication	0	3 (13)	4 (67)
Sedation (morphine)	0	24 (100)	6 (100)

SVD, spontaneous vaginal delivery; EmLSCS, emergency lower section caesarean section; CPAP, continuous positive airway pressure; CPR, cardiopulmonary resuscitation; EEG, electroencephalogram

3.2.3.2. Summary measures of cSO_2 and FTOE over time

Approximately 3% of the overall data was lost due to movement artefact or loss of probe contact with the skin. Table 3.6 displays summary measures of cSO_2 and FTOE over time in all infant groups at all time points. Due to limited data in the severe groups, only the mild and moderate groups and time points 6 to 24 were included in the statistical analysis. Five infants with mild HIE did not have SpO_2 values stored so FTOE were not available.

Figure 3.2 depicts changes in mean cSO_2 and FTOE over time in all infant groups. cSO_2 in all grades increases over the first 24 hours and then plateaus. FTOE decreases in all grades until 24 hours then plateaus.

Table 3.6: Summary measures of cSO_2 and FTOE over time by group.

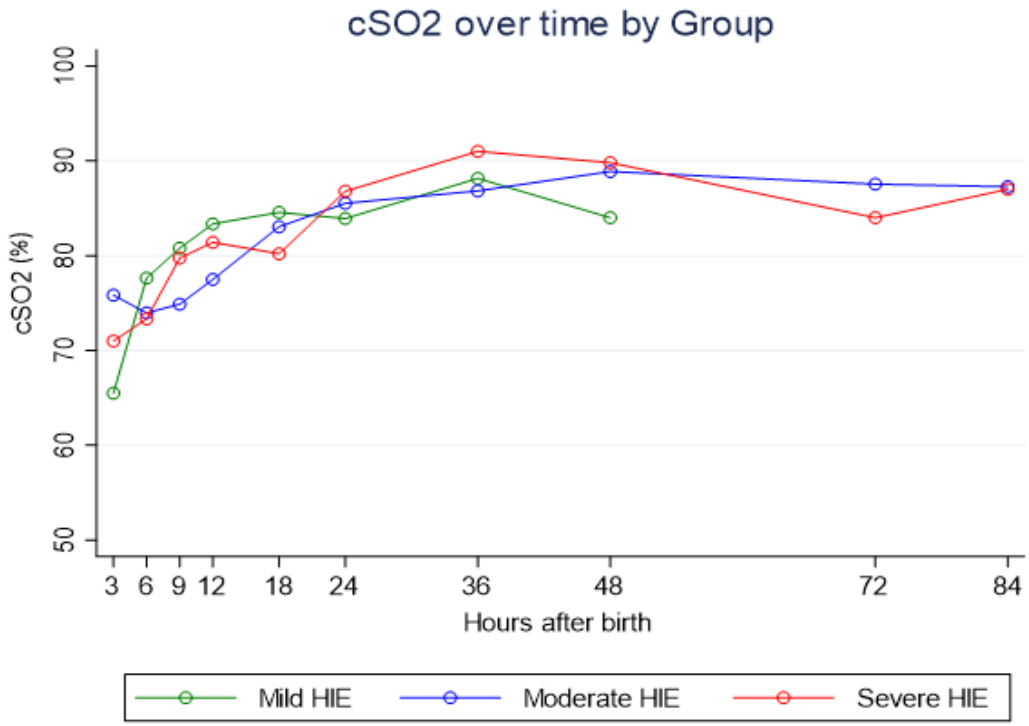
A. Summary measures of cSO_2 (%) over time by group

	Overall		Mild HIE - uncooled		Moderate HIE - cooled		Severe HIE - cooled	
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)
Hours after birth								
3	9	73.0 (13.1)	2	65.5 (13.4)	6	75.8 (14.3)	1	71.0
6	33	75.6 (10.0)	15	77.6 (9.0)	15	74.0 (11.8)	3	73.3 (1.5)
9	49	78.2 (9.5)	24	80.8 (7.1)	21	74.9 (11.6)	4	79.8 (5.4)
12	55	80.6 (8.9)	26	83.4 (7.7)	24	77.5 (9.8)	5	81.4 (6.8)
18	56	83.5 (8.0)	27	84.6 (7.5)	24	83.0 (8.4)	5	80.2 (9.8)
24	52	84.9 (7.3)	24	83.9 (7.5)	23	85.5 (7.4)	5	86.8 (7.1)
36	36	87.8 (6.4)	7	88.1 (5.9)	23	86.8 (6.6)	6	91.0 (5.9)
48	30	88.7 (6.2)	2	84.0 (1.4)	23	88.9 (6.2)	5	89.8 (7.1)
72	27	87.0 (7.4)	0		23	87.5 (7.2)	4	84.0 (9.1)
84	26	87.2 (7.7)	0		22	87.3 (7.4)	4	87.0 (10.9)

B. Summary measures of FTOE over time by group

	Overall		Mild HIE - uncooled		Moderate HIE - cooled		Severe HIE - cooled	
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)
Hours after birth								
3	6	0.26 (0.16)	1	0.42	4	0.23 (0.18)	1	0.24
6	26	0.22 (0.10)	11	0.21 (0.10)	12	0.22 (0.11)	3	0.24 (0.03)
9	38	0.21 (0.10)	17	0.18 (0.07)	18	0.24 (0.12)	3	0.19 (0.07)
12	46	0.18 (0.10)	21	0.16 (0.09)	21	0.21 (0.10)	4	0.13 (0.08)
18	47	0.15 (0.07)	22	0.15 (0.06)	22	0.16 (0.08)	3	0.13 (0.09)
24	42	0.14 (0.07)	19	0.14 (0.06)	20	0.14 (0.07)	3	0.14 (0.08)
36	28	0.11 (0.07)	5	0.11 (0.07)	19	0.12 (0.07)	4	0.07 (0.08)
48	24	0.11 (0.07)	2	0.14 (0.02)	19	0.11 (0.07)	3	0.11 (0.10)
72	23	0.12 (0.08)	0		19	0.12 (0.08)	4	0.13 (0.11)
84	22	0.11 (0.09)	0		18	0.11 (0.08)	4	0.08 (0.14)

A



B

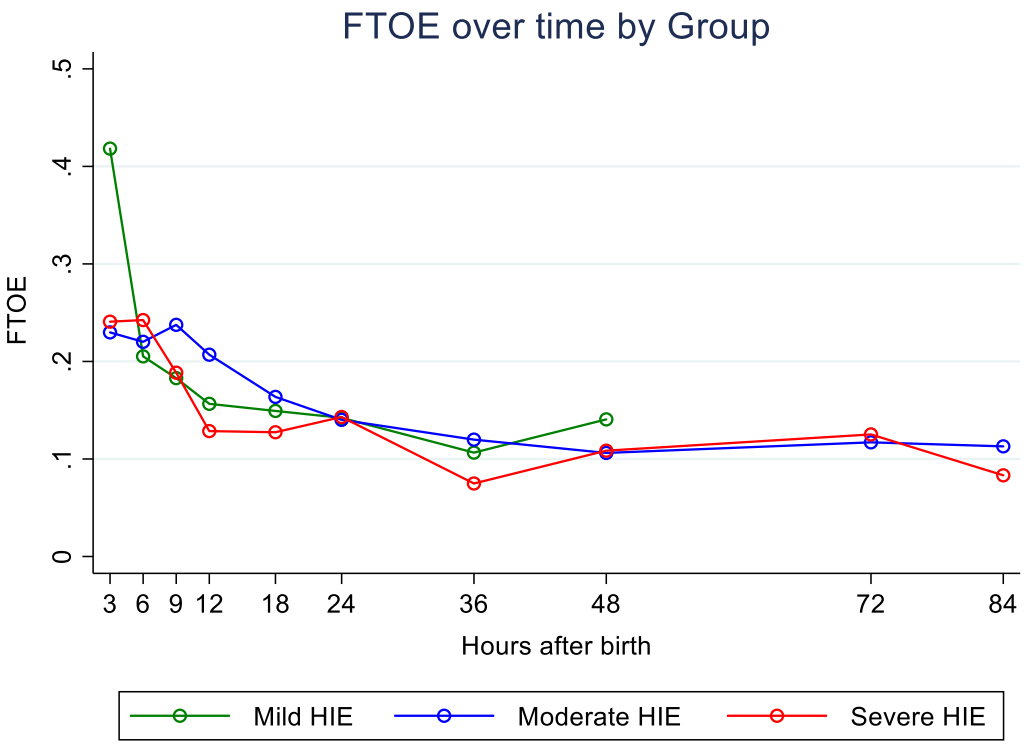


Figure 3.2: Mean cSO_2 (A) and FTOE (B) for each HIE group at each time point.

3.2.3.3. Evolution of cSO_2 over time

To assess the evolution of cSO_2 over time, a fixed quadratic time effect for each group and a random quadratic time effect was used. The mixed model results are presented in Table 3.7.

Mean cSO_2 increased in both groups over time with the rate of increase slowing down over time (decelerating positive curves).(Figure 3.3) The fitted curves were $79.298+0.796*(hours-6)-0.030(hours-6)^2$ for the mild group and $71.189+1.328*(hours-6)-0.030(hours-6)^2$ for the moderate group. The instantaneous linear rate of change at 6 hours after birth was significantly higher in the moderate group (1.328 vs 0.796 in the mild group, $p<0.001$). The rate of deceleration in both groups was not significantly different ($p>0.05$ for group*$(time-6)^2$ interaction) and hence not included in final model. Mean cSO_2 was significantly higher in the mild HIE group compared to the moderate HIE group at 6 hours (difference in means(95% CI): 8.1% (2.7 to 13.5%), p=0.003), 9 hours (difference in means(95% CI): 6.5% (1.6 to 11.4%), p=0.009) and 12 hours (difference in means(95% CI): 4.9% (0.4 to 9.4%), p=0.032). No significant differences were found between the two groups at 18 hours (difference in means(95% CI): 1.7% (-2.3 to 5.7%), p=0.401) and 24 hours (difference in means(95% CI): -1.5% (-5.6 to 2.6%), p=0.481).

Table 3.7: Results of mixed model analyses for cSO_2 and FTOE.

	Coefficient (95% CI)	p-value
cSO_2		
Intercept[a]	79.298 (75.309 to 83.287)	<0.001
Time[b]	0.796 (0.360 to 1.232)	<0.001
Time^2	-0.030 (-0.048 to -0.012)	0.001
Group		0.003
Mild HIE (Ref)	0	
Moderate HIE	-8.109 (-13.489 to -2.729)	
Time*Group		<0.001
Time*Mild HIE (Ref)	0	
Time*Moderate HIE	0.532 (0.277 to 0.788)	
FTOE		
Intercept[a]	0.171 (0.125 to 0.217)	<0.001
Time[b]	-0.002 (-0.004 to 0.001)	0.168
Group		0.016
Mild HIE (Ref)	0	
Moderate HIE	0.079 (0.014 to 0.143)	
Time*Group		0.003
Time*Mild HIE (Ref)	0	
Time*Moderate HIE	-0.005 (-0.008 to -0.002)	

[a] intercept represents time=6 hours after birth for mild HIE group;

[b]time is centred at 6 hours after birth

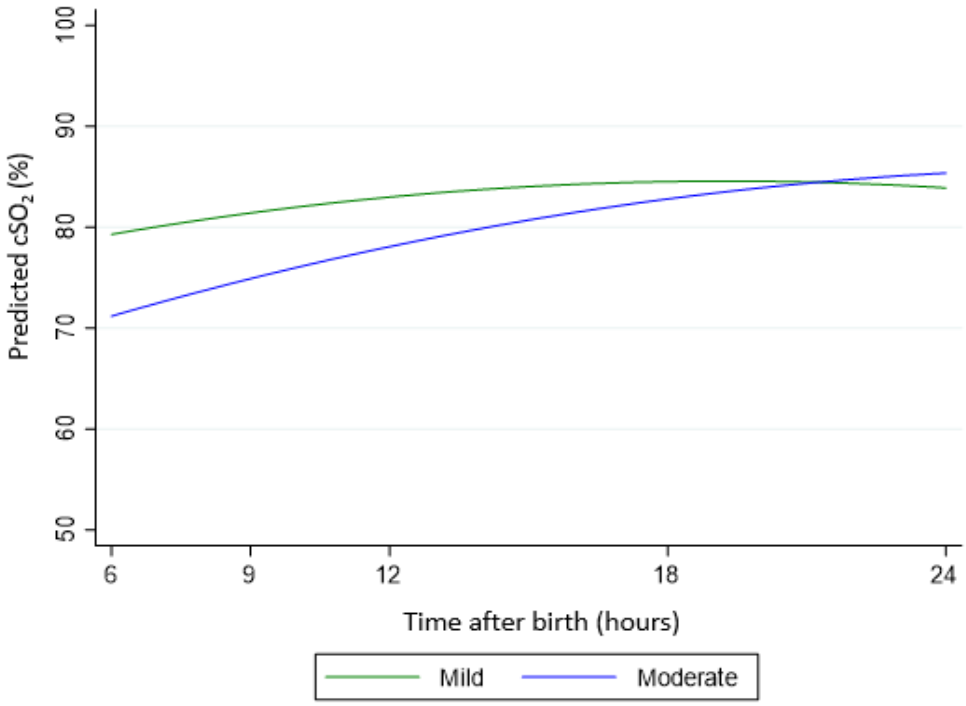


Figure 3.3: Predicted cSO_2 over time by HIE group.

3.2.3.4. Evolution of FTOE over time

To assess the evolution of FTOE over time, the best fitting model included a fixed linear time effect for each group and a random linear time effect. The mixed model results are presented in Table 3.7.

As is evident from Figure 3.4, mean FTOE decreased over time in both groups. The fitted lines were FTOE=0.171-0.002*(hours-6) for the mild group and FTOE=0.250-0.007*(hours-6) for the moderate group. The rate of change was faster in the moderate group (-0.007 vs -0.002 in the mild group, p=0.003) with bigger differences between the two groups at the earlier time points. Based on the fitted lines, mean FTOE was significantly higher in the moderate HIE group compared to the mild HIE group at both 6 hours (difference in means(95% CI): 0.079 (0.014 to 0.143), p=0.016) and 9 hours (difference in means(95% CI): 0.064 (0.007 to 0.121), p=0.029). No significant differences were found between the two groups at 12 hours (difference in means(95% CI): 0.049 (-0.002 to 0.099), p=0.060), 18 hours (difference in means(95% CI): 0.018 (-0.024 to 0.060), p=0.393) and 24 hours (difference in means(95% CI): -0.012 (-0.054 to 0.030), p=0.571).

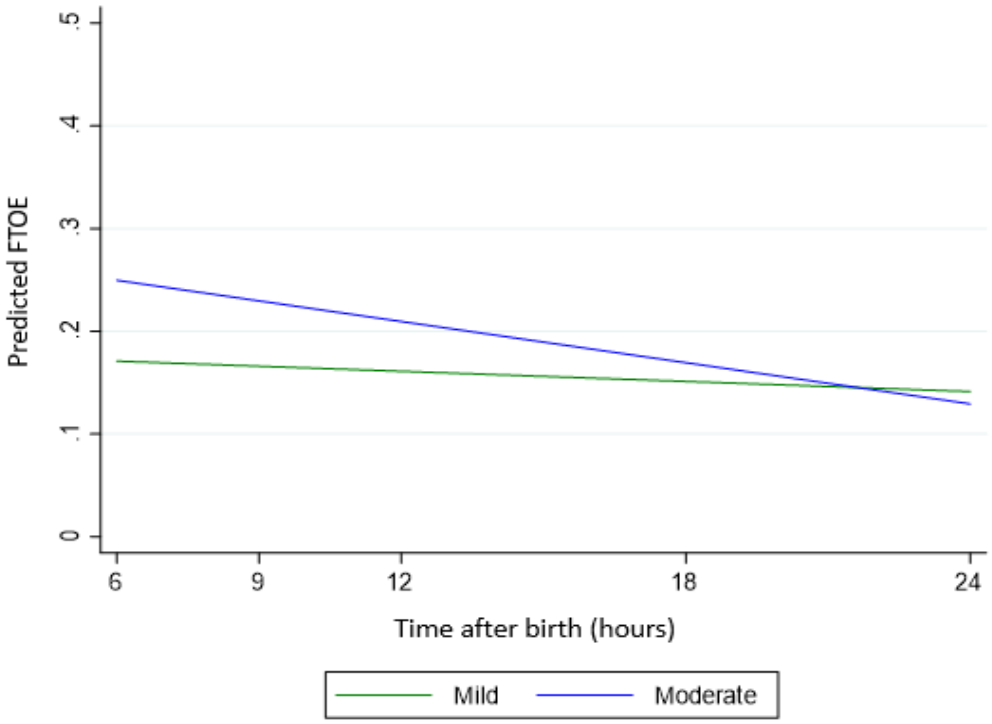


Figure 3.4: Predicted FTOE over time by HIE group.

3.2.4. Discussion

This study describes the evolution of NIRS in newborns with all grades of HIE. Infants with mild HIE have significantly higher predicted cSO_2 and lower FTOE 6 hours after delivery compared to infants undergoing TH for moderate HIE. cSO_2 in the mild group increases slowly over the first 12 hours and then plateaus. Although initially lower, cSO_2 in infants with moderate HIE increases over the first day and by 18 hours of age, both cSO_2 and FTOE are similar to uncooled infants with mild HIE. Although the numbers were small, cSO_2 in infants with severe HIE appears to increase almost linearly over the first 36 hours of age. In both the moderate and severe groups, cSO_2 and FTOE essentially remain unchanged during the rewarming phase.

We do not have control data for this cohort; however normative values exist in the literature. (258, 515) Our values in the moderate and severe groups are above the suggested cSO_2 values of 78% (+/-7) for full term, healthy infants on day 1 and 2 of

life using the same device and probe. Studies have also demonstrated a gradual decrease in cSO_2 over the 120 hours of life. (258) In our cohort, cSO_2 increases in all groups over the first 20 hours then plateaus, suggesting that encephalopathy alone, regardless of TH, may have an early effect on cerebral oxygenation.

Both animal and clinical studies have shown that the severity of the underlying encephalopathy has a major influence on CBF. (293, 516) In HIE, CBF is initially low and increases over the first 24 hours. This is likely due to a disruption in haemodynamic control including vasodilation, vasoparesis and impaired cardiac output. (291, 292) Whether this initial hypoperfusion is a protective strategy to reduce metabolic demand and thus further brain injury or whether it is as a result of neuronal injury and death is unclear. (517) Although one goal of hypothermia is to reduce cerebral metabolism and reduce cerebral blood flow in an attempt to minimise the effect of reperfusion injury, (517) hyperperfusion may still be seen in some infants. Wintermark et al. used MRI, specifically a pulsed arterial spin labelling sequence, to generate regional CBF maps. They showed that infants with brain injury on MRI displayed hypoperfusion on day of life 1, followed by hyperperfusion on days 2-3. They found that infants with early hyperperfusion had increased risk of brain injury. (293) Our results infer similar findings; infants with moderate and severe HIE have increasing cSO_2 values beyond 20 hours of age. It would appear that the severity of encephalopathy has an independent impact on cerebral oxygenation values. Infants with moderate HIE have lower cSO_2 initially compared to the mild group. The rate of change may also be important. Wintermark et al. showed that in a cohort of 7 infants undergoing TH for asphyxia, a greater increase in cSO_2 was seen in infants with severe HIE compared with those with moderate HIE. (266) This is true of our cohort. Infants with moderate HIE had a greater rate of increase in cSO_2 compared to the mild group and although inferential statistics were not possible in the severe group, their mean values suggest values higher than the moderate group.

TH is an important factor to consider. The goal of TH treatment in HIE is to reduce cerebral metabolism which results in decreased oxygen consumption. FTOE values in the mild group decrease slightly but generally remain stable over time. In the

moderate group receiving TH, predicted FTOE values are significantly higher than the mild group during the first 9 hours but by the end of the first day of life, FTOE values are similar to that of the mild group and continue to decrease further with cSO_2 increasing accordingly. By 24 hours, there is a trend towards a lower FTOE and higher cSO_2 in the moderate group. Although grade of encephalopathy plays an important role initially by determining the 'starting points', TH may have a protective effect on cerebral metabolism. Concerns have been raised that TH may also have an effect on reducing brain blood flow, specifically via resulting bradycardia, which may result in reduced cardiac output and subsequent decrease in oxygen delivery (5, 359, 518). Furthermore, $PaCO_2$ has a significant influence on the cerebral vasculature. Hypothermia affects our ability to accurately measure $PaCO_2$ thus raising concerns that $PaCO_2$ in infants may be lower than assumed, resulting in vasoconstriction and reduced cerebral blood flow. (519) However in our cohort, cSO_2 and FTOE remain relatively stable beyond the first day of life and during the rewarming period in the moderate and severe groups. This suggests preserved cerebral blood flow.

Sedation is routinely used in our unit for infants receiving TH due to the discomfort associated with low core temperatures. Seven infants also received anti-seizure medication for the treatment of seizures. Sedative agents may have a depressive effect on electrocortical activity, which in turn results in decreased cerebral metabolism. Both animal and clinical studies have demonstrated a decrease in FTOE following administration of sedative medication. (520, 521, 522, 523) In our practice, sedation is maintained at the lowest dose required to ensure comfort and is routinely weaned and discontinued towards the end of the TH and during the rewarming periods. Again, no change in cSO_2 or FTOE were noted at this time suggesting minimal effect of the sedation on cerebral NIRS values.

The effect of seizures on cSO_2 is unclear with varying alterations in cSO_2 described both between and within individual infants in the literature. (524, 525) Seizures are thought to both increase cerebral blood flow and metabolism (526) however, seizures have been shown to result in an increase, decrease or no change in cSO_2. (524, 525) Seven infants in our cohort had EEG seizures during the first 84 hours with

6 receiving anti-seizure medication. One infant did not have an EEG seizure but received anti-seizure medication for clinical concerns. Seizure location and duration varied in these infants, as did the change in NIRS values. As they had similar cSO_2 values to the remainder of the cohort at the selected time points, they were included in the analysis.

Objective, reliable biomarkers are required to identify infants at risk of brain injury in the crucial 6-hour therapeutic window. Our current methods primarily involve clinical assessment, which were never validated to examine infants as early as 6 hours after birth and may be influenced by many factors including mode of delivery, maternal analgesia and repeated examination. (2, 108, 113) Factors affecting assessment by an individual physician include a lack of confidence in one's ability to grade encephalopathy and fear of litigation that comes with missing an infant who may be suitable for intervention. (130) NIRS monitoring has many theoretical benefits in HIE as it can detect alterations in cerebral oxygenation and cerebral metabolism; changes which are pathognomonic of HIE. Unlike other monitoring modalities like electroencephalography, which require expert interpretation, NIRS can be implemented into clinical care with minimal training. It can be applied easily and quickly after delivery providing early objective information on cerebral oxygenation, metabolism and perfusion placing it in a prime position as a potential early bedside biomarker.

This study is limited by the small number of infants included, particularly in the early time measurements, and the varying number of data available at each time point. Obtaining early data in this cohort is challenging. (511) Only 26 infants were included at 6 hours of age however these numbers are similar to previously published studies examining NIRS at this early time point. (511) Our centre is a referral centre for TH and we receive infants with moderate and severe HIE who require TH. Included infants with mild HIE were all inborn which may have introduced an inclusion bias however, as per our clinical guidelines, all infants in our centre with mild HIE are admitted and we do not believe that this would have affected our results. A well-documented limitation of the INVOS neonatal sensor is that it truncates values to

between 15-95%. We are therefore unable to describe values above 95%, which may have limited our ability to distinguish between groups. Utilisation of a different device or probe may have resulted in different findings. In addition, data beyond 24 hours is limited for infants with mild HIE and therefore we cannot determine the clinical significance of NIRS values beyond this time frame.

Infants with mild HIE are a largely understudied population. Significant disability on follow-up has recently come to light, raising concerns and highlighting the need for clinical trials in this area. (115, 131) As a first step, we need to gain a better understanding of the process and evolution of the injury involved. Infants with mild HIE have less motor injury than infants with moderate or severe HIE but a similar risk of learning, emotional and behavioural issues which may not become apparent until school age and thus require longer term follow-up. (115, 117, 416) This certainly makes studies more challenging but necessary if we are to address this important question. Early identification of at risk infants would allow future clinical trials targeting potential interventions. The question of whether early cerebral NIRS, either alone, or in combination with other monitoring tools, can help identify these infants remains to be answered.

This study provides novel, early NIRS data in all grades of HIE and its evolution over time. In all grades, cSO_2 increases over the first 24 hours with a resultant decrease in FTOE. Although significant differences are evident between the uncooled mild group and the infants with moderate HIE receiving TH at the earlier time points, by 12 hours there is no difference in oxygen uptake and cSO_2 values are similar by 18 hours. While cSO_2 values in the mild group lie within suggested normative ranges, the trend of an increasing cSO_2 over time is different to previously published normative data suggesting an effect of the underlying encephalopathy itself. (258, 515) Further research on this group is required and correlation with long-term outcome is essential to determine whether early NIRS is helpful in the identification of infants at risk of brain injury.

4. Non-Invasive Continuous Cardiac Output Monitoring in Infants with Hypoxic Ischaemic Encephalopathy

4.1. Introduction

Hypoxic ischaemic encephalopathy (HIE) is a multi-organ pathology. While the majority of research has focused on brain injury and optimising neuroprotection, the impact of HIE and therapeutic hypothermia (TH) on the cardiovascular system is less well studied. Ischaemic changes and impaired ventricular function have been described following HIE, and impaired ventricular function has been shown to correlate with the grade of encephalopathy and outcome. (335, 347, 348, 355) Reduced cardiac output (CO) may further compound underlying brain injury due to reduced cerebral perfusion. (354) Therefore, it is important that relevant and continuous haemodynamic information is available to the clinician.

Current methods of determining cardiovascular function include blood pressure (BP) monitoring and point-of-care neonatal echocardiograms. BP has long been used as a surrogate for cardiovascular function however, changes in CO may occur despite a stable BP and BP is a poor surrogate marker of blood flow. (356, 357, 359) Echocardiography has become the primary method of measuring CO in infants in the neonatal unit. Although no study has directly compared echocardiogram-derived measures of CO with invasive measures such as thermodilution in the neonatal population, Alverson et al. has shown that values are similar to those previously measured during cardiac catheterisation. (310) A limitation of echocardiography however, is the need for trained personnel for recording and interpretation. Furthermore, it only provides a snapshot of haemodynamic function at a specific time point. Repeated clinical examination and monitoring of urine output also provide useful information regarding CO however ideally, monitoring should be non-invasive, accurate, reliable and, importantly, continuous.

Non-invasive methods of continuously monitoring CO are routinely used in adult intensive care units, and are now being evaluated in neonatal intensive care units. (359, 360, 361) Two different methods of measuring thoracic impedance and thus

CO are currently available: bioimpedance, of which electrical cardiometry (EC) is the most commonly used technique, and bioreactance (BR). (318)

BR is a technology which measures phase shifts in the aorta to detect changes in flow between systole and diastole. From this, stroke volume (SV) can be obtained and, in combination with heart rate (HR), CO can be calculated. BR technology has been validated in the adult population and values correlate with invasive measures of CO. (322, 323, 324, 325) It is less influenced by artefact and electrical noise than other non-invasive measures of CO and is unaffected by ventilation status. (327, 527) Its use has been assessed in infants and young children undergoing cardiac surgery. (326) In the neonatal population, it has been shown to correlate with echocardiogram-derived measures of left ventricular output. (328, 329) Its use in HIE has been limited to assessing the effect of TH on CO. (359)

Three studies have used non-invasive monitoring to assess the effect of therapeutic hypothermia on CO in infants with moderate and severe HIE-one using BR and two studies using EC. (359, 360, 361) No study has examined the ability of thoracic impedance to measure CO in infants with mild HIE or to describe the changes in CO over time in infants with all grades of HIE. Thus, the aim of this study was to describe early CO values in infants with all grades of HIE and to assess if its evolution over time differs between grades of HIE and compared with healthy term infants.

4.2. Methods

This was part of a larger prospective observational study conducted in Cork University Maternity Hospital, Cork, Ireland between November 2017 and March 2020, which recruited infants with all grades of HIE and healthy term controls. Infants with HIE had multi-modal monitoring during their stay in the neonatal unit to include electroencephalogram (EEG), near-infrared spectroscopy (NIRS), non-invasive cardiac output monitoring (NICOM), echocardiography, magnetic resonance imaging (MRI) and blood biomarkers.

NICOM was obtained using the Cheetah Starling NICOM ™ device (Cheetah Medical, USA) which uses BR technology. Four sensors are applied to the infant's thorax (left and right, upper and lower) in a manner that "boxes" the heart. The NICOM device averages measurements of CO, HR and SV every 60 seconds. Following the recording period, data was exported to Microsoft Excel. Mean CO and SV were divided by the infant's birth weight to provide measurements in mls/kg/minute (CO) and mls/kg (SV). HR was reported as beats/minute.

All infants had an echocardiogram performed during NICOM monitoring using the GE Vivid-I Portable Ultrasound (GE Healthcare, USA). Echocardiograms were analysed off-line by a neonatologist with echocardiography experience (E Dempsey) blinded to the study group. SV was calculated using the standard equation below.

$$\pi \, x \, \left(\frac{VOT}{2}\right)^2 \, x \, VTI$$

VOT, ventricular outflow tract; VTI, velocity time integral

CO in mls/kg/minute was then calculated as follows:

$$\frac{HR \, x \, SV}{birthweight}$$

Detailed functional echocardiography was not consistently performed, as the purpose of the echocardiograms was only to assess cardiac output in comparison to NICOM-derived CO.

4.2.1. Infants with HIE

Infants >36 weeks gestation at birth were included if they had evidence of HIE to include one or more of: an Apgar score of <5 at 5 minutes of age, requirement of continued resuscitation at 10 minutes of age or pH of <7.1/base deficit of >16/lactate of >9mmol/L on cord or first post-natal blood gas plus clinically evolving encephalopathy defined as the presence of abnormal neurological findings using the modified Sarnat score at 1 hour of age.

TH was commenced before 6 hours of age in all infants with moderate and severe HIE. Infants with mild HIE did not receive TH. It is practice in our unit that infants undergoing TH receive low dose morphine infusion (10-20mcg/kg/hour) which is titrated according to clinical response. Infants with mild HIE were initially commenced on IV fluids at a rate of 60mls/kg/day until lactate normalised and feeds were established. Infants with moderate and severe HIE were commenced on IV fluids at 40-60mls/kg/day and titrated to electrolytes and urine output.

Following parental consent, NICOM monitoring was commenced as soon as possible after birth and continued for approximately 24-36 hours in infants with mild HIE, and throughout TH and the rewarming phase in infants with moderate and severe HIE. 1-hour data epochs were extracted for analysis at 6, 9, 12, 18, 24, 36, 48, 72 and 84 hours of age for each infant where available.

Infants with HIE had an echocardiography assessment of CO performed in the first 48 hours of life and if NICOM monitoring continued beyond this time they had a second echocardiogram after 48 hours of age.

4.2.2. Healthy Term Infants

Healthy term infants were recruited on the post-natal ward (PNW). Infants >36 weeks gestation were eligible for recruitment if they did not require resuscitation at birth, had normal cord pH values and had an Apgar score of >8 at 5 minutes of age. These infants had NICOM recorded on the PNW at 6 and 24 hours of age for 1-2 hours duration.

Control infants had an echocardiogram performed on the PNW during one of the monitoring time points.

4.2.3. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 26.0, IBM Corp, U.S.A.) and Stata (version 15.1, StataCorp LP, U.S.A.). Continuous variables were

described using mean and standard deviation (SD) or median and interquartile range (IQR) and categorical variables using frequency and percentage.

To investigate and compare how CO, HR and SV changed over time (from 6 to 48 hours after birth) for the mild and moderate HIE groups, a mixed modelling approach was used. The optional functional form of the trajectory over time was identified from the family of polynomial functions (a straight line, a quadratic curve and a cubic curve). A bottom-up strategy was used, starting with an empty random intercepts model and then adding each fixed effect (linear, quadratic, cubic) followed by its corresponding random time effect (linear, quadratic, cubic), in turn. Model fit was evaluated using the deviance statistic (−2 log likelihood) and the Akaike Information Criterion. To investigate if changes over time differed by HIE group, the fixed effects of HIE group and the interactions of HIE group by time (linear, quadratic and cubic, as appropriate) were added to the mixed model in a sequential manner. Time was treated as a continuous variable in this analysis.

To investigate if changes in the NICOM measures (CO, HR and SV) between 6 and 24 hours differed between the mild HIE and control groups, random effects modelling was used. Each model included time, group and time*group as fixed effects and infant as a random effect. Time was treated as a categorical variable in this analysis.

One-way Analysis of Variance (ANOVA) was used to compare cardiac output, heart rate and stroke volume at 24 hours between the control, mild HIE and moderate HIE groups. If statistically significant differences were found, post-hoc pairwise comparisons were performed using Tukey's Honestly Significant Difference test.

All tests were two-sided and p-values <0.05 were considered statistically significant.

Bland-Altman analysis was used to compare echocardiography and NICOM measures of CO. As the data included repeated measures, bias and limits of agreement (LoA) were calculated using random effects modelling. (528, 529) The model included a constant term (no fixed effects) and a random effect for infants. The dependent

variable was the difference between the paired echocardiography and NICOM CO measurements. The intercept from the fitted model represented the mean bias and the standard deviation of the bias (SD) was calculated as the square root of the sum of the between and within-infant variances. Using these, the random effects limits of agreement were calculated using the conventional Bland-Altman limits of agreement formula (bias ± 1.96*SD). Limits of agreement provide an estimation of how precise the measurements are i.e. the repeatability and reproducibility. Percentage bias was calculated as (bias/mean value of the two methods) x 100. Percentage bias examines the mean difference between the measurements and gives an estimation of the accuracy between the methods. The percentage error refers to (1.96 x SD/mean value of the two methods) x 100 and examines the precision of NICOM and whether the two measurement techniques are interchangeable. A percentage error of 30% is clinically acceptable for interchangeability between the methods. (530)

4.3. Results

Sixty-one infants were included in this study (17 controls, 23 mild HIE, 17 moderate HIE, 4 severe HIE). Demographic and perinatal data are outlined in Table 4.1.

Table 4.1: Demographic and perinatal information of included infants.

	Controls n=17	**Mild n=23**	**Moderate n=17**	**Severe n=4**
GA, *weeks*	39.4 (37.6-40.4)	40.4 (38.3-40.9)	38.6 (37.6-40.0)	39.6 (38.4-40.6)
BW, *kg*	3.7 (3.4-3.8)	3.3 (3.0-3.7)	3.1 (2.8-3.6)	3.7 (3.4-3.9)
Sex: Male	6 (35)	15 (65)	9 (53)	2 (50)
ROM, *hours*	2.4 (1.2-12.2)	14.0 (3.5-27.6)	7.0 (4.1-16.7)	13.0 (4.0-13.0)
Onset of labour				
Spontaneous	7 (41)	14 (61)	8 (47)	4 (100)
Induction	8 (47)	7 (30)	7 (41)	
Pre-labour LSCS	2 (12)	2 (9)	2 (12)	
Mode of Delivery				
SVD	8 (47)	5 (22)	4 (24)	2 (50)
Instrumental	5 (29)	10 (43)	6 (35)	
Emergency LSCS	2 (12)	6 (26)	5 (29)	2 (50)
Pre-labour LSCS	2 (12)	2 (9)	2 (12)	
1 minute Apgar				
0-4	0	11 (48)	17 (100)	4 (100)
5-7	1 (6)	8 (35)		
8-10	16 (94)	4 (17)		
5 minute Apgar				
0-4		2 (9)	9 (53)	4 (100)
5-7		7 (30)	8 (47)	
8-10	17 (100)	14 (61)		
Biochemistry*				
Lowest pH		7.0 (6.9-7.1)	6.9 (6.8-7.1)	7.1 (6.9-7.1)
1st Postnatal Lactate		10.7 (5.4-13.3)	10.9 (7.6-13.7)	14.9 (9.1-17.6)
24 hour Lactate		2.4 (1.9–3.4)	1.7 (1.3–3.4)	2.5 (1.3–2.5)
48 hour Lactate		2.1 (1.8–2.1)	1.4 (1.1–2.1)	1.1
Medications				
Anti-seizure medications	0	0	3 (18)	2 (50)
Inotropes	0	0	1 (6)	2 (50)

**Biochemistry not recorded for control group.*
GA, gestational age; BW, birthweight; ROM, rupture of membranes; LSCS, lower section caesarean section; SVD, spontaneous vaginal delivery.
Categorical variables are represented as frequencies and percentage. Continuous variables are represented as median and interquartile range.

4.3.1. Summary measures of CO, HR and SV over time

Summary measures of CO (mls/kg/min), HR (beats/min) and SV (mls/kg) over time in all infant groups are outlined in Table 4.2. Four infants were included in the severe group. Early recordings were not possible in this group due to the challenges in obtaining early informed parental consent. Due to the limited data available in this group, they have been included in the descriptive analysis of the results below but omitted from further statistical analysis.

Figure 4.1 depicts changes in mean CO (mls/kg/min), HR (beats/min) and SV (mls/min) over time in all infant groups. Infants with mild HIE and controls have similar CO values over the first 24 hours. Infants with moderate and severe HIE have lower CO values which increase gradually throughout TH and again during rewarming. Although SV increases in both groups, during the rewarming phase, this increase is predominantly due to an increase in HR.

Table 4.2: Summary measures of CO, HR and SV over time per group.

A. Summary measures of cardiac output (mls/kg/min) over time by group

	Controls		Mild HIE - uncooled		Moderate HIE - cooled		Severe HIE - cooled	
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)
Hours after birth								
6	17	79.82 (15.58)	9	80.67 (15.08)	4	75.75 (12.56)		
9			13	82.10 (16.97)	4	73.74 (9.44)		
12			17	82.71 (18.70)	5	68.34 (9.26)		
18			20	86.92 (19.46)	8	73.42 (13.77)		
24	16	95.14 (16.37)	15	94.96 (32.52)	13	74.25 (10.33)		
36			10	93.38 (20.67)	13	84.39 (22.20)	3	82.17 (13.49)
48			5	99.04 (31.60)	14	89.88 (27.24)	1	99.76
72					14	90.36 (29.71)	3	84.78 (25.59)
84					14	100.74 (31.33)	3	102.36 (28.26)

B. Summary measures of heart rate (beats/min) over time by group

	Controls		Mild HIE - uncooled		Moderate HIE - cooled		Severe HIE - cooled	
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)
Hours after birth								
6	17	119.18 (8.89)	9	131.33 (6.93)	4	94.50 (11.27)		
9			13	126.73 (9.05)	4	93.00 (6.32)		
12			17	129.59 (11.59)	5	98.10 (6.91)		
18			20	129.00 (10.56)	8	100.94 (12.49)		
24	16	129.88 (10.26)	15	131.03 (14.57)	13	95.65 (5.55)		
36			10	126.50 (16.31)	13	101.46 (9.40)	3	131.67 (11.02)
48			5	126.60 (11.15)	14	101.43 (14.26)	1	123.00
72					14	100.32 (9.34)	3	103.33 (10.60)
84					14	114.14 (9.65)	3	121.00 (5.29)

C. Summary measures of stroke volume (mls/kg) over time by group

	Controls		Mild HIE - uncooled		Moderate HIE - cooled		Severe HIE - cooled	
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)
Hours after birth								
6	17	0.67 (0.12)	9	0.61 (0.13)	4	0.80 (0.07)		
9			13	0.65 (0.14)	4	0.78 (0.08)		
12			17	0.64 (0.15)	5	0.70 (0.10)		
18			20	0.67 (0.16)	8	0.73 (0.08)		
24	16	0.73 (0.13)	15	0.73 (0.25)	13	0.78 (0.13)		
36			10	0.74 (0.16)	13	0.82 (0.20)	3	0.62 (0.06)
48			5	0.77 (0.18)	14	0.87 (0.20)	1	0.81
72					14	0.89 (0.25)	3	0.81 (0.18)
84					14	0.88 (0.26)	3	0.85 (0.24)

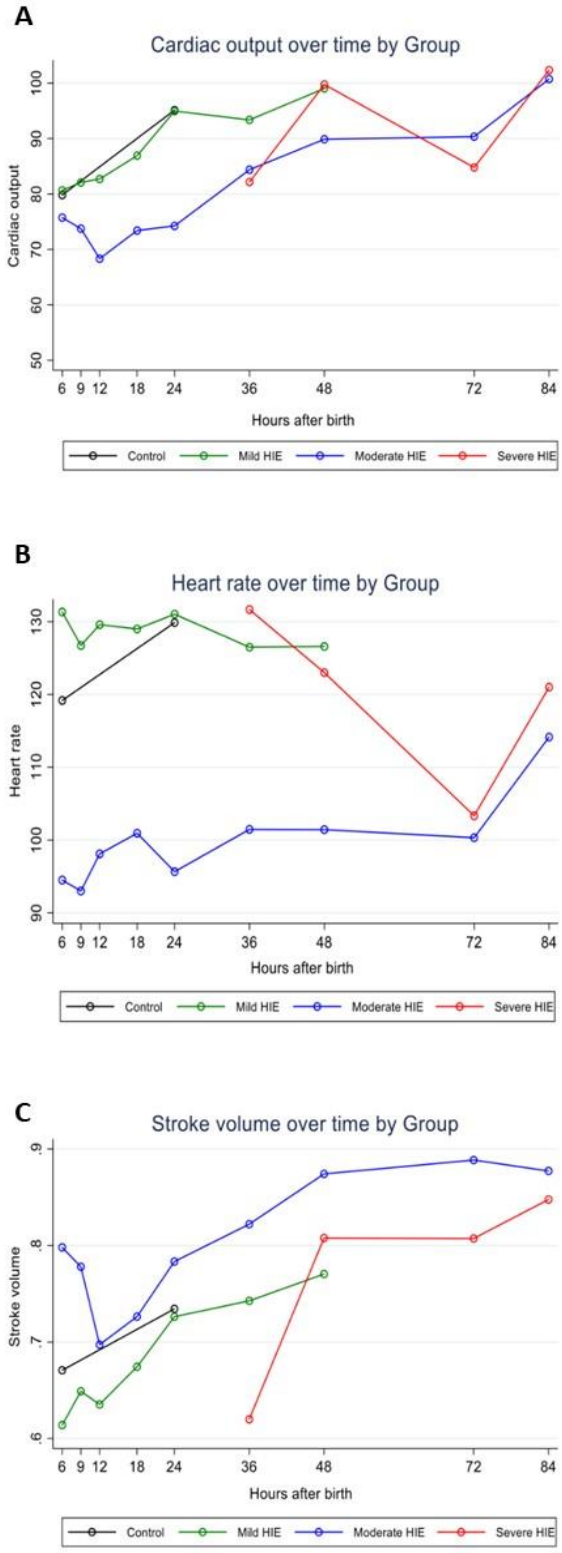


Figure 4.1: Mean values of cardiac output (A), heart rate (B) and stroke volume (C) over time by group.
CO is reported as mls/kg/minute; HR is reported as beats/minute; SV is reported as mls/kg

4.3.2. Evolution of CO, HR and SV over time

Figure 4.2 depicts evolution of CO, HR and SV over time in infants with mild and moderate HIE.

For cardiac output, the best fitting model included a fixed and random intercept, a fixed and random linear time effect and a fixed group effect. Changes over time did not differ between the two groups (p=0.683 for group*time interaction). The fitted lines were CO=79.5+0.5*(hours-6) for the mild group and CO=68.8+0.5*(hours-6) for the moderate group. CO increased over time in both the mild and moderate groups by 0.5mls/kg/min (95% CI:0.2, 0.9) for every hour increase in age (p=0.001). Across all timepoints, CO was on average 10.7mls/kg/min (95% CI:1.0, 20.4) higher in the mild HIE group (p=0.031).

For heart rate, the best fitting model was quadratic with fixed and random effects for the intercept, linear time and quadratic time, a fixed group effect and a fixed group*time effect. Mean heart rate increased in both groups over time with the rate of increase slowing down over time (decelerating positive curves). The fitted curves were 127.915+0.250*(hours-6)-0.009(hours-6)2 for the mild group and 89.525+0.696*(hours-6)-0.009(hours-6)2 for the moderate group. The instantaneous linear rate of change at 6 hours after birth was significantly higher in the moderate group (0.696 vs 0.250 in the mild group, p=0.001). The rate of deceleration in both groups was not significantly different (p>0.05 for group*(time-6)2 interaction and hence not included in final model).

For stroke volume, the best fitting model was linear with a fixed and random intercept, a fixed and random linear time effect and a fixed group effect. Changes over time did not differ between the two groups (p=0.249 for group*time interaction). The fitted lines were SV=0.618+0.004*(hours-6) for the mild group and SV=0.739+0.004*(hours-6) for the moderate group. SV increased over time in both the mild and moderate groups by 0.004mls/kg (95% CI:0.001, 0.006) for every hour increase in age (p=0.002). Across all timepoints, SV was on average 0.121mls/kg (95% CI:0.037, 0.205) higher in the moderate HIE group (p=0.005).

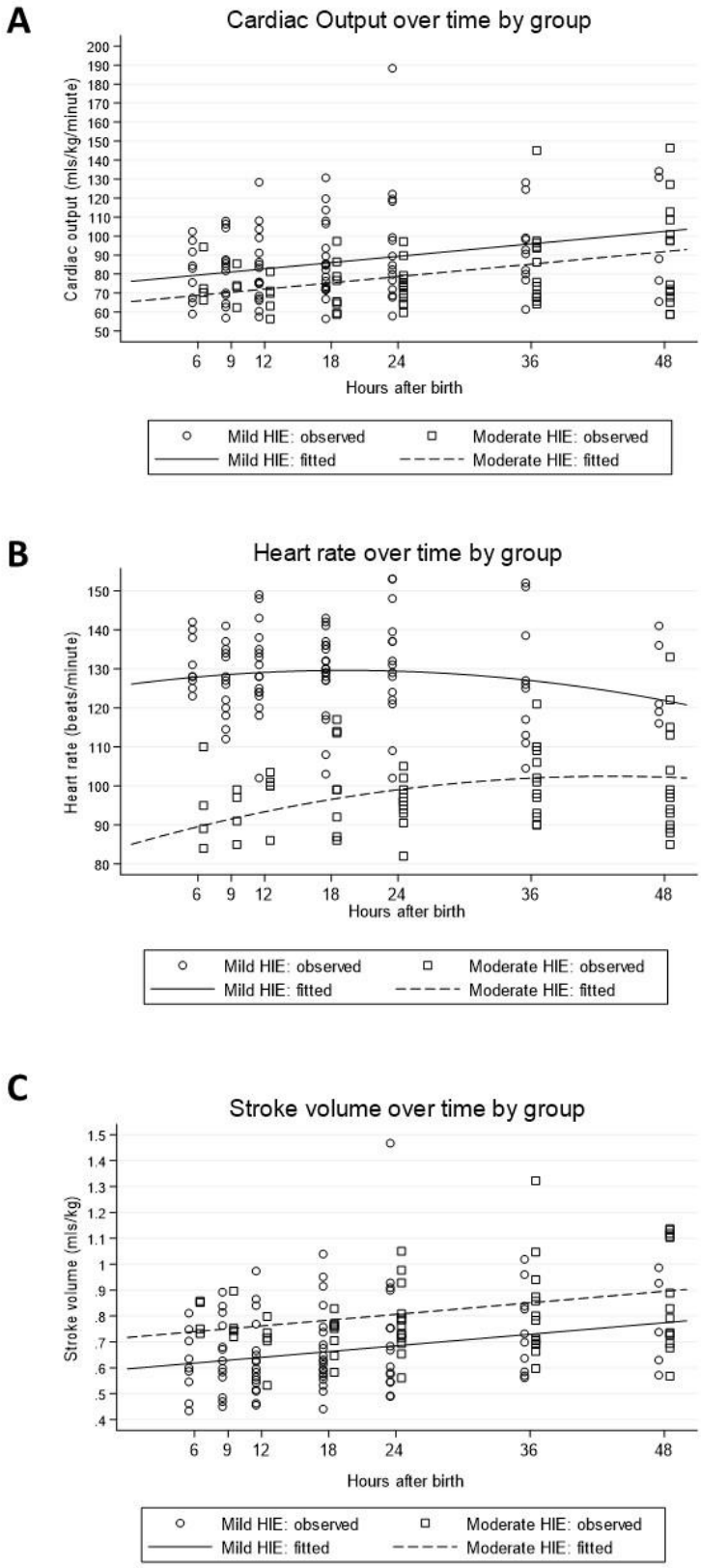


Figure 4.2: Change in cardiac output (A), heart rate (B) and stroke volume (C) over time in infants with mild HIE and moderate HIE.

4.3.3. Mild HIE and Controls at 6 and 24 hours

To examine this trend further, we assessed the cardiovascular changes in infants with mild HIE and controls at 6 and 24 hours of age. Changes in cardiac output over time did not differ between groups (p=0.947 for time*group). Cardiac output increased in both groups from 6 to 24 hours of age (p=0.001 for time) with CO on average 15.7mls/kg/min (95% CI: 6.4, 25.0) higher at 24 hours.(Figure 4.3 (A))

Changes in heart rate over time were different for the mild HIE and control groups (p=0.046 for time*group). In the control group, HR increased significantly from 6 to 24 hours (regression coefficient (95% CI): 11.0 (7.0, 15.0), p<0.001). There was no significant change over time in the mild HIE group (regression coefficient (95% CI): 3.5(-2.8, 9.7), p=0.274).

At 6 hours, infants with mild HIE had a significantly higher HR compared to the controls (regression coefficient (95% CI): 9.0(0.7, 17.4), p=0.034).(Figure 4.3 (B)) There was not a significant difference between the two groups at 24 hours (p=0.703).

Changes in SV over time did not differ between groups (p=0.427 for time*group). In both groups, SV increased between 6 and 24 hours (p=0.018) with SV on average 0.08ml/kg (95% CI: 0.01, 0.15) higher at 24 hours.(Figure 4.3 (C))

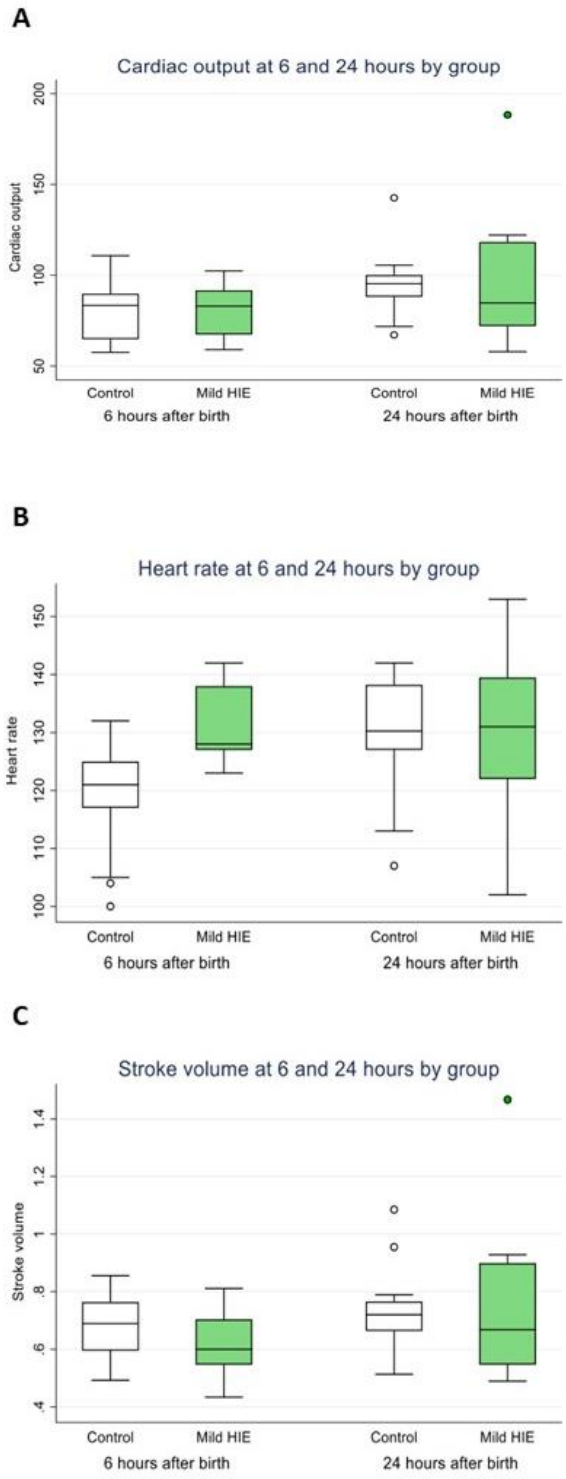


Figure 4.3: Evolution of cardiac output (A), heart rate (B) and stroke volume (C) over time in control and mild HIE groups.
CO is reported as mls/kg/minute; HR is reported as beats/minute; SV is reported as mls/kg

4.3.4. Controls, mild HIE and moderate HIE at 24 hours

Boxplots are presented in Figure 4.4 for cardiac output, heart rate and stroke volume at 24 hours for the control, mild HIE and moderate HIE groups.

Cardiac output at 24 hours was significantly different between the groups (p=0.025). Pairwise comparisons showed that CO was significantly lower in the moderate HIE group compared to both the mild HIE (adjusted p=0.046) and control group (adjusted p=0.040). No difference was found between the mild HIE and control group (adjusted p>0.999).

Heart rate at 24 hours was also significantly different between the groups (p<0.001). The differences were between the moderate HIE group and both the mild HIE (adjusted p<0.001) and control group (adjusted p<0.001) with heart rate being significantly lower in the moderate HIE group. No difference was found between infants with mild HIE and controls (adjusted p=0.953).

No differences in SV at 24 hours between groups were found (p=0.677).

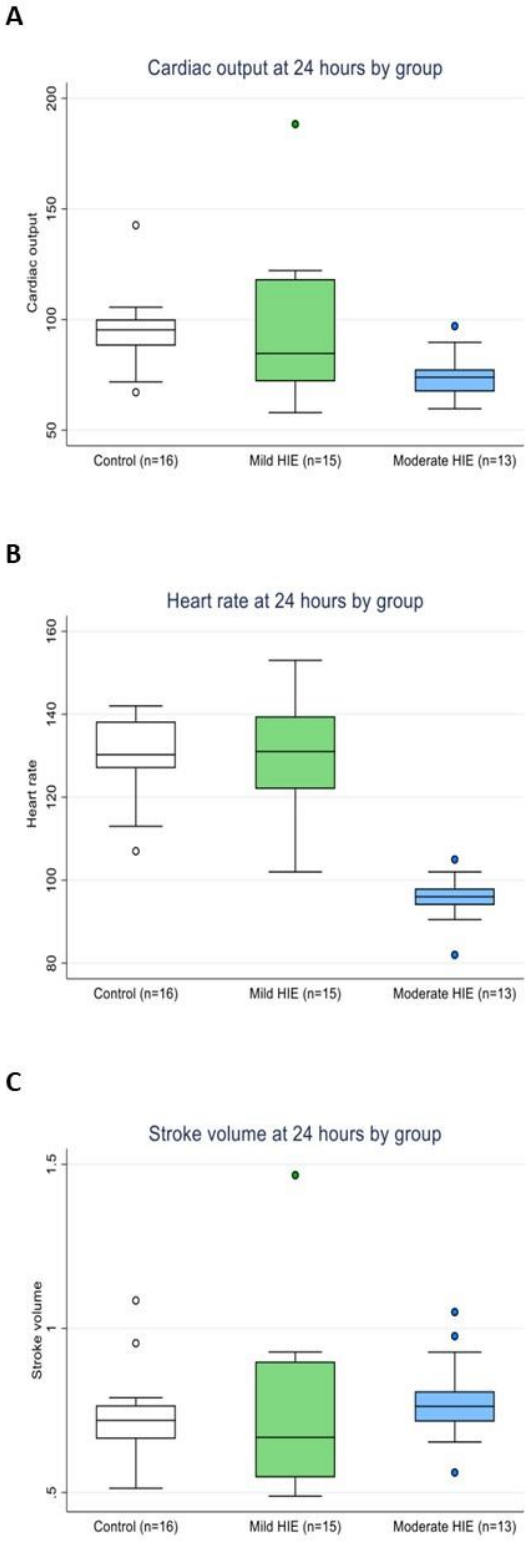


Figure 4.4: Cardiac output (A), heart rate (B) and stroke volume (C) at 24 hours of age in control, mild HIE and moderate groups.
CO is reported as mls/kg/minute; HR is reported as beats/minute; SV is reported as mls/kg

4.3.5. Assessment of agreement of echocardiography and NICOM measures of CO

Sixty-four echocardiogram-NICOM paired comparisons across 54 infants were included in the analysis (16 controls, 22 mild, 13 moderate and 3 severe). The Bland-Altman plot is presented in Figure 4.5. The bias and limits of agreement were 21.7mls/kg/min (95% limits of agreement -36.6 to 80.0 mls/kg/min). The percentage bias was 21.2% and the percentage error was 56.8%.

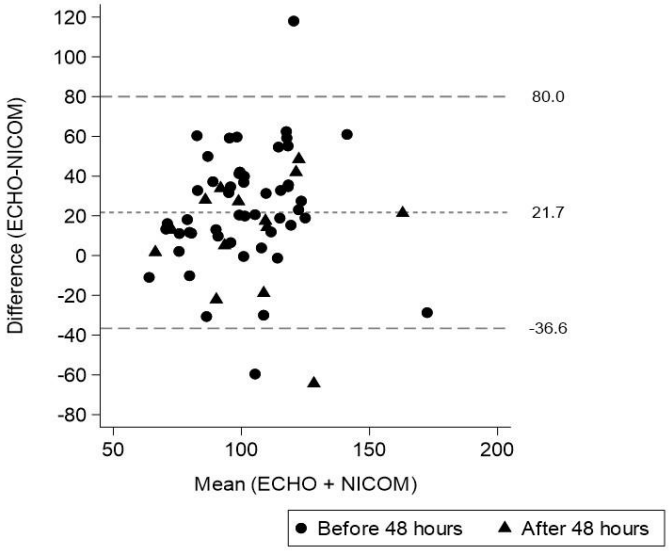


Figure 4.5: Bland-Altman analysis of echocardiography and NICOM measurements of CO (mls/kg/min).

4.4. Discussion

This is the first study to describe the evolution of CO using NICOM over time in all grades of HIE compared with healthy term controls. CO was similar in infants with mild HIE and controls, although infants with mild HIE had a significantly higher HR at 6 hours of age. Infants with moderate HIE had a significantly lower CO than the mild group at each time point whilst CO in both groups increased over time. The lower CO identified initially in the moderate group increased gradually over the TH period, mainly due to an increase in SV and a further increase in CO during the rewarming period was predominantly due to an increase in HR. These findings suggest an effect of both the underlying encephalopathy and subsequent treatment with TH, on cardiovascular haemodynamics.

In addition to its neurological implications, HIE may also result in significant haemodynamic alterations. The effect of HIE on cardiac function is 2-fold. Firstly, injury to the myocardium may result in ventricular dysfunction which may lead to reduced CO and pulmonary venous hypertension. (354) Secondly, pulmonary arterial hypertension may result from increased pulmonary vascular resistance secondary to hypoxia-induced vasoconstriction of the pulmonary vessels. (531) As cerebral autoregulation may also be impaired in infants with HIE, increased recognition of cardiovascular impairments and the subsequent effects on cerebral perfusion and thus, outcome is important. (532)

Infants with mild HIE had a significantly higher HR than controls at 6 hours. By 24 hours, CO, HR and SV were similar in both the mild and control groups. The normal physiological trend is that CO increases over the first 48 hours, predominantly due to an increase in SV and this increase coincides with increases in functional measurements of the right and left ventricles. (320, 533) HR is elevated at time of birth but reduces significantly in the first 15–30 minutes of life and gradually increases over the first week. (534) This is in keeping with our finding of CO, SV and HR increasing from 6 to 24 hours in the control group. The increased HR at 6 hours in infants with mild HIE may be explained by over-activation of the sympathetic nervous

system, consistent with previous findings. (2, 426) Although not significant, the slightly reduced SV may be due to reduced preload as a consequence of the increased HR or perhaps an increased afterload due to an effect of acute asphyxia on pulmonary and systemic vascular resistance. (331) However, detailed functional echocardiography was not performed at this early stage to allow us to determine this.

Infants with moderate HIE have a significantly lower CO over time when compared to infants with mild HIE. However CO increases steadily over time during TH, with a greater rise noted during rewarming. We found that SV increases gradually in infants with moderate and severe HIE during TH suggesting that the reduced SV seen was not due to TH alone. TH aims to reduce cerebral metabolic demand and thus reduce injury caused by secondary reperfusion. Bradycardia is a known side-effect. (5) In our cohort, HR remained stable throughout the cooling phase but increased during rewarming. Our group has previously demonstrated significant differences in measures of heart-rate variability (HRV) between infants with moderate HIE in the pre and post TH era suggesting increased activity of the parasympathetic nervous system which may also explain the reduced HR seen in our cohort during TH. (426) It is also important to consider the effect of medications such as morphine when interpreting our findings. Bradycardia is a known side-effect of morphine (535) and all infants with moderate and severe HIE received morphine during TH as per our unit's protocol, albeit at different doses and titrated according to clinical response.

Infants with moderate and severe HIE had a significant increase in HR and CO during the rewarming phase. This finding is in keeping with previously published studies. Forman et al. showed that NICOM measures of CO remained stable during TH in 20 infants with HIE but CO and HR significantly increased during rewarming. (359) Similarly, Wu et al. used EC to assess CO prior to and after rewarming in 20 infants with moderate and severe HIE and found that CO increased significantly during rewarming driven by a significant increase in HR. (360)

Eriksen et al. used EC to assess the effect of early TH on CO in a group of 15 infants with moderate and severe HIE compared with 10 healthy term controls. (361) Five infants with HIE had haemodynamic measurements recorded prior to initiation of TH. Compared with the control group, these infants had a lower CO and SV suggesting an effect of the original injury. CO reduced further once infants were cooled and this was largely due to a reduction in HR. All infants undergoing TH in our study had TH commenced prior to initiating NICOM monitoring so we cannot extrapolate whether the reduced CO was solely due to the effects of TH or whether it was compounded by the degree of encephalopathy. Activation of the parasympathetic nervous system is a hallmark of a moderate grade of encephalopathy evident by bradycardia. (2) In addition, perinatal asphyxia itself results in myocardial dysfunction. (331, 334, 347) Reduction in CO along with the increased pulmonary and systemic vascular resistance associated with acute asphyxia (331) may be a protective mechanism by which TH reduces the risk of cerebral hyperperfusion. However, maintenance of an appropriate CO is required to maintain adequate cerebral perfusion and oxygen delivery to avoid further brain injury. (354, 355) This delicate balance is crucial in optimising neuroprotection and so a continuous monitoring tool such as NICOM may be an important management tool. (351, 358, 359). Understanding the complex interaction between CO and cerebral blood flow in the future may allow us to individualise management of these infants.

NICOM has the potential to provide this information and has gained much interest in the neonatal population due to its ease of application and provision of a continuous insight into cardiac output. It is less affected by artefact than other non-invasive measures of CO and is not affected by ventilatory status. (321, 322, 323, 324, 325, 327) NICOM readings are not affected by reduced body temperature and this finding is also supported by previous studies in animals. (330, 359)

NICOM use in neonates remains predominantly in a research setting due to difficulties in validating its use against gold standard measurements of CO, which are invasive and challenging. Echocardiography remains the most accepted method of measuring cardiovascular function in infants in the neonatal unit. When compared

with echocardiogram-CO, NICOM consistently under reads echocardiography measurements by approximately 27-30%, highlighting its potential use as a trend monitor. (328, 329, 359) Our percentage bias of 21.2% is less than previously reported however, in keeping with previous studies, the percentage error of 56.8% exceeded the clinically acceptable percentage error of 30% implying that echocardiogram-CO and NICOM-CO are not interchangeable. (318) This may be explained as follows. Firstly, the NICOM system uses an algorithm developed to measure CO in adults and extrapolates it to neonatal measurements which may account for lower NICOM measurements. (330) Another consideration is that echocardiogram-CO relies on measurements of the aortic size and velocity of blood flow which can vary depending on the exact site of measurement. This may result in echocardiography measurements of CO slightly overestimating CO. (329).

Nonetheless, NICOM has considerable advantages. Predominantly, it does not require expert training to perform or interpret the measurements. Secondly, echocardiography only allows a window into cardiac function at a single snapshot in time. Cardiac function is dynamic. Cardiovascular dysfunction, particularly right ventricular dysfunction, is an independent risk factor for adverse outcome. (355) Alterations in BP, physical examination and urine output, although important clinical signs, are late markers of impaired myocardial function. NICOM has the potential to be a useful tool in the NICU as a trend monitor allowing for early recognition of infants at risk and thus early implementation of appropriate interventions to improve outcome. Furthermore, it may be helpful in monitoring the subsequent response to treatment. Non-invasive monitoring may also have important healthcare economic considerations. Echocardiograms cost approximately $1,000-$3,000 dollars per examination whereas NICOM sensors are much less expensive and may therefore provide a low-cost alternative to monitoring CO. (536)

This study is limited by small numbers of infants in each group and by varying durations of monitoring. Only four infants in our cohort had severe HIE making it difficult to interpret these findings but their trend in CO, HR and SV are similar to infants in the moderate group. Due to challenges associated with obtaining early

informed consent in this cohort, it was not possible to obtain CO measurements at all time points in all infants across the four groups. Furthermore, we do not have detailed functional echocardiography measurements on all infants and are therefore unable to determine the underlying pathophysiological mechanisms responsible for the differences seen in NICOM measurements. However, this is the first study to describe the early evolution of cardiac output changes in infants with all grades of HIE compared to controls. Long-term follow-up is currently underway which will allow us to further examine whether these changes in CO are important in the prediction of outcome.

In conclusion, infants with mild HIE have a higher HR but similar CO to healthy infants at 6 hours of age and by 24 hours, there was no difference in either parameter. Infants with moderate HIE undergoing TH have a significantly lower CO and HR when compared with the mild and control groups. During TH, CO increases gradually over time driven by a gradual increase in SV, possibly reflecting improved diastolic function, and by a significant increase in HR during the rewarming phase. Cardiovascular compromise is a known complication of HIE and is an independent risk factor for adverse outcome. Improved and accessible methods of monitoring cardiovascular function are required. NICOM may provide a non-invasive, continuous, low-cost alternative to monitoring CO in infants with HIE however further research is warranted.

5. Multi-modal Monitoring of Infants with Hypoxic Ischaemic Encephalopathy within 12 hours of birth and Prediction of Short-term Outcome

5.1. Introduction

Despite advancements in maternal and neonatal care, rates of Hypoxic Ischaemic Encephalopathy (HIE) remain static. With 2 million infants affected each year, it is one of the leading causes of disease burden worldwide. (4, 6) Despite a significant improvement in outcomes following the introduction of therapeutic hypothermia (TH) for infants with moderate-severe HIE, (5) approximately 40% of infants with HIE will have neurodevelopmental impairment. (5, 110, 412)

Mild HIE accounts for 40% of all cases of HIE. (114) Currently not eligible for TH, 20% will have adverse events in the short-term and 25% will have significant disability on long-term follow-up. (113, 115) DuPont et al. found that in their cohort, 5 of the 60 infants diagnosed initially as having mild HIE later had seizures. (2) It is unclear as to whether these infants were incorrectly diagnosed at the outset or whether the encephalopathy progressed. (113)

HIE is an evolving process with a dynamic spectrum of neurological findings, making it challenging to definitively assign a grade in the early timeframe. Clinical examination is subjective, is influenced by many maternal and neonatal factors and has only been validated if performed continually over the first 24-72 hours. (2, 108, 112, 113) This, coupled with the requirement to initiate TH within the therapeutic window of 6 hours, provides a significant challenge for clinicians. (143) Early objective biomarkers are required. Readily available bedside measures include electroencephalography (EEG), near-infrared spectroscopy (NIRS) and non-invasive cardiac output monitoring (NICOM).

Continuous, multi-channel EEG (cEEG) provides a functional measure of brain health and injury, and has long been used in HIE management. Pre-TH, amplitude-integrated EGG (aEEG) was the most useful tool in the prediction of outcome in infants with HIE

and for this reason was incorporated into the inclusion criteria for some of the TH trials. (71, 159, 418) cEEG and aEEG remain helpful; however, with TH the timing of their optimum predictive ability has now shifted to 24-48 hours. (196, 419, 420, 537) Certainly, a normal EEG as early as 6 hours predicts a normal outcome but in the case of an abnormal EEG, the recovery and time taken to return to normal is important. (121, 182, 193) Furthermore, absence of discontinuity or burst-suppression alone does not imply a normal outcome. We have previously shown that infants with mild HIE have more subtle abnormalities such as a predominance of slow-wave activity and lack of sleep-wake cycling. (538)

Cerebral NIRS has many theoretical advantages as a potential biomarker. It provides real-time, easy-to-interpret information on cerebral oxygenation (cSO_2) and oxygen extraction at the bedside. cSO_2 at 24 hours is predictive of neurodevelopmental outcome; however, information on its use as an early biomarker is limited. (511)

NICOM use has evolved in the neonatal unit. (318) This modality gives a measure of cardiac output (CO), stroke volume (SV) and heart rate (HR) measured non-invasively by electrodes placed on the thorax. It may provide dynamic, continuous information on haemodynamic status and its evolution over time. (321, 322, 323, 324, 325, 326, 327) NICOM provides potential insight into the impact of HIE on cardiac function and the potential impact of TH. (359) No study has examined its ability to predict outcome in HIE.

HIE is a multi-organ pathology. It stands to reason that the best biomarker should incorporate various measures of organ function to improve prediction of infants who have more severe injury and thus have worse neurodevelopmental outcome. TH must be commenced within 6 hours of birth to be effective, (5, 143) however some infants who inadvertently miss the therapeutic window may still benefit from TH up to 12 hours. (31) We hypothesised that a combination of monitoring techniques, a multi-modal approach, might improve our early ability to predict outcome. The study aim was to determine the ability of early EEG, NIRS and NICOM (at 6 and 12 hours of

age), individually and in combination to predict short-term MRI outcome in infants with all grades of HIE.

5.2. Methods

This prospective observational study was conducted at Cork University Maternity Hospital, Cork, Ireland from November 2017 to March 2020. Inclusion criteria was as follows:

Infants >36 weeks gestation with one or more of:

- Apgar <5 at 5 minutes,
- Postnatal resuscitation >10 minutes (positive pressure ventilation on-going),
- pH <7.1 or base deficit >16 or lactate >9mmol on cord or first post-natal blood sample,

AND

- Clinically evolving encephalopathy defined as the presence of abnormal neurological findings on the modified Sarnat score performed at approximately 1 hour of age. (2, 116)

Exclusion criteria included congenital abnormalities, inborn errors of metabolism, congenital infections or confirmed sepsis.

EEG, NIRS and NICOM monitoring were commenced as soon as possible after delivery following informed, written parental consent.

All monitoring modalities were time-synched at the beginning of the recording and reviewed throughout the recording period. 1-hour epochs of data were selected at 6 and 12 hours of age for analysis. These two time points were selected due to their clinical significance. TH should be commenced within 6 hours of life and infants who inadvertently miss the therapeutic window may still benefit up to 12 hours of age.(2, 15, 24)

5.2.1. EEG

EEG monitoring was performed using the NicoletOne EEG System (Natus, USA), Nihon Kohden (Nihon Kohden, Japan) or Lifelines iEEG (Lifelines Neuro, UK) machines. 1-hour epochs of EEG were pruned and artefacts were manually annotated and removed.

EEGs were independently reviewed by two neonatal neurophysiologists (G Boylan, S Mathieson) blinded to outcome. EEGs were qualitatively assessed and graded based on our previously published grading system.(Table 5.1) (121)

Quantitative EEG analysis was performed using the NEURAL (**N**eonatal **E**eg feat**UR**e set in m**A**th**L**ab) software package (version 0.4.3) which provided multiple measures of EEG spectral power. (237)

Table 5.1: Classification of EEG Background Activity.

Grade	Findings	Description
0	Normal EEG Findings	Continuous background pattern with normal physiological features such as anterior slow waves
1	Normal/Mild Findings	Continuous background pattern with slightly abnormal activity (e.g. Mild asymmetry, mild voltage depression, or poorly defined sleep-wake cycle)
2	Moderate abnormalities	Discontinuous activity with an interburst interval of <10 seconds, no clear sleep-wake cycle, or clear asymmetry or asynchrony)
3	Major abnormalities	Discontinuous activity with an interburst interval of 10-60 seconds, severe attenuation of background patterns, or no sleep-wake cycle.
4	Inactive EEG findings	Background activity of < 10uV or severe discontinuity with an interburst interval of >60 seconds

5.2.2. NIRS

Cerebral NIRS monitoring was performed with the INVOS 5100 oximeter and the neonatal OxyAlert™ NIRSensor (Covidien, USA) placed on the right frontal area. SpO_2 data was measured with the Nellcor SpO_2 Neonatal Sensor (Covidien, USA) and the IntelliVue MP70 (Philips Healthcare, The Netherlands) and stored with the EEG signals. The NIRS and SpO_2 signals were cleaned and analysed as outlined previously in Chapter 3 (see section 3.2.2.1). FTOE was then calculated using the following standard formula for each infant.

$$\frac{(SpO_2 - cSO_2)}{SpO_2}$$

Nine infants did not have SpO_2 values stored so FTOE is not available for these infants.

For each infant, median cSO_2 and FTOE values at each time point were calculated.

5.2.3. NICOM

Non-invasive measures of CO, SV and HR were obtained using the Cheetah Starling NICOM ™ device (Cheetah Medical, USA) which uses bioreactance technology. Four sensors are applied to the infant's thorax (left/right, upper/lower) in a manner that "boxes" the heart. The NICOM device averages measurements of CO, HR and SV every 60 seconds.

For each infant, median CO, HR and SV values at each time point were calculated.

5.2.4. MRI

Brain MRIs were performed on a 1.5 T GE scanner (GE Medical Systems, USA), in the first week of life when the infant was stable for transfer. Infants were transferred to the MRI department via an MR Diagnostics Incubator System nomag® IC MR-conditional incubator with integrated coil for newborns. MRIs were independently analysed by a neonatal radiologist and a neonatal neurologist (M Moore, B Walsh) blinded to outcome using the Barkovich scoring system. Moderate-Severe MRI injury

was defined as a Barkovich score of ≥2 in the deep grey matter and/or a score of ≥3 in the watershed area. (384, 539)

5.2.5. Outcome

Abnormal outcome: Abnormal outcome was classified as an abnormal MRI (using a Barkovich score ≥1) and/or death of the infant within the first week after birth.

Moderate-severe outcome: We also assessed moderate-severe outcome defined as moderate-severe MRI injury (Barkovich score of ≥2 in the deep grey matter and/or a score of ≥3 in the watershed area) and/or death of the infant within the first week after birth.

5.2.6. Statistical analysis

Demographical data is displayed as median and interquartile range (IQR) for continuous variables and number and percentage for categorical variables. Demographic variables were compared between outcome groups using the Mann-Whitney U test for continuous variables and Fisher's Exact test for categorical variables.

Univariable analysis using the Mann-Whitney U test was performed to assess the ability of individual variables measured at 6 and 12 hours to predict abnormal outcome and moderate-severe outcome. Receiver operating characteristic (ROC) curves were generated and the area under the curve (AUC) and its corresponding 95% confidence interval (CI) were calculated. Youden's index (index=sensitivity+specificity-1) was used to find the optimal sensitivity-specificity cut-off point on the ROC curve and the corresponding sensitivity and specificity were estimated.

Multivariable logistic regression was used to assess whether combining variables improved prediction. Variables with a p-value <0.25 in the univariable analysis were eligible for inclusion in the model. As quantitative EEG measures of spectral power

(FB1 – FB4) are highly correlated, the best individual frequency band (FB) measure based on the univariable analysis was selected for inclusion.

Due to small numbers of infants with NICOM monitoring, they were not included in analysis to assess prediction of moderate-severe outcome.

All tests were two-sided and a p-value <0.05 was considered to be statistically significant. IBM SPSS Statistics (version 27.0, IBM Corp., USA) was used for the statistical analysis except for the testing of equality of the AUCs from the best univariable model with the multivariable model which was done using the Stata roccomp package (version 17.0, StataCorp LP, USA).

5.3. Results

5.3.1. Participants

Fifty-seven infants with HIE were included in this analysis (27 mild, 24 moderate, 6 severe). Thirty-four infants had monitoring data for at least one monitoring modality available at 6 hours and 56 infants had data available at 12 hours. Median gestational age for the entire group was 39.9 weeks (IQR 38.1–40.7) and median birthweight was 3.4 kgs (IQR 3.0-3.7). Demographic and perinatal information by outcome group is outlined in Table 5.2.

Table 5.2: Demographic information of infants included.

	Normal Outcome (n=39) n(%)*	**Abnormal Outcome (n=18) n(%)***	**p-value**
Demographics			
Gestational Age (weeks): median(IQR)	39.9 (38.3-40.7)	39.3 (37.7-40.7)	0.332[1]
Birth Weight (kgs): median(IQR)	3.35 (2.97-3.83)	3.36 (2.93-3.55)	0.391[1]
Gender (male)	23 (59)	10 (56)	1.000[2]
Perinatal Data			
Grade of HIE			**0.018**[2]
Mild	21 (54)	6 (33)	
Moderate	17 (44)	7 (39)	
Severe	1 (3)	5 (28)	
Mode of Delivery			0.515[2]
SVD	10 (26)	6 (33)	
Instrumental	16 (41)	4 (22)	
EmLSCS	11 (28)	6 (33)	
Pre-labour LSCS	2 (5)	2 (11)	
PPV at 10 minutes	14 (36)	4 (22)	0.237[2]
Apgar <5 at 10 minutes[3]	4 (10)	7 (39)	**0.029**[2]
Therapeutic Hypothermia	18 (46)	12 (67)	0.168[2]
EEG Seizures	1 (3)	7 (39)	**0.001**[2]
Inotropes	0	3 (17)	**0.028**[2]
Biochemical Data			
Lowest pH**: median(IQR)	7.00 (6.86-7.09)	6.98 (6.82-7.09)	0.508[1]
Lowest BE**: median(IQR)	-15.0 (-16.2 to -9.8)	-13.8 (-15.9 to -9.3)	0.625[1]
Highest Lactate**: median(IQR)	10.1 (7.0-13.2)	13.6 (6.7-15.4)	0.137[1]

SVD, spontaneous vaginal delivery; LSCS, lower section caesarean section; EmLSCS, emergency LSCS; PPV, positive pressure ventilation; BE, base excess

**unless stated otherwise*

***Worst pH, base deficit, lactate on either umbilical cord blood or first post-natal blood gas.*

[1]p-values derived from Mann-Whitney U Test.

[2]p-values derived from Fisher's Exact Test. Values in bold indicate a p-value <0.05.

[3]n=26 in normal group and n=14 in abnormal group

5.3.2. Outcome

Three infants died, two of whom did not have an MRI scan. MRIs were performed on median day of life 5 (IQR 3-5). MRI outcome overall and by HIE group is outlined in Table 5.3. Approximately 22% of infants with mild HIE had MRI injury with these infants demonstrating watershed injury only. Twenty-nine percent of the infants with moderate HIE had MRI abnormalities, predominantly watershed in origin. Thirteen percent of infants with moderate HIE had moderate-severe injury on MRI (n=3). Infants with severe HIE had, for the most part, involvement of both the watershed and basal ganglia and demonstrated moderate-severe injury. Two infants had MRI abnormalities noted that did not meet the Barkovich criteria. These infants both had small punctate white matter lesions outside of the watershed areas.

Table 5.3: Short-term outcome and grade of MRI injury according to degree of HIE.

	Overall (n=57) n(%)	**Mild (n=27) n(%)**	**Moderate (n=24) n(%)**	**Severe (n=6) n(%)**	**p-value**
Barkovich Scoring +/- Death					0.018[1]
Normal outcome	39 (68)	21 (78)	17 (71)	1 (17)	
Abnormal outcome	18 (32)	6 (22)	7 (29)	5 (83)	
Moderate-Severe Barkovich Scoring +/- Death					<0.001[1]
Normal outcome	47 (83)	26 (96)	20 (83)	1 (17)	
Abnormal outcome	10 (18)	1 (4)	4 (17)	5 (83)	
Abnormal outcome					
Death pre-MRI	2	0	1	1	
Death post-MRI	1	0	0	1	
Injury Consistent with Barkovich Classification	16	6	6	4[2]	
Moderate-severe MRI Injury	8	1	3	4[2]	
MRI injury					
Any	18	7	7	4[2]	
Type of injury					
Watershed injury only	12	6	5	1	
Basal ganglia injury only	1	0	1	0	
Both watershed and basal ganglia injury	3	0	0	3[2]	

[1]*p-values derived from Fisher's Exact Test for normal vs abnormal outcome across HIE groups*

[2]*includes infant who died*

5.3.3. Univariable Analysis

The results of the univariable analysis investigating the predictive ability of the individual variables at 6 and 12 hours are outlined in Table 5.4 and Table 5.5.

5.3.3.1. 6 hours

At 6 hours, none of the variables significantly predicted an abnormal outcome or a moderate-severe outcome.

5.3.3.2. 12 hours

At 12 hours, quantitative EEG measures (spectral power) and qualitative EEG grades were the best predictors of both an abnormal outcome and a moderate-severe outcome with their predictive ability being better for the moderate-severe outcome. For the abnormal outcome, spectral power (FB4) was the best individual predictor with an AUC of 0.68 (95% CI: 0.53–0.84).

For the moderate-severe outcome, qualitative EEG grade was the best individual predictor with an AUC of 0.84 (95% CI: 0.70–0.98).

Table 5.4: Ability of variables measured at 6 hours to predict abnormal outcome.

		n (normal/abnormal)	Normal Outcome	Abnormal Outcome	p-value	AUC	95% CI	Sensitivity	Specificity
Barkovich Score +/- Death									
NIRS	cSO_2	33 (21/12)	76.5 (66.5 - 85.0)	78.0 (72.3 - 81.4)	0.754	0.54	0.34-0.73	75	48
	FTOE	26 (16/10)	0.19 (0.13 - 0.30)	0.21 (0.16 - 0.28)	0.623	0.56	0.34-0.79	100	25
NICOM	CO	13 (7/6)	70.3 (64.9 - 84.4)	87.3 (71.1 - 98.8)	0.138	0.76	0.50-1.00	100	43
	HR	13 (7/6)	127 (89 - 140)	127 (116 - 133)	0.836	0.54	0.20-0.87	0	71
	SV	13 (7/6)	0.63 (0.46 - 0.85)	0.73 (0.58 - 0.77)	0.836	0.55	0.22-0.88	0	71
EEG	FB1	34 (22/12)	175.4 (81.3 - 279.0)	133.6 (82.9 - 334.3)	0.709	0.54	0.33-0.75	58	59
	FB2	34 (22/12)	12.0 (7.1 - 15.8)	8.8 (5.5 - 16.5)	0.606	0.56	0.35-0.77	58	64
	FB3	34 (22/12)	5.3 (3.7 - 8.0)	4.9 (3.6 - 8.9)	0.901	0.52	0.30-0.73	25	91
	FB4	34 (22/12)	3.1 (2.5 - 5.7)	3.6 (1.7 - 5.0)	0.873	0.52	0.31-0.73	67	59
Qualitative EEG Grade		34 (22/12)	1.0 (0.8 - 3.0)	1.0 (1.0 - 2.8)	0.817	0.53	0.32-0.73	92	23
Moderate-Severe Barkovich Score +/- Death									
NIRS	cSO_2	33 (28/5)	78.0 (68.0 - 85.0)	73.0 (69.5 - 77.3)	0.478	0.60	0.41-0.80	80	61
	FTOE	26 (21/5)	0.17 (0.13 - 0.29)	0.24 (0.21 - 0.29)	0.278	0.67	0.47-0.87	100	52
EEG	FB1	34 (29/5)	193 (96 - 295)	80 (26 - 261)	0.135	0.72	0.44-0.99	80	66
	FB2	34 (29/5)	12.0 (7.0 - 16.3)	6.1 (2.9 - 13.0)	0.179	0.70	0.43-0.97	60	83
	FB3	34 (29/5)	5.4 (3.9 - 8.1)	3.5 (2.0 - 7.2)	0.232	0.68	0.39-0.97	60	83
	FB4	34 (29/5)	3.3 (2.6 - 5.2)	1.6 (1.1 - 5.1)	0.273	0.66	0.36-0.97	60	90
Qualitative EEG Grade		34 (29/5)	1.0 (1.0 - 2.5)	2.0 (1.0 - 3.5)	0.213	0.68	0.44-0.92	60	66

cSO_2, cerebral oxygenation; FTOE, fractional tissue oxygen extraction; CO, cardiac output; HR, heart rate; SV, stroke volume; FB, frequency band. p-values derived from Mann-Whitney U test. Sensitivities and specificities based on optimising Youden's Index.

Table 5.5: Ability of variables measured at 12 hours to predict abnormal outcome.

		n (normal/abnormal)	Normal Outcome	Abnormal Outcome	p-value	AUC	95% CI	Sensitivity	Specificity
Barkovich Score +/- Death									
NIRS	cSO_2	55 (38/17)	80.5 (74.5 - 88.3)	80.0 (76.0 - 85.0)	0.682	0.54	0.38-0.69	88	32
	FTOE	46 (32/14)	0.15 (0.09 - 0.25)	0.17 (0.11 - 0.24)	0.599	0.55	0.38-0.72	100	22
NICOM	CO	22 (14/8)	74.9 (66.8 - 86.6)	82.3 (60.2 - 95.9)	0.868	0.53	0.25-0.81	63	71
	HR	22 (14/8)	125 (102 - 133)	126 (108 - 141)	0.570	0.58	0.32-0.83	38	86
	SV	22 (14/8)	0.64 (0.56 - 0.72)	0.57 (0.52 - 0.79)	0.616	0.57	0.30-0.85	63	71
EEG	FB1	56 (38/18)	210.8 (91.4 - 282.8)	85.2 (36.4 - 263.9)	**0.046**	0.67	0.50-0.83	50	87
	FB2	56 (38/18)	11.4 (6.5 - 15.4)	8.5 (3.0 - 14.0)	0.065	0.65	0.49-0.81	44	87
	FB3	56 (38/18)	5.7 (4.4 - 7.0)	4.2 (1.8 - 6.9)	0.150	0.62	0.45-0.79	50	79
	FB4	56 (38/18)	3.7 (2.3 - 6.5)	2.3 (0.8 - 4.1)	**0.028**	0.68	0.53-0.84	50	89
Qualitative EEG Grade		56 (38/18)	1.0 (1.0 – 2.0)	1.5 (1.0 – 2.5)	0.062	0.64	0.48-0.81	22	100
Moderate-Severe Barkovich Score +/- Death									
NIRS	cSO_2	55 (46/9)	82.0 (75.0 - 88.0)	77.0 (75.0 - 84.0)	0.284	0.61	0.43-0.80	89	46
	FTOE	46 (38/8)	0.16 (0.09 - 0.24)	0.19 (0.09 - 0.26)	0.540	0.57	0.35-0.79	50	74
EEG	FB1	56 (46/10)	210.8 (91.4 - 282.8)	42.2 (3.8 - 150.2)	**0.002**	0.82	0.64-1.00	70	96
	FB2	56 (46/10)	11.5 (7.4 - 15.2)	3.7 (0.4 - 11.8)	**0.005**	0.79	0.59-0.98	70	89
	FB3	56 (46/10)	5.8 (4.4 - 7.2)	2.3 (0.3 - 6.0)	**0.007**	0.77	0.58-0.97	70	91
	FB4	56 (46/10)	3.6 (2.3 - 6.5)	0.9 (0.4 - 3.5)	**0.003**	0.80	0.63-0.98	60	98
Qualitative EEG Grade		56 (46/10)	1.0 (1.0 – 2.0)	2.0 (1.8 – 4.0	**<0.001**	0.84	0.70-0.98	80	74

cSO_2, cerebral oxygenation; FTOE, fractional tissue oxygen extraction; CO, cardiac output; HR, heart rate; SV, stroke volume; FB, frequency band p-values derived from Mann-Whitney U test. Sensitivities and specificities based on optimising Youden's Index. Values in bold indicate p<0.05.

5.3.4. Multivariable Analysis

5.3.4.1. 6 hours

For abnormal outcome, only one variable had a p-value <0.25 in the univariable analysis and hence no multivariable analysis was performed.

For moderate-severe outcome, spectral power (FB1) and qualitative EEG grade were included in the multivariable model. Combining the variables did not improve the prediction of an abnormal outcome (AUC (95% CI): 0.67 (0.41–0.93)).

5.3.4.2. 12 hours

For abnormal outcome, spectral power (FB4) and qualitative EEG grade were included in the multivariable model. Combining the variables yielded an AUC of 0.70 (95% CI: 0.55–0.85), which was not a statistically significant improvement (p=0.690) over a model containing spectral power (FB4) only.

For moderate-severe outcome, spectral power (FB1) and qualitative EEG grade were included in the multivariable model. Combining the variables yielded an AUC of 0.88 (95% CI: 0.76–1.00), which was not a statistically significant improvement (p=0.201) over a model containing spectral power (FB1) only.

5.4. Discussion

Early identification of infants at risk of brain injury and thus adverse neurodevelopmental outcome remains central to the management of infants with suspected HIE. It is the first and most important step in the treatment pathway and forms the basis of all subsequent intervention strategies and clinical trials. Any potential biomarker needs to be accurate, accessible, easily interpreted and timely. Currently available objective tools, such as pH and lactate, focus our attention on the infants that require closer monitoring but are not sufficiently sensitive or specific to

identify infants at risk. (59, 94) Other available tools including clinical examination and Apgar scores are subjective and have many confounders. (2, 60, 108, 112) This, coupled with the dynamic history of HIE, make selection of infants for treatment clinically challenging, especially amongst those infants with possible mild/moderate HIE.

As HIE is a multi-organ pathology, we sought to combine measures of multi-organ physiological dysfunction in an attempt to provide a better overview of the HIE process. This is the first study to combine currently available monitoring devices that provide real-time information at the bedside.

Twenty-two percent of the infants with mild, 29% of those with moderate HIE and 83% of infants with severe HIE had an adverse short-term outcome as defined by MRI abnormality and/or death. This rate of MRI abnormality is slightly lower than previously documented studies. However, previous studies included infants who received TH for mild encephalopathy either selectively or inadvertently raising the possibility that those infants were higher on the spectrum of severity. (113, 118, 119) In addition, many of these studies use 3T MRI scanners which are more sensitive to subtle injury. In contrast to previous studies, there was a significant difference in the pattern of injury between infants with different grades of HIE. (118, 119) Infants in the mild and moderate groups had predominately white matter and watershed injuries whereas infants with severe HIE had moderate-severe MRI injury and the majority had involvement of the basal ganglia.

At 6 hours, no marker significantly predicted short-term outcome. Information on early, continuous haemodynamic monitoring in HIE is limited and studies examining the use of non-invasive cardiac output monitoring to date have focused on infants with moderate and severe HIE and the effect of TH on CO. (359, 360, 361) Our limited number of infants with available NICOM recordings at these time points are too small to determine whether or not NICOM is helpful in prediction of outcome. Previous echocardiography studies however have demonstrated impaired left ventricular function and reduced SV following birth asphyxia which correlates with severity of

encephalopathy. (335, 347, 348) This myocardial dysfunction appears to be transient and improves over time. Further research in this area is warranted.

Interestingly, early cSO_2 and FTOE were not associated with abnormal outcome in our cohort. A paucity of data exists on the use of early FTOE and cSO_2 to predict outcome in infants with HIE. (511) The ability of early NIRS to predict outcome is conflicting, although most studies agree that a high cSO_2 and a lower FTOE is predictive of long-term neurodevelopmental outcome beyond 24 hours of age. (299, 511)

EEG is an important monitoring tool in infants with HIE. Its predictive ability, although delayed with TH, is important. A normal EEG predicts a normal 2 year outcome and time to recovery of sleep-wake cycling is a helpful tool when discussing outcome with parents. (121) Studies have demonstrated that the recovery of the EEG is as important as the initial injury in the prediction of outcome (121, 182, 193) and with the introduction of TH, is now most predictive at 48 hours. (196, 419, 420, 537) cEEG is uncompressed and discrepancies have been demonstrated between cEEG and aEEG in the same infant. (200) cEEG may be more helpful in detecting subtle differences, which may be useful in predicting outcome. Infants with an abnormal outcome demonstrate reduced power and a higher grade of EEG severity at 12 hours suggesting a delayed recovery of the EEG in these infants.

The main disadvantage of cEEG is that it requires expert interpretation that is not always readily available. Quantitative analysis of the EEG overcomes this by providing an objective, scalable, automated description of the EEG thus allowing us to quantify the EEG features that would otherwise be difficult to define on visual assessment alone. We have previously identified significant differences in EEG power in infants with mild HIE compared with controls. (538) For this reason, we focused on spectral power for this analysis. Across all frequency bands, the EEG power at 12 hours was significantly lower in the infants with an abnormal outcome. This is not surprising as discontinuity and low voltage activity are hallmark features of HIE and their prevalence and severity increases with increasing grade of HIE, thus resulting in

reduced overall power. Quantitative EEG features had similar predictive abilities to the qualitative grading score. Sensitivity of quantitative EEG measures ranged from 44-50% for abnormal outcome and 60-70% for moderate-severe abnormal outcome with specificities of 79-89% and 89-98% respectively. As many units do not have 24-hour access to a neonatal neurologist or neurophysiologist, an automated algorithm may be useful in interpreting the cEEG and predicting outcome just as automated seizure detection algorithms have been shown to improve seizure recognition. (233)

Interestingly, combining different markers of brain health did not improve prediction of abnormal short-term outcome. This may be due to the different phases in the underlying pathology at the different time points in the injury pathway. The different modalities identify different features of brain health and may, instead, be important at different time-points in the evolution.

This study is limited by a small sample size. We were unable to truly assess the ability of NICOM to predict outcome as only 13 infants had NICOM monitoring available at 6 hours and 22 infants at 12 hours. Furthermore, poor MRI outcome does not always indicate abnormal neurodevelopmental outcome. Long-term neurodevelopmental follow-up of this cohort is currently underway. A further limitation is that our cohort includes infants receiving TH and those who did not. Although TH improves outcome, its impact on outcome is not homogenous as 40% of infants continue to have neurodevelopmental impairment. Our sample size was too small to assess multi-modal monitoring based on grade of encephalopathy or TH status, however we found no difference in rates of TH between the infants with a normal outcome and those with an abnormal outcome.

This is the first study to combine different markers of brain health and assess their individual and combined ability to predict short-term outcome. EEG remains a key tool in the monitoring of infants with HIE and prediction of outcome. Although not predictive at 6 hours, both qualitative and quantitative EEG features successively predicted short-term outcome in infants with all grades of HIE at 12 hours.

Quantitative EEG algorithms may be useful in the future to aid in the prediction of infants at risk of brain injury.

6. Conclusion

Hypoxic Ischaemic Encephalopathy (HIE) remains a significant challenge for clinicians as, despite significant improvements in treatments, approximately 40% of these infants continue to have neurodevelopmental impairment. (5, 110, 412) Reasons for this are likely to be multifactorial.

Firstly, our current methods of grading encephalopathy are suboptimal. Clinical assessment tools validated to assess infants over *the first week of life* have been incorporated into clinical decision-making and are currently being used to inform therapeutic interventions *in the first 6 hours*. (2, 108) Not only is the timeline an issue, but clinical assessment is subjective and has been shown to misclassify infants at an early age. (113)

Furthermore, the optimal 6-hour therapeutic window described in animal models was determined with a known and exact time of hypoxic ischaemia. (142, 540, 541) The real world is considerably less precise. Although the exact timing of the initial insult is unknown in many cases, the 6 hour window from birth has been accepted as the maximum amount of time within which interventions should be implemented, (5) allowing little time to resuscitate, stabilise and identify infants at risk of brain injury. In a certain proportion, the sentinel event may have occurred long before delivery placing these infants well outside the timeframe for intervention (70) and may explain why in many cases, despite therapeutic hypothermia (TH), infants may progress to significant disability.

There is also a growing body of evidence that infants with mild HIE are at risk of adverse outcome. (115) At present, no therapeutic intervention has been proven to improve outcome in these infants and current strategies involve early supportive management only. Despite this, many centres have moved towards providing TH to these infants due to the fear of misdiagnosis. (130) Clinicians are not confident in their ability to accurately distinguish between infants with mild and moderate encephalopathy. Added to this is the fear that the condition may evolve and infants

with mild HIE may progress to moderate HIE at a later stage and thus the clinical team may miss the opportunity for intervention. This fear is not unfounded. Studies have shown infants originally classified as having either a normal or mildly encephalopathic examination later developing seizures or abnormal MRI findings consistent with moderate encephalopathy. (113, 118) Medico-legal cases have also been taken against clinicians for not offering TH to infants who may have benefited from the treatment. Further research is required to investigate the use of TH in infants with mild HIE but prior to this, improved methods of disease stratification are required to reduce numbers needed to power such a study and to reduce the risk of adverse events in infants in whom intervention would not be necessary.

Improved understanding of the underlying evolution of injury and changes in physiological biomarkers may allow us to better stratify infants at risk. Earlier detection would ultimately allow for earlier intervention and implementation of adjunctive therapies. Multi-modal, non-invasive assessments have the potential to bring objectivity to our current assessment tools.

As part of this PhD, I have conducted original, prospective research on infants with hypoxic ischaemic encephalopathy, recruiting 61 infants with HIE and 21 healthy term controls. Of the infants with HIE, 30 were mild, 25 were moderate and 6 had severe HIE. These infants were recruited as soon as possible after delivery allowing for the early commencement of continuous monitoring including continuous multi-channel EEG, near-infrared spectroscopy and non-invasive cardiac output monitoring. Infants with mild HIE had recordings until 24 hours if possible, or until the EEG background had normalized. For infants receiving therapeutic hypothermia, monitoring was continued for the duration of hypothermia treatment and rewarming and extended as clinically necessary, often until seizures had ceased or the EEG had recovered.

In addition, relevant clinical data was collected from the neonatal and maternal charts regarding the pregnancy, delivery details and post-natal course in the neonatal unit. Neurological examination was performed at 1 hour of age using the modified

Sarnat score. Grading of encephalopathy was based on clinical history and early examination. Following recruitment, information was reviewed by two independent clinicians and consensus was reached regarding the final grade of encephalopathy.

EEG has always played a key role in the management of infants with HIE. Sarnat et al. first realised its value and incorporated it into their original grading system as it provides continuous, immediate information about brain activity. (2) A limitation of continuous EEG is the requirement for expert application and interpretation and many have suggested the use of aEEG as a user-friendly alternative. However, discrepancies exist between interpretation of cEEG and aEEG and even among expert reviewers, inter-observer agreement is poor. (200) Continuous EEG remains the gold standard neurophysiological test.

Due to previous studies suggesting that infants with mild HIE had a normal outcome, the main emphasis has been on delineating between mild and moderate grades of encephalopathy for the purpose of implementing TH with many studies and grading systems combining normal and mild EEG features. (71, 182, 208) We wanted to further describe the differences in EEG features between infants with mild encephalopathy and normal term controls (Chapter 2: Multichannel EEG Abnormalities during the first 6 hours in Infants with Mild Hypoxic Ischaemic Encephalopathy). EEGs were visually assessed by two neonatal neurophysiologists. Infants with mild HIE displayed both an absence of normal EEG features and the frequent presence of abnormal features. Approximately 72% of infants with mild HIE had some abnormal feature on their EEG. Absence of sleep wake cycling is a well-documented disturbance in infants with HIE and has been associated with poor neurodevelopmental outcome. We found that only 53% of infants with mild HIE had normal sleep-wake cycling (SWC) in the first 6 hours after birth. Infants with mild HIE had excessive delta activity and abnormal sharp waves. These features were not present in the control group and all infants in the control group demonstrated normal SWC. The necessity of expert input into the application and interpretation of continuous EEG has limited its widespread use and availability. Quantitative EEG analysis may overcome this as it provides a scalable, continuous and objective

assessment of the EEG. Our analysis showed significant differences in spectral shape between infants with mild HIE and healthy term controls with 91% of spectral power in the EEGs of infants with mild HIE in the delta band (<4 Hz). Both spectral flatness and spectral difference were significantly lower in the delta and theta frequency bands for the mild HIE group compared with non-HIE group.

Chapter 3 (Near-Infrared Spectroscopy in Infants with Hypoxic Ischaemic Encephalopathy) examines the evolution of NIRS in infants with all grades of HIE. We began by systematically reviewing the available literature on the ability of NIRS to predict outcome in the first 6 hours after birth. Although cSO_2 and FTOE significantly predict adverse neurodevelopmental outcome beyond 24 hours of age, (271, 294, 299) its use as an early biomarker was less described. We identified 7 studies encompassing 161 infants who had NIRS monitoring in the first 6 hours of life and available short or long-term outcome. Although some studies described a trend of higher cSO_2 at 6 hours in infants with an adverse outcome, in the majority this was not significant until beyond 24 hours. Varying monitoring devices and probes were used to predict varying methods of outcome and we concluded that small sample sizes and varying methodology made it difficult to generalise results. Our review highlighted a paucity of information on NIRS in the early 6-hour timeframe after birth and also on its evolution over time in infants with mild HIE.

For this reason, we examined cSO_2 and FTOE in infants with HIE. This is the first study to describe evolution of NIRS over time in all grades of HIE. cSO_2 increased over the first 24 hours in all groups regardless of TH status. Although we did not have control data, the trajectory of cSO_2 and FTOE in the mild group was different to previously published norms using the same device and probe configuration suggesting an effect of the underlying encephalopathy on cerebral oxygenation. (258, 515) Infants with mild HIE had significantly higher cSO_2 and lower FTOE over the first 9-12 hours of life however beyond this, they were on par with their cooled moderate counterparts: a group receiving a proven neuroprotective intervention.

While most HIE research to date has focused on different markers of brain health (EEG, NIRS, MRI) we know that HIE is a multi-organ pathology with many systems interconnected and influencing each other. We sought to examine haemodynamic markers as cardiac output and blood flow may have a direct impact on cerebral perfusion (Chapter 4: Non-Invasive Continuous Cardiac Output Monitoring in Infants with Hypoxic Ischaemic Encephalopathy). (354, 355)

Cardiac output and myocardial function may be transiently affected in HIE. Previous studies examining functional echocardiography in infants with HIE demonstrate left ventricular dysfunction, reduced cardiac output and myocardial ischaemia. (334, 347) For the most part, this appears to be transient but there is a paucity of real-time information. Echocardiography is not available in all units, requires expert performance and interpretation and provides only a snapshot in time. Non-invasive measures of monitoring cardiac output (CO) would allow for trend monitoring at the bedside. NICOM is feasible in the neonatal unit and is unaffected by TH. (352) Across all time points, CO was lower in infants with moderate HIE compared to those with mild HIE. This was predominantly due to a lower heart rate (HR). Stroke volume (SV) was maintained in infants with moderate HIE and gradually increased over 6-72 hours despite TH. Infants with mild HIE had a similar CO and SV to a control group of healthy, term infants over the first 24 hours. Infants in the mild HIE group had a higher HR to the control group at 6 hours but this difference was not evident by 24 hours.

Finally, in Chapter 5 (Multi-modal Monitoring of Infants with Hypoxic Ischaemic Encephalopathy within 12 hours of birth and Prediction of Short-term Outcome), we sought to examine whether these physiological monitoring modalities could aid in prognostication either independently or in combination. We defined abnormal short-term outcome as a composite outcome of MRI abnormality and/or death. Twenty-two percent of infants with mild HIE, 29% of those with moderate HIE and 83% of those with severe HIE had an abnormal outcome. We chose 6 and 12 hours of age as TH should be commenced within 6 hours from birth and in infants who inadvertently miss the 6-hour window, benefit has been described up to 12 hours so we believed

that these were two helpful and important time points. At 6 hours, none of the neuro-monitoring variables were significantly associated with outcome. At 12 hours, both qualitative and quantitative EEG markers were significantly associated with abnormal outcome with sensitivities of 22-50% for abnormal outcome and 60-80% for moderate-severe abnormal outcome with specificities of 79-100% and 74-98% respectively. Combining different modalities did not improve prediction of outcome. EEG has the ability to predict abnormal outcome as early as 12 hours after birth. Quantitative EEG markers showed similar predictive ability to qualitative EEG features and may have greater sensitivity for prediction of MRI findings.

6.1. Overview of Main Findings of this Thesis

Multichannel EEG Abnormalities during the first 6 hours in Infants with Mild Hypoxic Ischaemic Encephalopathy:

- Seventy-two percent of infants with mild HIE have some abnormal features on their continuous EEG in the first 6 hours of life.

- Visual assessment revealed absence of normal features and presence of abnormal features in the cEEG of infants with mild HIE; 48% had diffuse slow waves, 47% had absent/poor sleep-wake cycling and 19% were excessively discontinuous. All infants in the control group demonstrated normal, mixed-frequency activity and sleep-wake cycling.

- Quantitative analysis revealed significant differences in spectral shape between infants with mild HIE and controls. Spectral edge frequency in infants with mild HIE was 5.2 Hz compared with 6.3 Hz in the control group ($p=0.008$). Both spectral flatness and spectral difference were significantly lower in the delta and theta bands for the mild EEGs compared with controls.

- Clear differences exist in the EEGs of infants with mild HIE and control infants before 6 hours of age. These differences may aid in the objective identification of infants with mild HIE who may be most at risk of abnormal outcome.

Evolution of Early Cerebral NIRS in Hypoxic Ischaemic Encephalopathy:

- Information on early cerebral NIRS and its evolution over time is lacking in infants with all grades of HIE.

- In our cohort, cSO_2 increased and FTOE decreased over the first 24 hours in all grades of HIE regardless of TH status.

- Compared to the moderate group, infants with mild HIE had significantly higher cSO_2 at 6hrs (p=0.003), 9hrs (p=0.009) and 12hrs (p=0.032) and lower FTOE at 6hrs (p=0.016) and 9hrs (0.029).

- Beyond 18 hours, no differences were seen between the moderate group receiving TH and the uncooled mild group.

Non-invasive, Continuous Cardiac Output Monitoring in Infants with Hypoxic Ischaemic Encephalopathy:

- Infants with mild HIE have a higher heart rate at 6 hours of age compared with controls but no difference was seen in stroke volume or cardiac output.

- At 24 hours, no difference was seen between infants with mild HIE and controls.

- Infants with moderate HIE undergoing therapeutic hypothermia have a significantly lower cardiac output compared with mild HIE and control groups.

Heart rate is significantly reduced but stroke volume is maintained and gradually increases from 6-72 hours despite therapeutic hypothermia.

Multi-modal Monitoring of Infants with Hypoxic Ischaemic Encephalopathy within 12 hours of Birth and Prediction of Short-term Outcome:

- Twenty-two percent of infants with mild HIE, 29% of infants with moderate HIE and 83% of those with severe HIE had an abnormal short-term outcome (abnormal MRI findings using Barkovich scoring system and/or death of the infant in the first week).

- At 6 hours, none of the EEG, NIRS or NICOM measures, either independently or in combination, predicted short-term outcome.

- At 12 hours of age, both qualitative EEG grade and quantitative EEG features of spectral power significantly predicted abnormal short-term outcome in infants with all grades of HIE.

- Quantitative EEG algorithms may be useful to aid in the prediction of infants at risk of brain injury.

6.2. Strengths and Limitations

This is the first study to describe early, multi-modal monitoring in infants with all grades of HIE and the evolution of physiological biomarkers over the first 4 days of life. Certain limitations of this work have been discussed throughout the thesis and primarily relate to the small sample size. Although we included a total of 61 infants with HIE, sub-group analysis assessing grade of encephalopathy were based on small sample sizes. Research in this population as a whole is challenging due to the unpredictable occurrence of HIE and its relatively low prevalence (0.3%), resulting in single centre studies remaining small. Obtaining early informed parental consent is

also challenging. Our unit is a tertiary neonatal unit which services a larger hinterland of the South West Hospital Group in Ireland. Infants requiring therapeutic hypothermia are therefore transferred to our unit, often long before maternal transfer can be arranged. In these cases, informed consent may not be possible until one or both parents arrive in the unit resulting in a delay in the initiation of monitoring.

Another limitation is the varying levels of therapeutic interventions across the group. Infants with moderate and severe HIE receive therapeutic hypothermia, a proven neuroprotective intervention, whereas infants with mild HIE receive supportive management only. This limits our ability to make direct comparisons between the groups however it reflects the real-life difficulty with conducting research on infants with mild HIE in the era of therapeutic hypothermia. Despite this, infants with mild HIE have similar long-term outcomes to infants with moderate HIE that have been cooled highlighting the importance of further research in this area.

Chapter 2 (Multichannel EEG Abnormalities during the first 6 hours in Infants with Mild Hypoxic Ischaemic Encephalopathy) is limited by its retrospective design however, each cohort had strict enrolment criteria and we included clinical and EEG grading of HIE. Prospective enrolment of infants with mild HIE would require a multi-centre approach to recruit sufficient numbers.

As a primary aim of the thesis included combining monitoring modalities to predict outcome, time synchronisation of the data was crucial. All devices were time-synchronised at the beginning of each recording and monitored during the recording period. Data from each device was subsequently time matched off-line. Although we do not believe that this affected our results, combining all of the signal outputs into a single recording device would perhaps allow for more uniform sampling of the data in future.

Despite these limitations, we believe that the strengths of this project outweigh the limitations allowing us to achieve the primary aims of the thesis. We identified

significant differences in the EEGs of infants with mild HIE compared with controls and succeeded in describing early NIRS and NICOM values and their evolution in infants with all grades of HIE. This is also the first cohort combining all of these monitoring modalities in the same group of infants. Infants were recruited according to clearly defined and previously published inclusion criteria. (512, 513) Infants were prospectively enrolled in the immediate post-natal period allowing for the early initiation of monitoring. Comprehensive data was carefully collected and all infants had short-term outcome data available. Long-term neurodevelopmental follow-up of these infants is also currently underway.

6.3. Future and ongoing research generated by this thesis

HIE remains a clinical challenge with many questions yet to be answered. TH has certainly improved outcome although, clearly, it does not provide the same benefit to every infant. (5) Improved understanding of the underlying evolution and mechanism of injury has allowed for investigation of more targeted therapies focusing on different phases of the injury pathway. (35) Animal models provide significant insight into potential treatment strategies however, timing the injury in the clinical setting is challenging. We are operating in a non-ideal, imperfect world and are striving for precision within tests that are inherently limited.

Future research in this area should be considered under two main categories: timing of injury and prediction of outcome.

The timing of the original injury is crucial, as it is the timestamp from which every intervention is measured. We know that TH is effective if initiated within the first 6 hours of injury and becomes less effective with time. (27, 34, 150) If there is a known sentinel event around the time of delivery, for example, a uterine rupture or a cord prolapse, our time zero becomes clear. However, in many infants this injury may have occurred hours, days or even weeks before delivery and likely explains why some infants do not benefit from TH. In these infants, alternative therapies such as stem

cells, which target the tertiary phase of injury, may be more efficacious. (35) Fetal heart monitoring and CTG are not sensitive or specific enough to identify injury. (542) Early MRI may be helpful. Injury may be evident on diffusion-weighted sequences from the first day following the injury and is most obvious on days 2-3. Following this, there is a period of "pseudo-normalisation" which occurs around day 6-8. (362, 374, 375) Hypoxic injury is not evident on conventional T1 and T2 weighted images until the first to second week after the injury. (376) These timeframes may allow for crude estimation of how old the injury is. MRI on the first day of life, however, is difficult for many reasons. Infants often require stabilization and medical intervention. Most units do not have access to an MRI scanner in the NICU and transfer of the infant is therefore required for the purpose of the scan. Technical challenges may arise if the infant is ventilated and undergoing therapeutic hypothermia. I am currently undertaking a Neonatal Neurology Fellowship with the Brigham and Women's Hospital and Harvard University, Boston, MA, USA to examine patterns of MR injury in the era of TH. We are currently recruiting a cohort of infants with HIE and healthy term controls. Infants in both groups have MRIs in the first and second week of life and at 4, 6 and 12 weeks, along with a standardized neurodevelopmental assessment at 12 months. This will allow us to assess the temporal evolution of hypoxic ischaemic brain injury and its impact both on brain development and subsequent neurodevelopmental outcome. We also plan to determine whether obtaining an early MRI scan in infants with HIE is feasible and helpful. This project will involve serial, daily MRI scans in an in-NICU MRI scanner over the first 4 days of life. This will allow us to assess the nature and timing of brain injury and its evolution over time.

The second area for research encompasses prediction of outcome. Early, accurate, accessible identification of infants at risk of adverse neurodevelopmental outcome would allow for selective and targeted intervention therapies and rehabilitation strategies. This would have many benefits clinically and economically. Firstly, at the bedside, it would allow for improved prognostication and would facilitate informed discussion with parents around expected outcome. Secondly identifying infants at risk of poor outcome would allow for more appropriate use of limited resources such as physiotherapy, occupational therapy and speech and language therapies. Early

intervention and rehabilitation improves outcome. Early biomarkers would allow for improved stratification and prioritization of infants at risk. Finally, objective biomarkers would inform future research and would aid in the selection of infants who may benefit the most, reducing the numbers required to power studies and truly examining the effect of different interventions on targeted groups.

Infants with severe HIE tend to have a poor outcome and infants with a normal examination and normal EEG do well. (2) The difficulty lies in the spectrum in between and huge debate surrounds infants with mild HIE in particular.

The definition of mild HIE is particularly challenging. Examination scores are subjective and may grade infants differently depending on which scoring system is used. (111) We have identified clear, objective differences between the EEG features of infants with mild HIE and controls. Two-year follow-up of the final cohort is underway and we plan to correlate these findings with outcome to assess whether these differences are helpful in prognostication. Regarding the management of infants with mild HIE, ultimately an RCT is required to decide whether infants with mild HIE would benefit from TH and this is time sensitive as many centres have moved to cooling infants with mild HIE and thus losing equipoise. (131) We are also collaborating with our colleagues in the Brigham and Women's Hospital, Boston, MA, USA, a centre which offers TH to all infants with mild HIE, to conduct a comparative study examining the safety profile and side-effects of TH in infants with mild HIE. The outcomes of these two studies would be beneficial in informing the development of future RCTs.

NIRS monitoring has many theoretical benefits yet to date has not been successful in early prediction of outcome. Certainly, beyond 24 hours of age, its role becomes clearer. (299) We have seen significant differences in cSO_2 and FTOE as early as 6 hours between infants with mild and moderate HIE and their trajectory are different both to one another and to previously published values in healthy term infants yet we have failed to correlate this difference with short-term outcome. However, by looking solely at absolute values and an average of these values over a specific

timeframe, we lose the inherent variability and fluctuations within the signal itself, similar to the differences between the compressed aEEG and continuous multi-channel EEG. Our group has begun to develop a quantitative feature set to objectively describe the NIRS signal and has found significant features that identify brain injury in preterm infants. (508) We plan to expand our exploratory analysis to infants with HIE to determine whether quantitative analysis of the NIRS signal is helpful in predicting infants at risk of brain injury.

Seizures are common following HIE and seizure burden is an independent risk for poor neurodevelopmental outcome. (222) Little data currently exists on the effect of seizures on cerebral perfusion, particularly in neonates, with some case reports describing an increase in cSO_2 and others a decrease at the time of the seizure. (524, 525) Eight infants in our cohort had EEG seizures of varying origin and morphology. We plan to examine the effect of seizures on cSO_2 and FTOE.

Our group has also received funding from the Health Research Board, Ireland under the NEPTuNE study to continue to examine the impact of neonatal seizures on the development of later postnatal epilepsy. (543) EEGs from this cohort will be combined with EEGs from previous cohorts to determine the true incidence of postnatal epilepsy following electrographic confirmed neonatal seizures.

The evolution of CO, HR and SV as measured by NICOM is novel data. This is the first study to describe the use of NICOM in infants with all grades of HIE. NICOM monitoring in this population is feasible and due to its ease of application and interpretation could have potential clinical utility in the NICU. Although small in numbers, these measures demonstrated potential and represent a possible area for future research.

Autoregulation is the process by which infants maintain constant cerebral perfusion despite fluctuations in cerebral blood flow. This protective process may be lost in infants with HIE. Other groups have used NIRS and BP monitoring to identify an optimal mean arterial blood pressure range within which cerebral perfusion is

maintained. (276, 544) As mentioned previously however, BP is a poor marker of blood flow. We are currently examining the association of NIRS and NICOM to determine whether CO as measured by NICOM is a better identifier of autoregulation.

Although this thesis focused primarily on physiological biomarkers, our group has published previously on the merits of biochemical biomarkers. (545, 546, 547) Examining a large cohort of infants with perinatal asphyxia and subsequent HIE compared with healthy term controls, altered expression of micro RNA, specifically miR-374a-5p, miR-376c-3p and miR-181b-5p, was noted in the umbilical cord blood of infants with perinatal asphyxia and HIE. (548) In addition we have previously reported the metabolomic profiles of infants with HIE, and that a combination of clinical markers and cord alanine can improve prediction. (95, 549) As part of this study, post-natal blood samples were also collected from these infants. This thesis focused primarily on multi-modal physiological monitoring in HIE and as such, biomarker analysis was beyond the scope of this thesis. However, these post-natal samples will be used to explore post-natal expression of these biomarkers; both miRNA and candidate metabolite pathways over the first 48 hours and determine whether they may be useful in the early identification of infants with HIE.

6.4. Conclusion

This is the first study to combine all of these monitoring modalities in infants with all grades of HIE soon after birth. Multi-modal monitoring is feasible and this thesis has succeeded in providing novel insights into the underlying physiology and evolution of injury in infants with all grades of HIE. This study has provided numerous opportunities for future research and I personally look forward to continuing to answer the important questions and strive to ultimately improve the care and treatment that we provide to infants with HIE and their families.

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Appendices

A. MONItOr Ethical Approval

UCC
Tel: + 353-21-490 1901
Fax: + 353-21-490 1919
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee
Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Our Ref ECM 5 (5) 04/07/17

10th August 2017

Professor Eugene Dempsey
Consultant Neonatologist
Neonatal Unit
Ground Floor
Cork University Maternity Hospital
Wilton
Cork

Re: The Monitor Study: Multimodal assessment of newborns at risk of neonatal hypoxic ischaemic encephalopathy.

Dear Professor Dempsey

The Clinical Research Ethics Committee of the Cork Teaching Hospitals reviewed your correspondence at its recent meeting held on 9th August 2017.

The following documents have been approved:

- Cover letter dated 12th June 2017
- Application form signed 12th June 2017
- Study protocol version 1.0 dated 12th June 2017
- Parent information leaflet version 1.0 dated 12th June 2017: Correct page numbering prior to use
- Consent form version 1.0 dated 12th June 2017
- CV for chief investigator.

Permission is granted to begin this study.

The date of this letter is the date of authorization of the trial.

Please keep a copy of this signed approval letter in your study master file for audit purposes

Yours sincerely

Professor Michael G Molloy
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice

B. MONItOr Parent Information Leaflet for Infants with HIE

The MONItOr Study

Multimodal Assessment of Newborns at Risk of Neonatal Hypoxic Ischaemic Encephalopathy

Parent Information Leaflet

You are being invited to give your permission for your baby to take part in a clinical research study which will look at assessing infants who may be at risk of neonatal encephalopathy. Before you decide whether to participate, it is important for you to understand why this research is being done and what it will involve.

This process is known as informed consent. This leaflet gives detailed information about the research study, which will be discussed with you. Once you understand it fully, you will be asked to sign a consent form if you are happy for your baby to take part. A copy of this information leaflet will be given to you to keep.

Your baby's participation in this study is entirely voluntary. If you decide not to take part, your baby will continue to receive the high standard of care you would expect. You are free to withdraw your baby at any time, without giving a reason.

The rest of this leaflet explains the study in more detail and describes what the study will mean for you and your baby. Please take your time to decide. If anything is unclear, or if you would like more information, please feel free to ask any questions – we will be happy to answer them.

What is the study about?

Some babies require resuscitation when they don't cry or move after delivery. This may occur when a baby has had a difficult or stressful time during labour or delivery, associated with interference in the blood and oxygen delivery to the baby. Sometimes this can lead to what's known as hypoxic-ischaemic encephalopathy (HIE). HIE can affect all organs, but most importantly the brain and the heart.

At the moment, we have no single test to tell whether your baby has had a significant injury or not. By conducting a series of investigations, we are trying to establish the degree of the injury, if present, and the long-term impact on development so that we can intervene sooner and improve the outcome. By collecting blood samples, we are hoping to find chemical and protein markers which can tell us very soon after birth which babies have suffered a significant injury.

To assess the brain structure and activity, your baby will have EEG monitoring, brain oxygenation monitoring, continuous carbon dioxide (CO_2) measurements and magnetic resonance scans (MRI/MRS).

To assess the heart function, we are going to use non-invasive cardiac output monitoring and an echocardiogram. The study will be concluded with a standardised developmental assessment of your baby at approximately 18 to 24 months of age.

What will happen to my baby in this study?

During hospitalisation, babies will have blood tests done as part of their routine care after birth. Immediately after birth your baby had blood tests performed for their routine care. The research team also stored an extra 1 ml of blood at this time. This was done as it is important to look at very early biochemical markers of outcome, which change rapidly over time.

With your permission, we wish to store these blood samples for later analysis of biochemical components which may in the future help doctors to decide on the appropriate care of infants with HIE. If you do not wish to consent to the study, the 1ml of blood collected from your baby will be discarded.

Your baby's progress after birth will be recorded and additional tests will be performed after admission to the Neonatal Unit. These tests assess the activity and structure of the brain and heart and include electroencephalogram (EEG), near infrared spectroscopy (NIRS), non-invasive cardiac output monitoring (NICOM), echocardiogram (ECHO), continuous CO2 monitoring, magnetic resonance imaging and spectroscopy scans. Details of these tests are provided below.

What is Electroencephalogram (EEG)?

We would like to do an EEG, which is the best test ("gold standard") routinely performed in clinical practice to assess the brain activity in infants at risk of brain injury.

EEG measures tiny signals from the brain and can be recorded using small attachments to your baby's head (similar to those that record the heart activity from the chest).

These small attachments are connected to a machine by short wires which record the brain signals ("brain waves"). They take about 15 minutes to apply, with no discomfort, and after this they will be left in place for a minimum of 6 hours up to a maximum of 84 hours as clinically indicated.

Our EEG system also uses video to help us study movement patterns during sleep. This video recording is strictly confidential and will not be used for any other purposes.

What is Near Infrared Spectroscopy (NIRS)?

We would like to study the changes in the oxygen supply to your baby's brain.

NIRS is a widely used test that records the oxygen level in a baby's brain.

Similar to the EEG attachments, the NIRS probe (small disk) will also be applied to your baby's forehead and will be left in place for a minimum of 6 hours up to a maximum of 84 hours as clinically indicated.

What are Non-invasive Cardiac Output Monitoring (NICOM) and Echocardiogram (ECHO)?

To assess the heart function of your baby we are using a machine called a NICOM. This requires only sticker electrodes to be applied to your baby's skin. These stickers are connected to the NICOM machine by wires.

We will also perform up to two ultrasound examinations of the heart (ECHO). No adverse effects are expected as a result of both these tests.

What is transcutaneous CO2 monitoring?

Babies will be monitored with a transcutaneous carbon dioxide monitor, which is a heated sensor that measures carbon dioxide (CO_2) levels in the blood. This is an indicator of how well your baby is breathing.

The carbon dioxide is measured through the skin so that it is not necessary to keep taking blood from your baby to measure his/her carbon dioxide.

All these tests are harmless and painless to your baby.

The attachments used are very safe, non-invasive, and cannot damage your baby in any way as they do not send out any electrical signals. They are used routinely in Neonatal Intensive Care Units worldwide.

Your baby will have to be handled a little more than usual in order to place the attachments but we can assure you that we will apply these electrodes/monitors carefully and slowly to ensure that your baby is disturbed as little as possible.

What is Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS)?

As part of this research, your baby will have a MRI scan done to assess if there is any brain injury present. MRI is routinely used in clinical practice for infants with HIE.

We will also do a special scan called MRS, to detect any chemical changes in your baby's brain.

MRI and MRS are non-invasive and painless scans, with no side effects involved.

For both of these scans your baby will be placed in a special incubator with continuous monitoring and transported to the MRI scanner. The scans will take about 50 minutes to be completed.

Follow up?

A very important part of the study will be the assessment of your baby's long-term development and growth. The **developmental assessment** will be done using standardised neurodevelopmental tests appropriate for your child's age between **18** and **24 months** of age. We may also ask your child to play a simple game on a touchscreen device (BabyScreen App) which we are piloting as a method of assessing your baby's learning and problem solving ability. We would be grateful if you would allow us to contact you to arrange this follow up at the appropriate time.

Why is this study important?

This is the first study combining all these investigations in the same group of infants with hypoxic-ischaemic injury, trying to identify markers that predict the occurrence of adverse events and poor developmental outcome.

How many babies will take part in this research study?

Enrolment for this study will be done over approximately **18 months** and there will be approximately 100 babies in total enrolled.

Why has my baby been invited to take part?

The group that we would like to study are babies that are born after 36 weeks' gestation, with concerns of interrupted oxygen supply to the brain during delivery.

Are there any risks to my baby by entering the study?

No, there should not be any risk to your baby. **All the tests that will be performed are routine tests in the NICU. The research team will apply and perform the tests very carefully in order not to disturb your baby. In the neonatal unit in Cork University Maternity Hospital, we have been using these devices as part of clinical observational studies for a long time.**

Will I be allowed to see my baby during this investigation?

Of course, we will encourage you to see your baby as much as you can while your baby is in the Neonatal Care Unit.

Will my baby benefit from the study?

There may or may not be direct benefit to your baby from taking part in this study. We cannot promise that the study will benefit your baby but the information that we gather may improve the care of other babies in the future who have this problem.

What are my options?

We are very grateful to you for considering you and your child's participation in this study but we understand if you decide not to enroll. The decision to take part is completely up to you; you are under no obligation to participate. If you do decide to take part, you will be asked to sign a consent form and your child will be asked to sign an assent form. You will be given a copy of both to keep. If you decide to take part initially and then change your mind, you are free to withdraw your child from the study at any stage without giving any reason. This will not affect the medical care of you or your child at any point.

What happens to the information, recordings and samples collected?

By participating in this study, information from you and your child (also called "personal data") will be collected for the purposes mentioned above in this Parent Information Leaflet. This personal data includes, for example:

- Information that directly identifies you and your child (such as your name, and your year of birth);
- Information on you and your child's health and medical condition including your medical history;
- Information contained in the baby's routine blood samples and the results after analysis.

Will my baby's records be kept confidential?

To ensure confidentiality, the data generated during the study is **coded** with the unique Study ID Number that your child will be allocated once recruited to the MONItOr Study. Any information that leaves the clinical site will only be labelled with your child's ID Number. Every person that has access to your uncoded data (that is kept at Cork University Maternity Hospital) is subject to professional secrecy and confidentiality.

Data that directly identifies you or your child (uncoded data) is stored in a locked filing cabinet separate to study documentation. Only study personnel can match your child's name to the unique Study ID Number. This data will

solely be accessible to the study research team, the study Sponsor and their representatives to check if the study is conducted properly and that your rights are being respected. If this occurs all personal information made available for inspection will be handled in the strictest confidence and in accordance with legal data protection requirements. All information collected about your child will be kept private and confidential and will be stored in a secure web based database. The information will be pseudo-anonymised and any information that could identify your child **will not be stored** on the database.

What happens to information from the study?

University College Cork (UCC) is the study's Sponsor and will act as the data controller for this study. Any personal data which you provide to the University will be treated with the highest standards of security and confidentiality, in accordance with Irish and European Data Protection legislation.

Any personal data you provide to us during the course of this study will be processed fairly and lawfully. Signing the Informed Consent Form means that your personal data will be used for the purposes outlined in this Parent Information Leaflet (PIL).

The clinical site, the study investigators and the members of the study team will use your personal data within the scope defined above. The General Data Protection Regulation allows us to process your data because you have provided your consent. You have a right to request access to this information and to have a copy of it. You have the right to have any incorrect or inaccurate data deleted, unless this request makes it impossible or very hard to conduct the study.

You are entitled to withdraw your consent at any time. If you do withdraw your consent, we will keep your child's coded personal data collected prior to withdrawal, for processing along with other data collected as part of the study to preserve the integrity of the study.

Your personal information will be stored securely at the INFANT Centre. An anonymised database (where participants are assigned unique numbers and there is no identifiable information) will also be stored on the researcher's

password protected computers at UCC. All information management will adhere to the Data Protection Act and GDPR.

For how long will this data be kept?

We will keep identifiable information about your child from this study for at least 25 years after the study has concluded, as per UCC's General Disposal Authority 1 - Academic Administration: Research Document. We would like your permission to allow us to store your child's de-identified data and the recordings for future related research for a period of 25 years. Further research analyses may be performed but this will be subject to further ethics approval if applicable.

If you have any complaints in connection with our processing of your personal data, you can contact UCC's Data Protection Officer (DPO):

Office of Corporate & Legal Affairs,
University College Cork,
Western Road, Cork
E: foi@ucc.ie Tel: +353 21 4903949

You also have the right to lodge a complaint with the Data Protection Commission if you are unhappy with our processing of your personal data. Details of how to lodge a complaint can be found on the Data Protection Commission's website (www.dataprotection.ie), or by telephoning 1890 252 231.

What will happen to the results of this research study?

The MONItOr study will be managed by the staff of the Department of Paediatrics and Child Health, University College Cork. Results of this research study may be published in medical journals and presented at scientific meetings. Further research analyses may be performed but only by the study investigators and subject to further ethics approval if applicable.
Your baby's name or anything else identifiable to your baby will not be released or published. A copy of the signed informed consent form will be included in your baby's medical notes.

Who will access my/my child's personal data?

During or after the study, the study personnel, the sponsor representative and the Research Ethics Committee (REC) will be allowed access to your child's uncoded medical records, which identify him/her by name. This is so that they can check that the study is being carried out correctly and that your and your child's rights are being respected and also to ensure the accuracy of the information collected and registered.

Who has reviewed this study?

All research in Ireland is carefully reviewed by an independent group of people, called a Research Ethics Committee, to protect your baby's safety, rights, wellbeing and dignity. This study has been approved by the Cork Research Ethics Committee (CREC).

What will happen if I do not wish my baby to carry on in this study?

You are free to withdraw your baby at any time, without giving a reason. This will not affect the high level of care you will expect for your baby. We would however like your permission to use the information that has already been collected up to the point of withdrawal.

Thank you for taking the time to read this information leaflet. Should you require any further information please feel free to ask your doctor, named below:

Principal Investigator

Professor Eugene Dempsey, Consultant Neonatologist

Cork University Maternity Hospital, Wilton, Cork

021 4920525

C. MONItOr Consent Form for Infants with HIE

Consent Form

Multim**o**dal Assessment of **N**ewborns at R**i**sk of Ne**o**natal Hypoxic Ischaemic Encephalopathy –The **MONItOr** Study

Baby's Name	Baby's Study Number

Principal Investigator: Prof. Eugene Dempsey

Please **initial** box

1. I confirm that I have read and understood the Parent Information Leaflet. The study has also been explained to me and I have had the opportunity to ask questions and have had them answered satisfactorily. ☐
2. I understand that my baby's participation in this study is entirely voluntary. I am free to withdraw my baby from the study at any time, without giving any reason, and without the medical care or legal rights of my baby being affected. ☐
3. I agree that if I choose to withdraw my baby from the study that I will give the researchers involved permission to keep and use data or samples collected before this withdrawal. I understand that I can withdraw this permission at any time. ☐
4. I understand that sections of my baby's and my medical notes and data collected may be looked at by research staff involved in the study or the Ethics Committee. This will allow them to check that the study has been run safely. I give permission for these individuals to have access to my and my baby's records. ☐
5. I consent that anonymised data collected for this study may be used, now or in the future, for research analysis without my further permission, but subject to approval by a Research Ethics Committee ☐
6. I agree that blood samples can be collected from my baby and stored for future analysis to assess biochemical markers for hypoxic-ischemic injury . ☐
7. I agree to my baby having Electroencephalogram (EEG), transcutaneous CO2, Near Infrared Spectrometry (NIRS), Non-invasive cardiac output (NICOM) monitoring performed. ☐
8. I agree for my baby to have echocardiography and a MRI/MRS scan as part of this study. ☐
9. I agree to my contact details being retained by the research personnel and that I can be contacted in the future to arrange for my baby to have neurodevelopmental follow up. ☐
10. I agree to my baby's GP being informed of my baby's part in this research study. ☐

Parent(s)/Guardian(s) Name *print*	Date	Signature
Parent(s)/Guardian(s) Name *print*	Date	Signature
Name of Researcher/Informant *print*	Date	Signature
Name of Witness (if required)	Date	Signature

Version 4.0 16th JUL 2018

1 copy to parent(s); 1 Copy to Investigator Site File; 1 Copy (scanned) to baby's notes

D. MONItOr Parent Information Leaflet for Control Infants

The MONItOr Study

Multimodal Assessment of Newborns at Risk of Neonatal Hypoxic Ischaemic Encephalopathy

Parent Information Leaflet for Control Group (Post Natal Ward)

You are being invited to give your permission for your baby to take part in a clinical research study. Before you decide whether to participate, it is important for you to understand why this research is being done and what it will involve.

This process is known as informed consent. This leaflet gives detailed information about the research study, which will be discussed with you. Once you understand it fully, you will be asked to sign a consent form if you are happy for your baby to take part. A copy of this information leaflet will be given to you to keep.

Your baby's participation in this study is entirely voluntary. If you decide not to take part, your baby will continue to receive the high standard of care you would expect. You are free to withdraw your baby at any time, without giving a reason.

The rest of this leaflet explains the study in more detail and describes what the study will mean for you and your baby. Please take your time to decide. If anything is unclear, or if you would like more information, please feel free to ask any questions – we will be happy to answer them.

What is the MONItOr study?

The MONItOr study is a research study currently taking place in this hospital looking at infants who need resuscitation after birth. This happens in about 2% of deliveries. Some of these infants may be at risk of long term problems and need intervention in the first few days of life. At the moment, we have no good reliable test which can give us this information straight after birth. Through this study, we hope to develop a blood test which can tell us very soon after birth which babies have suffered significant injury so that we can intervene and improve their outcome. We hope that in the future, a simple blood test at delivery will be able to give doctors and parents more information within hours of a baby's birth.

In some of these infants, heart and brain function is also affected. We assess the heart of these babies with a test called non-invasive cardiac output monitoring (NICOM) and also perform an ultrasound of the heart, called an echocardiogram (ECHO). All of these tests are painless and have no adverse effects.

In order to help babies who have difficulties after birth, it is also important for us to study babies who have had normal, uneventful deliveries so that we can compare their blood samples and heart function directly to those of babies who did have difficulties. These babies act as **controls** and we compare them to infants who are unwell. We are asking for your baby to be a control infant for the study because your baby had an uneventful, normal delivery. Enrolment for this study will be done over approximately 18 months and there will be approximately 40 babies enrolled in the control group of this study.

What will happen to my baby in this study?

Your baby's progress after birth will be recorded along with relevant information from your medical notes regarding your pregnancy, labour and delivery details. Information about your baby's progress will be documented in your baby's medical notes and will be recorded for this study. All information

collected about you and your baby during the study will be pseudoanonymised; your baby will only be identified using a unique study number, not your baby's name or address.

With your permission, we will assess the heart function of your baby, in order to gain data on normal heart function. We are assessing the heart with non-invasive cardiac output monitoring, for which we are using a machine called NICOM. This requires 4 sticker electrodes to be applied to your baby's skin. These stickers are connected to the NICOM machine by wires. This is a painless procedure and will not cause your baby any discomfort. We will also perform an ultrasound examination of your baby's heart (ECHO).

If you are happy for your baby to have a heel-prick test as part of the study, we will take 0.09mls (less than 1 fiftieth of a teaspoon) of blood. Some of this blood sample will be analysed immediately for levels of certain blood components and the rest will be stored as a dried blood spot. We would like your permission to store this dried blood spot so that later we can measure chemicals and proteins in the blood sample to allow us to compare their levels to the levels of babies who need resuscitation at birth.

As part of the study, we will ask you to meet us again when your baby is 18 - 24 months of age so that we can record their progress and development. You will be contacted and an appointment will be made for a follow up assessment at a time convenient for you and your family. This visit will happen in Cork University Hospital. This follow up appointment will take approximately 1.5 hours, during which time your baby's development will be assessed using standardized neurodevelopmental tests appropriate for your child's age and results will be discussed with you. We will also ask your child to play a simple 10-15 minute puzzle on a touchscreen device (BabyScreen App) which we are piloting as a method of assessing your baby's learning and problem solving ability.

You can agree to take part in some or all parts of the study and your permission will be recorded on your consent form.

What are my options?

We are very grateful to you for considering you and your child's participation in this study but we understand if you decide not to enroll. The decision to take part is completely up to you; you are under no obligation to participate. If you do decide to take part, you will be asked to sign a consent form. You will be given a copy to keep. If you decide to take part initially and then change your mind, you are free to withdraw your child from the study at any stage without giving any reason. This will not affect the medical care of you or your child at any point.

Are there any risks to my baby by entering this study?

No, there should not be any risk to your baby. All the tests that will be performed are routine tests. The research team will apply and perform the tests very carefully in order not to disturb your baby. In the neonatal unit in Cork University Maternity Hospital, we have been using these devices as part of clinical observational studies for a long time.

What happens to the information, recordings and samples collected?

By participating in this study, information from you and your child (also called "personal data") will be collected for the purposes mentioned above in this Parent Information Leaflet. This personal data includes, for example:

- Information that directly identifies you and your child (such as your name, and your year of birth);
- Information on you and your child's health and medical condition including your medical history;
- Information contained in the baby's routine blood samples and the results after analysis.

Will my baby's records be kept confidential?

To ensure confidentiality, the data generated during the study is **coded** with the unique Study ID Number that your child will be allocated once recruited to the MONItOr Study. Any information that leaves the clinical site will only be labelled with your child's ID Number. Every person that has access to your uncoded data (that is kept at Cork University Maternity Hospital) is subject to professional secrecy and confidentiality.

Data that directly identifies you or your child (uncoded data) is stored in a locked filing cabinet separate to study documentation. Only study personnel can match your child's name to the unique Study ID Number. This data will solely be accessible to the study research team, the study Sponsor and their representatives to check if the study is conducted properly and that your rights are being respected. If this occurs all personal information made available for inspection will be handled in the strictest confidence and in accordance with legal data protection requirements. All information collected about your child will be kept private and confidential and will be stored in a secure web based database. The information will be pseudo-anonymised and any information that could identify your child **will not be stored** on the database.

What happens to information from the study?

University College Cork (UCC) is the study's Sponsor and will act as the data controller for this study. Any personal data which you provide to the University will be treated with the highest standards of security and confidentiality, in accordance with Irish and European Data Protection legislation.

Any personal data you provide to us during the course of this study will be processed fairly and lawfully. Signing the Informed Consent Form means that your personal data will be used for the purposes outlined in this Parent Information Leaflet (PIL).

The clinical site, the study investigators and the members of the study team will use your personal data within the scope defined above. The General Data Protection Regulation allows us to process your data because you have provided your consent. You have a right to request access to this information and to have a copy of it. You have the right to have any incorrect or inaccurate data deleted, unless this request makes it impossible or very hard to conduct the study.

You are entitled to withdraw your consent at any time. If you do withdraw your consent, we will keep your child's coded personal data collected prior to withdrawal, for processing along with other data collected as part of the study to preserve the integrity of the study.
Your personal information will be stored securely at the INFANT Centre. An anonymised database (where participants are assigned unique numbers and there is no identifiable information) will also be stored on the researcher's password protected computers at UCC. All information management will adhere to the Data Protection Act and GDPR.

Who will access my/my child's personal data?

During or after the study, the study personnel, the sponsor representative and the Research Ethics Committee (REC) will be allowed access to your child's uncoded medical records, which identify him/her by name. This is so that they can check that the study is being carried out correctly and that your and your child's rights are being respected and also to ensure the accuracy of the information collected and registered.

For how long will this data be kept?

We will keep identifiable information about your child from this study for at least 25 years after the study has concluded, as per UCC's General Disposal Authority 1 - Academic Administration: Research Document. We would like your permission to allow us to store your child's de-identified data and the recordings for future related research for a period of 25 years. Further research analyses may be performed but this will be subject to further ethics approval if applicable.

If you have any complaints in connection with our processing of your personal data, you can contact UCC's Data Protection Officer (DPO):

Office of Corporate & Legal Affairs,
University College Cork,
Western Road, Cork
E: foi@ucc.ie Tel: +353 21 4903949

You also have the right to lodge a complaint with the Data Protection Commission if you are unhappy with our processing of your personal data. Details of how to lodge a complaint can be found on the Data Protection Commission's website (www.dataprotection.ie), or by telephoning 1890 252 231.

What will happen to the results of this research study?

The MONItOr study will be managed by the staff of the Department of Paediatrics and Child Health, University College Cork. Results of this research study may be published in medical journals and presented at scientific meetings. Further research analyses may be performed but only by the study investigators and subject to further ethics approval if applicable.
Your baby's name or anything else identifiable to your baby will not be released or published. A copy of the signed informed consent form will be included in your baby's medical notes.

Who has reviewed this study?

All research in Ireland is carefully reviewed by an independent group of people, called a Research Ethics Committee, to protect your baby's safety, rights, wellbeing and dignity. This study has been approved by the Cork Research Ethics Committee (CREC).

Thank you for taking the time to read this information leaflet. Should you require any further information please feel free to ask your doctor, named below:

Principal Investigator

Professor Eugene Dempsey,

Consultant Neonatologist

Cork University Maternity Hospital,

Wilton,

Cork

Tel: 021 4920525

E. MONItOr Consent form for Control Infants

Consent Form for Control Group (Post-Natal Ward)

Multimodal Assessment of Newborns at Risk of Neonatal Hypoxic Ischaemic Encephalopathy –The **MONItOr** Study

Baby's Name	Baby's Study Number

Principal Investigator: Prof. Eugene Dempsey

Please **initial** box

1. I confirm that I have read and understood the **Parent Information Leaflet for the Control Group (Post-Natal Ward)**. The study has also been explained to me and I have had the opportunity to ask questions and have had them answered satisfactorily. .I understand why the research is being done and any risks involved. ☐
2. I understand that my baby's participation in this study is entirely voluntary. In the event that I withdraw my child from the study, I agree that the coded personal data collected prior to withdrawal may still be processed along with other data collected as part of the study to preserve the integrity of the study. I am free to withdraw my baby from the study at any time, without giving any reason, and without the medical care or legal rights of my baby being affected. ☐
3. I agree that if I choose to withdraw my baby from the study that I will give the researchers involved permission to keep and use data or samples collected before this withdrawal. I understand that I can withdraw this permission at any time. ☐
4. I give permission for my/my child's medical records to be reviewed and information to be taken from them to be analysed in confidence by the study team. ☐
5. I give permission for all anonymised information collected from my child to be used now or stored for possible future related research *without my further consent being required* but subject to approval of a Research Ethics Committee. ☐
6. I agree that blood samples can be collected from my baby and that these may be stored for future analysis directly related to this study. ☐
7. I agree for my baby to have non-invasive cardiac output (NICOM) monitoring and echocardiography performed. ☐
8. I understand that the sponsors and Investigators have such insurance as is required by law in the event of injury resulting from this research. ☐
9. I am aware that confidentiality of records concerning my own and my child's involvement in this study will be maintained according to national and EU Data Protection Laws. When required by law, the records of this study may be reviewed by government agencies, ethics committee and sponsors of the study. ☐
10. I agree to my contact details being retained by the research personnel and that I can be contacted in the future to arrange for my baby to have neurodevelopmental follow up. ☐
11. I agree to my baby's GP being informed of my baby's part in this research study. ☐

Version 1.0 12 AUG 2019

1 copy to parent(s); 1 Copy to Investigator Site File; 1 Copy (scanned) to baby's notes

Consent Form for Control Group (Post-Natal Ward)

Multimodal Assessment of Newborns at Risk of Neonatal Hypoxic Ischaemic Encephalopathy –The **MONItOr** Study

_____	_____	_____
Parent(s)/Guardian(s) Name *print*	Date	Signature
_____	_____	_____
Parent(s)/Guardian(s) Name *print*	Date	Signature
_____	_____	_____
Name of Researcher/Informant *print*	Date	Signature
_____	_____	_____
Name of Witness (if required)	Date	Signature

Version 1.0 12 AUG 2019

1 copy to parent(s); 1 Copy to Investigator Site File; 1 Copy (scanned) to baby's notes

F. Modified Sarnat Score

Stage	Normal	Mild Stage 1	Moderate/Stage 2	Severe/Stage 3
1. Level of Consciousness	Normal	Hyper-alert/Irritable	Lethargic/Obtunded	Stupor/Coma
2. Spontaneous Activity	Normal	Normal	Decreased	Absent
3. Muscle Tone	Normal	Normal	Mild Hypotonia	Flaccid
4. Posture	Normal	Mild Distal Flexion	Strong Distal Flexion	Decerebrate
5. Primitive Reflexes				
Suck	Normal	Weak	Weak/Absent	Absent
Moro	Normal	Strong/Low Threshold	Weak/Incomplete/ High Threshold	Absent
6. Autonomic Function				
Pupils	Normal	Mydriasis	Miosis	Unequal/Fixed/ Dilated/Poor Reflex
Heart Rate	Normal	Tachycardia	Bradycardia	Variable
Respirations	Normal	Normal	Periodic Breathing	Apnea

G. International Prospective Register of Systematic Reviews

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

Citation

Aisling Garvey, Andreea Pavel, Deirdre Murray, Geraldine Boylan, Eugene Dempsey. Does early (<6 hours of age) near-infrared spectroscopy (NIRS) monitoring in neonates with hypoxic-ischaemic encephalopathy (HIE) predict outcome? a systematic review and meta-analysis. PROSPERO 2019 CRD42019127907 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019127907

Review question

To determine whether early Near-infrared Spectroscopy (NIRS) monitoring (<6 hours of age) can predict neonatal outcome as defined by 1. Grade of Encephalopathy (defined clinically using Thompson or Sarnat score, or electrophysiologically as mild, moderate or severe) 2. Brain MRI findings 3. Neurodevelopmental Outcome at 1-2 years of age.

Searches

Sources to be searched:

• PubMed/MEDLINE

• Web of Science

• Scopus

• The Cochrane Library

• Embase

The following terms will be used for the PubMed Database and adapted for the above databases.

1. Neonatal:

("infant, newborn"[MeSH Terms] OR ("infant"[All Fields] AND "newborn"[All Fields]) OR "newborn infant"[All Fields] OR "newborn"[All Fields]) OR ("infant"[MeSH Terms] OR "infant"[All Fields]) OR ("infant, newborn"[MeSH Terms] OR ("infant"[All Fields] AND "newborn"[All Fields]) OR "newborn infant"[All Fields] OR "neonate"[All Fields])

2. HIE:

"hypoxia ischaemia" OR "hypoxic ischemia" OR "hypoxic ischaemia" OR "hypoxic ischemic" OR "hypoxic ischaemic" OR "HIE" OR "asphyxia*" OR “anoxia” [MeSH] OR "encephalopathy" OR "asphyxia neonatorum"[MeSH] OR "Hypoxia--?Ischemia, Brain"[MeSH] OR “neonatal encephalopathy”

3. NIRS:

“spectroscopy, near-infrared”[MeSH]; OR (“INVOS* or EQUANOX* or FORESIGHT* or FORE-SIGHT* or CerOX* or Cer-OX* or IN-Spectra or INSpectra* or Niro*”) OR “NIRS*” OR “near IR spectroscop*” OR “near* infrared* spectroscop*” OR “(cerebr* or regional* or tissue* or brain*) (oxymet* or oximet*)” OR “(cerebr* or regional* or tissue* or brain*) (oxygen* or oxygen*)” OR “StO2 or rscO2 or rSO2 or SctO2” OR “FTOE”

Databases will be searched for articles published in the English language over the last 30 years (1988 to 2018). All studies will be indexed in EndNote.

Types of study to be included
Retrospective and prospective observational studies, randomised and quasi-randomised controlled trials.

Condition or domain being studied
Globally, it is estimated that 1 million babies die each year from HIE, and for each baby that dies from HIE, another will survive with significant disability. HIE is diagnosed based on clinical exam, Apgar scores and level of acidosis. Clinical examination is subjective and based on a guideline that was developed for examining babies at 24hours of life.

Therapeutic hypothermia (TH) is an effective treatment, but must be commenced within 6 hours of delivery. In this narrow time frame, the population who would benefit from treatment must be identified, stabilised and commenced on TH. TH has been studied only for moderate-severe HIE based on trials which excluded mild grades due to their perceived low level of disability. However, recent studies have now shown that up to 30% of babies with mild HIE have significant disability at 2 years and at 5 years, their cognitive outcome is similar to those children with moderate grade HIE at birth. Early biomarkers are necessary to identify infants who may benefit from therapeutic intervention.

Participants/population
Infants born ?36+0 weeks gestation with a clinical or electroencephalographic (EEG) diagnosis of Hypoxic Ischaemic Encephalopathy (all grades) who have had NIRS monitoring commenced within 6 hours of life.

Intervention(s), exposure(s)
Infants with Hypoxic Ischaemic Encephalopathy defined clinically by Thompson or Sarnat scoring, or electrophysiologically using amplitude-integrated EEG (aEEG) or continuous EEG (cEEG).

Comparator(s)/control
Not applicable

Context
All studies performed in a hospital setting.

Main outcome(s)
Main outcome(s)

1. Thompson/Sarnat standardised clinical score on admission, at 24 hours of life, at 48 hours and at discharge from hospital.

2. Death

3. MRI in the first week of life.

4. Standardised Neurodevelopmental Outcome at 1-2 years and at 5 years of age.

Additional outcome(s)
Not applicable

Data extraction (selection and coding)
A literature search will be performed by two independent reviewers (AG and AMP) to ensure a complete search of the literature. Following the literature search, all papers will be indexed to EndNote. Titles and abstracts will be reviewed to reject duplicate papers and papers that are not relevant to our research question. Publications from the same study population will be linked and the publication with more robust outcome data will be chosen. A second phase of screening will assess the papers for inclusion/exclusion criteria. All remaining papers will be reviewed by two independent reviewers (AG and AMP). Any discrepancies with the authors will be resolved by a third reviewer (ED). Two reviewers will independently complete the full data extraction form and risk of bias assessment.

The following data will be extracted from all the included studies:

- Authors, year and journal of publication

- Study type: observational cohort study or clinical randomized or quasi-randomised controlled trials

- Study population with demographics

- Assessment method of HIE diagnosis and severity

- Therapeutic hypothermia or not; other treatments used.

- Physiological monitoring performed before 6 hours of age (NIRS)

- Brain MRI assessment

- Short and long term outcomes, including mortality.

Authors will be contacted to request any missing data necessary.

Inclusion criteria:

1. Observational cohort study or clinical randomized or quasi-randomised controlled trials

2. Human study

3. Newborn infants born at ?36 weeks gestational age

4. Neonatal HIE defined clinically, using Thompson or Sarnat scoring, AND/OR electrophysiologically, using amplitude-integrated (aEEG) or continuous EEG (cEEG)

5. NIRS monitoring commenced before 6 hours of age

Exclusion criteria:

1. HIE diagnosis and grade not described

2. No monitoring performed before 6 hours of age

Risk of bias (quality) assessment

Studies will be critically appraised using The Joanna Briggs Institute Critical Appraisal Tools for cohort, randomised and quasi-randomised studies.

Strategy for data synthesis

The systematic review will include a narrative of all the studies included, detailing the early findings of NIRS monitoring.

If the studies are similar enough clinically and methodologically, the results of the studies will be pooled in a meta-analysis. Data from each study will be summarised in a 2X2 table (true-positives, false-positives, false-negatives and true negatives) and the sensitivity and specificity for each study calculated. A coupled forest plot displaying the sensitivity and specificity and corresponding 95% CIs for each study and a summary plot displaying the sensitivity-specificity point for each study will be created. Heterogeneity among studies will be investigated by visual inspection of these plots. If no substantial heterogeneity is observed, pooled sensitivity and specificity estimates and corresponding 95% CIs will be calculated for studies with a common threshold using the bivariate random effects regression model or the univariate random effects model (if the number of studies is small). If different thresholds are reported in the individual studies, a hierarchical summary receiver operating characteristic (HSROC) curve will be created and a global summary of test accuracy calculated. The analysis will be performed using Stata (version 13.0, StataCorp LP, College Station, TX, USA).

Analysis of subgroups or subsets
HIE grade: mild, moderate, severe

HIE treatment: cooled, uncooled

HIE outcome: MRI in first 2 weeks of life

Neurodevelopmental outcome at 1 to 2 years

Contact details for further information
Aisling Garvey
aisling.garvey@ucc.ie

Organisational affiliation of the review
INFANT Centre
infantcentre.ie

Review team members and their organisational affiliations
Dr Aisling Garvey. INFANT Centre
Dr Andreea Pavel. INFANT Centre
Professor Deirdre Murray. INFANT Centre
Professor Geraldine Boylan. INFANT Centre
Professor Eugene Dempsey. INFANT Centre

Type and method of review
Systematic review

Anticipated or actual start date
18 March 2019

Anticipated completion date
15 April 2019

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National Childrens Research Centre, Dublin

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Conflicts of interest
Yes

Language
English

Country
Ireland

Stage of review
Review Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Electroencephalography; Humans; Hypothermia, Induced; Hypoxia-Ischemia, Brain; Infant, Newborn; Spectroscopy, Near-Infrared

Date of registration in PROSPERO
09 April 2019

Date of first submission
08 March 2019

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	Yes	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.
The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions
09 April 2019