Early prediction of hypoxic ischaemic encephalopathy in newborn infants in a resource-limited setting

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Declaration

I, Alan Richard Horn, hereby declare that this thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisors and contributions from others as outlined in the acknowledgements. The assistance I received with study management, data collection and manuscript review from the co-authors of the publications that form part of this thesis is described at the beginning of each relevant chapter.

Neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other university.

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Abstract

Hypoxic ischaemic encephalopathy (HIE) after birth is an important cause of neonatal morbidity and mortality, particularly in resource-limited regions. Therapeutic hypothermia initiated within the first 6 hours of life, in settings that can offer neonatal intensive care, is a therapy that can reduce death or severe disability in newborn infants with moderate or severe HIE. Therapeutic hypothermia has not been shown to be safe or effective in low-resource settings where neonatal intensive care is not available; however, there are situations such as in some centres in South Africa, where limited neonatal intensive care (NICU) is available against a background of moderate neonatal mortality rates, relatively low socio-economic conditions and limited capacity for long-term follow-up. In such settings, accurate case definition and early prediction of HIE and outcome may assist with the appropriate allocation of resources. The amplitude-integrated electro-encephalogram (aEEG) is an ideal tool to use for prediction of outcome and the need for cooling, but it's availability is limited, particularly at primary and secondary hospitals.

Clinicians and health administrators in resource-limited areas are faced with the following issues:

(i) How predictive of moderate-severe HIE are the commonly used bedside predictors?

(ii) What is the incidence of HIE and how should it be defined?

(iii) Is therapeutic hypothermia feasible in terms of practicality, affordability and expertise?

(iv) How can infants with moderate-severe HIE be identified early enough to benefit from hypothermia?

(v) Can infants who will not recover, despite hypothermia, be identified early?

This thesis examines these issues in the Southern Cape Peninsula of South Africa and analyses new data that assist with planning medical management, the prediction of outcome, benchmarking, and the design of future studies in similar settings. When the research commenced, therapeutic hypothermia was already an established standard of care in the region. At the time, the basic method used was servo-controlled cooling with gel-packs, but a formal assessment of the core temperature profile of this method was required.

Chapter 1 presents the rationale and outline of the thesis. The burden of disease of HIE is described in the context of different resource settings around the world with detailed reference to South Africa and the setting of the research in the Southern Cape Peninsula. Chapter 2 presents a summary and discussion of the literature relevant to the research questions and methods. Chapter 3 is a systematic literature review of published studies of clinical or serum predictors of moderate-severe HIE. Subsequently the thesis includes four main related themes of work that are presented as separate journal publications. The first theme (Chapter 4) is a retrospective folder review investigating how the incidence of HIE in our region can vary with regard to the differing criteria for intrapartum hypoxia and determining how the severity of encephalopathy varies with different grading systems. The second theme (Chapter 5) is a case series study evaluating the basic method of cooling used in the region. This evaluation facilitates the reliable interpretation of the subsequent short-term outcomes of infants managed in this way. The third theme (Chapter 6) is a prospective cohort study of 60 infants with HIE, that determines whether early clinical signs can identify infants who have an abnormal aEEG at 6 hours and who may therefore benefit from therapeutic hypothermia. The fourth theme (Chapter 7) describes a subgroup analysis of cooled infants and determines whether specific early clinical signs can assist in identifying infants with moderate-severe HIE who are so severely affected that treatment is futile. Our outcome marker was a persistently severely abnormal aEEG background at 48 hours.

The systematic literature review found a high risk of bias in the studies identifying clinically applicable predictors of moderate-severe HIE. The addition of clinical assessment to serum predictors increased prediction specificity, but the most useful studies defined diagnostic thresholds that still required prospective validation. The retrospective folder review showed that the incidence of HIE varied more than two-fold in the same population, depending on which defining criteria were used; the incidence of moderate-severe HIE varied from 1.5–3.7 per 1 000 live births. Our cooling method achieved a mean rectal temperature of 33.9 ± 0.3 °C throughout cooling; a core temperature of ≤ 34 °C was achieved within a median time of 45 minutes. The prospective cohort study found that an abnormal 6-hour aEEG frequently occurs in the absence of specific individual clinical signs of moderate HIE. However, a receiver operating characteristic curve analysis of the Thompson scores showed that a score of ≥ 7 at age 3–5 hours is a sensitive predictor of either an abnormal 6-hour aEEG or moderate-severe encephalopathy presenting within 72 hours of birth. The subgroup analysis found that a Thompson score of ≥ 16,

assessed at 3–5 hours, identified a sub-group of infants who showed a persistently severely abnormal aEEG at 48 hours despite cooling with high specificity but low sensitivity.

In Chapter 8, the thesis concludes that consensus definitions of HIE are lacking; such consensus definitions are vital for benchmarking and comparing regions and interventions accurately. The incidence of HIE in the Southern Cape Peninsula is lower than expected compared to the neonatal mortality rate; these discrepancies are likely to have been influenced by the definitions that were used. The servo-controlled gel-pack cooling method used during the prospective clinical study achieved acceptable core temperatures and was feasible. The results of the prospective study suggest that the Thompson score may be an appropriate tool to assist with triaging, selecting and excluding of infants for therapeutic hypothermia. However, the predictive threshold Thompson scores must be validated as pre-specified thresholds in prospective studies before they can be reliably applied to clinical practice.

This thesis describes the only large, systematic study of infants with HIE in such a setting that includes aEEG analysis and population-based data; these data have relevance to the application of therapeutic hypothermia in both high- and low-resource settings. These studies demonstrate the critical importance of clear and consistent criteria for diagnosing HIE, and outline some of the challenges that need to be overcome for optimising neuroprotective therapies now and in the future.

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I would like to acknowledge and thank the following people: Prof David Woods, Prof Atties Malan, Prof Vincent Harrison and Prof Clarissa Pieper, for encouraging me in the direction of this research; Dr Ilse Els for her encouragement and support; Prof Denis Azzopardi and Prof John Wyatt, for insightful comments in the very early stages; Dr Clare Thompson for advice regarding the use of the Thompson HIE score; Dr Alida Nel for assistance with patient recruitment; Ms Nicola Maxwell for assistance with creating the database; Mr Clinton Johnson for his assistance with the sourcing of aEEG monitors and for providing some of the training on those devices; Ms Lauren Lewis for her very efficient production of the maps of the Western Cape; Mr Henri Carrara for his patience and insight while guiding me through the process of some aspects of statistical analysis and the use of STATA in general; Ms Gabeba Abass for administrative and morale support; Dr Michael Harrison, Dr Lucy Linley, Dr Candice Nelson, Dr Moegammad Raban, Dr Yaseen Joolay, Dr Natasha Rhoda, and Dr Lloyd Tooke for their morale support, their assistance with patient care and data collection, and their comments on the manuscripts arising from this research; Dr Manigandan Chandrasekaran for his role in the blinded aEEG assessments and his comments on the relevant manuscripts; the nurses and registrars at the research sites who assisted with the care and assessment of the neonates; and the parents and newborns for participating in this research.

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Abbreviations

ACOG	American College of Obstetricians and Gynecologists
ADP	Adenosine diphosphate
aEEG	Amplitude-integrated electroencephalogram
ATP	Adenosine triphosphate
BS	Burst-suppression
CI	Confidence interval
CLV	Continuous low voltage
CNV	Continuous normal voltage
CP	Cerebral palsy
CPAP	Continuous positive airway pressure;
CTG	Cardiotocograph
DNV	Discontinuous normal voltage
DOR	Diagnostic odds ratio
EEG	Electroencephalogram
EPP	Exchangeable phosphate pool
FT	Flat trace
GSH	Groote Schuur Hospital
HIE	Hypoxic ischemic encephalopathy
ICE	Infant Cooling Evaluation
ICPTF	International Cerebral Palsy Task Force
ILCOR	International Liaison Committee on Resuscitation
LR	Likelihood ratio
MMH	Mowbray Maternity Hospital
MOU	Midwife Obstetric Unit
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MSEG	Modified Sarnat encephalopathy grade
NE	Neonatal encephalopathy
NICE	National Institute for Health and Clinical Excellence
NICHD	National Institute of Child Health and Human Development
NICU	Neonatal intensive care unit
NMR	Neonatal mortality rates
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase

NSH	New Somerset Hospital
NTP	Nucleotide triphosphate
OR	Odds ratio
PCr	Phosphocreatine
Pi	Inorganic phosphate
PMNS	Peninsula Maternal and Neonatal Service
PPV	Positive predictive value
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
QUADAS-2	Revised quality assessment tool for diagnostic accuracy studies
ROC	Receiver operating characteristic
RR	Risk ratio
SD	Standard deviation
SWC	Sleep wake cycling
WHO	World Health Organisation

Chapter 1 Introduction

1.1 Problem statement and rationale

Hypoxic ischemic encephalopathy (HIE) is a potentially preventable condition of the newborn infant that can result in death or disability in 60–70% of infants with moderate or severe (moderate-severe) encephalopathy [1-4]. Neonatal encephalopathy (NE) is a non-specific term describing the clinical manifestation of disordered brain function in term infants during the early neonatal period [5]. The etiology of NE includes genetic, congenital, neurological, metabolic, traumatic, hypoxic and infective conditions. The disorder is termed HIE if intrapartum hypoxia is the presumptive cause of the encephalopathy [6, 7].

Clinical trials carried out in high-resource settings with a sophisticated level of neonatal intensive care (NICU) have shown that therapeutic hypothermia prevents death or severe disability in one of every six to seven infants with moderate-severe HIE [4]. However, the incidence of HIE is highest in the regions with the least resources [8]. Therapeutic hypothermia has not been shown to be safe or effective in low-resource settings where NICU is not available and significant challenges surrounding the provision of basic neonatal care in these settings need to be addressed before any neuroprotective therapy can be recommended [9]. South Africa, however, is classified by the World Bank as an upper middle income developing country [10]: it can be considered a “mid-resource” setting. In parts of South Africa, such as the Southern Cape Peninsula, there are NICUs that can provide the level of intensive care required for therapeutic hypothermia. However the limited budget and limited bed capacity present difficulties in ensuring that the resources are appropriately used. In order for hypothermia to be most effective, it should be commenced before the age of 6 hours [11]. Early identification of infants with moderate-severe HIE is therefore crucial and uniform case definitions of HIE will allow meaningful epidemiological comparisons between populations.

The key challenges include:

(i) identification of infants with moderate-severe HIE using existing bedside predictors;

(ii) benchmarking the incidence of HIE according to various accepted diagnostic criteria for HIE;

(iii) determining whether a basic inexpensive method of inducing and maintaining therapeutic hypothermia is feasible in this setting;

(iv) identification of infants with moderate-severe HIE early enough to benefit from hypothermia; and

(v) identification of infants who are so severely affected that they will not have a normal outcome, despite treatment with hypothermia.

An important additional challenge in the Southern Cape Peninsula of South Africa, is that a high proportion of mothers travel from other provinces (particularly from the Eastern Cape) and are temporarily resident in peri-urban regions of Cape Town around the time of the birth of their infants: the mothers believe that they will receive superior perinatal care in the Peninsula and most return to their hometowns after the birth of their infants [12]. This transient migratory pattern creates significant difficulties in obtaining sufficient long-term follow-up data.

The amplitude-integrated electroencephalogram (aEEG) is a tool that could be used as a proxy for long-term outcome. The aEEG predicts neurological outcome with high sensitivity and specificity at 3 and 6 hours [13]. At age 6 hours, it can determine eligibility for hypothermia [14-16] and at age 48 hours, it identifies infants who will have a poor outcome, despite therapeutic hypothermia [17]. However, an aEEG is expensive, requires technical expertise and is unlikely to be available in all resource-limited settings, particularly in primary health care settings where referral of infants takes place. If early clinical signs can be correlated with subsequent aEEG findings, they may provide a means of identifying infants with moderate-severe HIE sufficiently early to enable them to benefit from hypothermia. Similarly, correlation of early clinical signs with severely abnormal aEEG at 48 hours may provide a means of identifying infants who will have a poor outcome, despite receiving therapeutic hypothermia.

1.2 Context

1.2.1 The burden of disease of hypoxic ischemic encephalopathy and the need for benchmarking

Throughout the world, HIE is recognised as an important cause of neonatal morbidity and mortality [8, 18]. Globally, the proportion of child deaths occurring during the neonatal period, increased to over 41% during the last decade [19]. The interpretation of data from studies of HIE incidence is hindered by the lack of uniform criteria and accurate up-to-date population data.

A recent review [20] cites old data from three population studies [21-23] in the United Kingdom, Australia and Sweden between 1991 and 1996. The pooled incidence of HIE was estimated at 1.5 per 1 000 live births, but different definitions of HIE were used in each study.

The International Cerebral Palsy Task Force (ICPTF) and the American College of Obstetricians and Gynecologists (ACOG) require blood gas values or multiple intrapartum anomalies as evidence of intrapartum hypoxia [24, 25]; these data are frequently not available in resource-limited settings [8, 26]. Although these criteria may be important in the medico-legal setting, from an epidemiological perspective, the actual incidence of HIE may be underestimated if such stringent criteria are required. In a recent review of worldwide neonatal mortality rates (NMR) and NE rates, Lawn et al. used the term “intrapartum-related impairment” to describe impaired survivors following birth asphyxia [8]. They described five categories of NMR, ranging from Category One with very low mortality (≤ 5 per 1 000 live births) and a NE incidence of 1.9 per 1 000 live births, to Category Five with very high mortality (≥ 45 per 1 000 live births) and a NE incidence of 26.5 per 1 000 live births). The countries with the least resources had the highest NMR and the highest NE incidence. However, these data showed wide ranges and some discrepancies which may relate to the lack of uniform diagnostic criteria for NE and HIE: a NMR of 6–15 per 1 000 live births was associated with a NE incidence of 4.7–8.7 per 1 000 live births while a NE incidence of 3.6–10.2 per 1 000 live births was recorded for regions with a NMR of 16–30 per 1 000 live births [8].

There is a paucity of published data, particularly population-based data, on the incidence of HIE in Sub-Saharan Africa. A South African hospital-based study carried out in 2009 found an incidence of 8.3 per 1 000 live births [27]. A population-based study in the Southern Cape Peninsula in 2002, reported HIE in 3.6 per 1 000

live births and moderate-severe HIE in 1.7 per 1 000 live births [28]. A more recent clinic-based study within the same region determined an HIE incidence of 9 per 1 000 live births [29]. Although different criteria were used to define HIE in each study, these data suggest that the incidence of NE in South Africa, particularly in the Southern Cape Peninsula, is comparable to the low or moderate mortality rate categories described by Lawn et al. and is substantially lower than in most other regions of Africa and South Asia (classified as Category Four or Category Five) where the NMR generally exceeds 30 per 1 000 live births and the mean NE incidence ranges from 13.4 to 26.5 per 1 000 live births [8].

1.2.2 The Southern Cape Peninsula and the Peninsula Maternity and Neonatal Service: geographic considerations and resource limitations

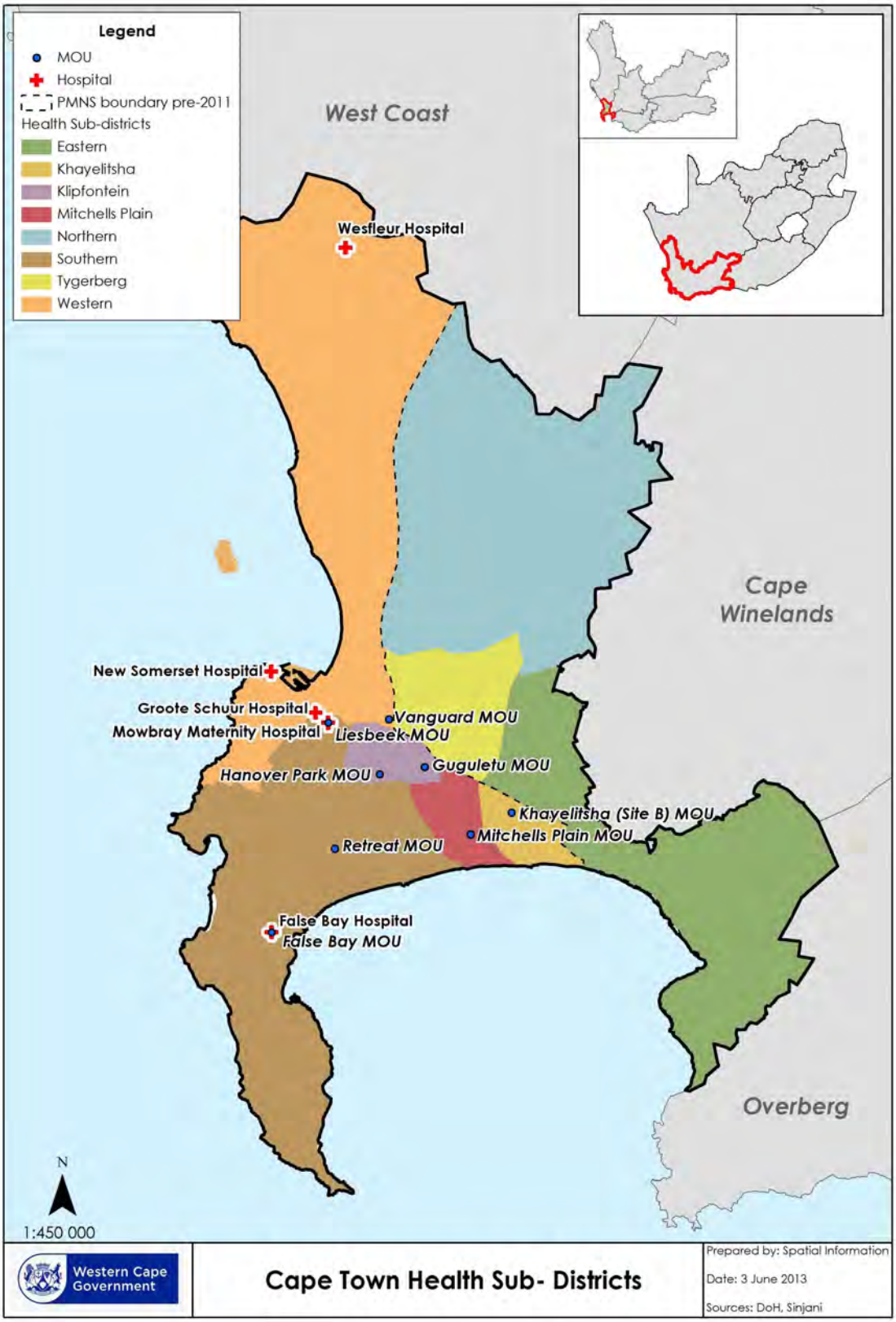
The Western Cape province is one of nine provinces in South Africa (Figure 1.1). The Western Cape is divided into six district municipalities. The City of Cape Town is the only metropolitan district and covers the smallest area: 2 502 km^2 compared to the provincial total of 129 462 km^2. The City of Cape Town is divided into eight health sub-districts (Figure 1.2). In 2011, the Western Cape had a population of 5.5 million, representing the highest population density in South Africa [30].

The Western Cape Department of Health is primarily responsible for providing medical care to the 75.6% of the population who do not have private medical health insurance [31]. The socio-economic challenges in the region are illustrated by the findings of the South African National Census of 2011: only 63.5 % of households use electricity for heating, cooking and lighting while 90.5% of households have access to a flush or chemical toilet [30]. Despite these limitations, the Western Cape is the only province to have had all its districts fall within in the highest socio-economic quintile since 2001 [31]. Futhermore, the NMR in the Western Cape was the lowest in South Africa, at 5.1 per 1 000 live births in 2010. The National South African NMR in 2010 was 11.8 per 1 000 live births with an individual provincial range of 5.1–16.3 per 1 000 live births [32]. Although the NMR and socio-economic indicators suggest that there are less resource limitations in the Western Cape than in the rest of South Africa, the Western Cape is moderately resource-limited region by global standards [10]. These limitations are further compounded by the resource inequalities between the sub-districts of the region itself [33].

The contextual implications of the above data must be considered when conducting epidemiological or comparative research in the Western Cape and South Africa.

Figure 1.1 The nine provinces of South Africa

Figure 1.2 The Health Sub-Districts of the Western Cape



For the purpose of this thesis, the Southern Cape Peninsula is defined as the area within the City of Cape Town Metropolitan district that was supported by the Peninsula Maternal and Neonatal Service (PMNS) at the time of the research. This area includes the following health sub-districts: Southern, Western, Klipfontein, Mitchell's Plain, and Khayelithsha (Figure 1.2). The PMNS was set up in 1980 to function as a well-defined urban/peri-urban perinatal service [34]. At the time of data collection for this thesis, the PMNS consisted of one tertiary hospital, two secondary hospitals, two primary care hospitals and eight Midwife Obstetric Units (MOUs): Groote Schuur Hospital (GSH), Mowbray Maternity Hospital (MMH), New Somerset Hospital (NSH), Falsebay Hospital (containing False Bay MOU), Wesfleur Hospital, Vanguard Drive MOU, Hanover Park MOU, Gugulethu MOU, Mitchell's Plain MOU, Khayelitsha MOU, Liesbeek MOU (based at MMH), and Retreat MOU respectively (Figure 1.2). Birth data for the entire PMNS, including home births in the region, are entered into the Perinatal Problem Identification Programme database, maintained at MMH. These data provide denominator figures that can be used to calculate the incidence of specific perinatal pathologies in the PMNS.

Limited regional demographic data is kept in a central database at MMH. Data from September 2008 to March 2009 shows a caesarean section rate of 20.1%, a low birth weight rate of 15.8% and 4% of deliveries occur at home or en-route to hospital (personal communication, Dr Candice Nelson). The high incidence of low birth weight and birth before arrival at hospital is in keeping with socio-economic limitations and limited access to healthcare centres respectively.

GSH, MMH and NSH all have facilities for neonatal ventilation, including blood gas analysis, blood pressure monitoring and administration of inotropic agents.

1.2.3 Therapeutic hypothermia in the Southern Cape Peninsula

In 2007, when research for this thesis was being considered, therapeutic hypothermia was already an established standard of care at the participating hospitals in the Southern Cape Peninsula. The method involved a modification of techniques previously described in studies that were ongoing [35] or already published at the time [14, 36]. However, the ability of the cooling method to achieve and maintain appropriate core temperatures needed to be established before studies involving short- or long-term outcomes could be meaningfully interpreted.

1.3 Aims, objectives and overview

In this section, I present the aims and objectives, an overview of the contents of the rest of the thesis, and a declaration of the ethical approvals.

1.3.1 Aims and objectives

The overall aim of this thesis is to develop and evaluate strategies for the early diagnosis and management of moderate-severe HIE within the resource constraints of the Southern Cape Peninsula. The role of basic clinical assessment as a predictor of moderate-severe HIE and subsequent outcome after cooling is explored; a systematic review of bedside predictors of moderate-severe HIE that could be used in our context is performed; a study of the incidence of HIE in the region is carried out; and, an evaluation of the basic cooling method already in use at the research sites is done, to facilitate reasonable inferences regarding the outcomes.

The specific aims and objectives are listed below:

(i) To conduct a systematic review to identify and examine studies describing the ability of clinical assessments or serological biomarkers, measured before age 6 hours and defined by threshold values, to identify moderate-severe HIE occurring during the first week of life in term newborn infants.

(ii) To conduct a retrospective folder review at the three referral hospitals in the Southern Cape peninsula – GSH, NSH, and MMH (all infants with HIE in the region are normally referred to one of these hospitals).

The specific objectives were:

a) to investigate how the incidence of HIE in the Southern Cape Peninsula of South Africa varies with respect to different criteria for intrapartum hypoxia; and

b) to determine how encephalopathy severity varies with the grading system used.

(iii) To perform an observational cohort study at MMH, evaluating the method of cooling in use at the hospitals in the Southern Cape Peninsula.

The specific objectives were to determine whether:

a) the target rectal temperature was achieved within an acceptable time;

b) rectal temperatures were maintained in an acceptable range; and

c) the method of monitoring a proxy core temperature was accurate.

(iv) To conduct a prospective cohort study of infants who were evaluated for therapeutic hypothermia at the three referral hospitals in the Southern Cape peninsula.

The primary objective was to determine the threshold Thompson HIE score at age 3–5 hours that predicted an abnormal 6-hour aEEG.

The secondary objectives were to determine the following:

a) the predictive value of modified Sarnat encephalopathy grading (MSEG) at age 3–5 hours for an abnormal 6-hour aEEG;

b) the predictive value of specific clinical signs at age 3–5 hours for an abnormal 6-hour aEEG – the specific clinical signs were those that were used as entry criteria in the cooling trials with abnormal aEEG as an additional criterion; and

c) the ability of the Thompson score threshold to predict moderate-severe encephalopathy presenting within 72 hours of birth.

(v) To perform a secondary analysis of the sub-group of cooled infants who formed part of the above study to identify clinical predictors of a poor outcome, despite therapeutic hypothermia.

The specific objectives were:

a) to determine the Thompson score at age 3–5 hours that best predicted death or a severely abnormal aEEG at 48 hours in cooled infants with HIE; and

b) to determine if specific individual clinical signs at age 3–5 hours predict death or a severely abnormal aEEG at 48 hours.

1.3.2 Overview

References are presented at the end of each chapter and abbreviations are written out in full at first mention in all chapters to ensure uniformity with the chapters included in publication format. Referencing has been standardised to the Vancouver style throughout.

In Chapter 2, the literature relating to the research questions and methodology is summarised and critically appraised. The chapter begins by addressing the difficulties in defining HIE. The discussion is limited to aspects of intrapartum monitoring, early clinical assessment and serum parameters immediately available at birth: these are the data that are most likely to be readily accessible at the time that management decisions need to be made. The pathophysiology and biochemical pathways are briefly reviewed in order to contextualise the subsequent descriptions of clinical assessment, aEEG and neuroprotection adequately.

The studies determining the ability of clinical signs to predict abnormal aEEG are central to this thesis; a discussion of the clinical presentation and commonly used methods of grading and scoring HIE is presented and the prognostic abilities of these approaches are reviewed. This is followed by a summary of the technical and prognostic concepts of the aEEG. The chapter concludes with a section on neuroprotection that describes the rationale behind the use and method of cooling as a standard of care at the time of the prospective cohort study.

Chapter 3 is a systematic literature review addressing the first objective.

Chapters 4−7 comprise four published papers addressing the last four objectives above, in sequence. The papers are included (with minor amendments addressing the recommendations of the examiners of the thesis) with the permission of the publishers and the University of Cape Town Doctoral Degrees Board. Details of the contributions of the authors, the publication status and the abstract in the format required by the journal are presented at the beginning of each chapter. The main text of the papers has been reformatted to maintain stylistic continuity, including renumbering of Tables and Figures with the Chapter number as prefix.

The sequence in which the papers appear in this thesis is as follows:

Chapter 4.

Horn AR, Swingler GH, Myer L, Harrison MC, Linley LL, Nelson C, et al. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. *J Perinat Med* 2013; 41(2):211–7.

Chapter 5.

Horn AR, Harrison MC, Linley LL. Evaluating a simple method of neuroprotective hypothermia for newborn infants. *J Trop Pediatr* 2010, 56(3):172–7.

Chapter 6.

Horn AR, Swingler GH, Myer L, Linley LL, Raban MS, Joolay Y, et al. Early clinical signs in neonates with hypoxic ischemic encephalopathy predict an abnormal amplitude-integrated electroencephalogram at age 6 hours. *BMC Pediatr* 2013,13(1):52.

Chapter 7.

Horn AR, Swingler GH, Myer L, Linley LL, Chandresekaran M, Robertson NJ. Early clinical predictors of a severely abnormal amplitude-integrated electroencephalogram at 48 hours in cooled neonates. *Acta Paediatr* 2013 May 30. doi: 10.1111/apa.12306. [Epub ahead of print]

Chapter 8 presents and discusses the findings of Chapters 3–7, with respect to the original rationale and aims of the thesis. The contributions to new knowledge in the field and implications for clinical practice are also described. Relevant conclusions are drawn. Limitations pertaining to the individual studies and the research setting of all the studies are discussed and suggestions for possible further research are made.

The Appendices contain the data collection sheets and consent forms relevant to the included publications.

1.3.3 Ethical approval

The research included in this thesis was approved by the University of Cape Town Health Sciences Faculty Human Research Ethics Committee: chapter 5 is approved under number REC REF 175/2008 and chapters 4,6 and 7 are all approved under REC REF 240/2007. The research conforms to the principles of the 2008 Declaration of Helsinki [37]. A parent or legal guardian was required to give informed written consent for data collection in prospective studies. All data was kept in a password-protected database and was de-identified during analysis. All infants received the existing standard of care during the research.

1.4 References

1. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

2. Toh V, Rajadurai VS. Term infants with hypoxic ischaemic encephalopathy: poor neurodevelopmental outcome despite standard neonatal intensive care. *J Trop Pediatr* 1999;45(4):229-32.

3. Mwakyusa SD, Manji KP, Massawe AW. The hypoxic ischaemic encephalopathy score in predicting neurodevelopmental outcomes among infants with birth asphyxia at the Muhimbili National Hospital, Dar-es-Salaam, Tanzania. *J Trop Pediatr* 2009;55(1):8-14.

4. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for Neonatal Hypoxic Ischemic Encephalopathy: An Updated Systematic Review and Meta-analysis. *Arch Pediatr Adolesc Med* 2012;166(6):558-66.

5. Nelson KB, Leviton A. How much of neonatal encephalopathy is due to birth asphyxia? *Am J Dis Child* 1991;145(11):1325-31.

6. Hankins GD, Speer M. Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. *Obstet Gynecol* 2003;102(3):628-36.

7. Amiel-Tison C. Cerebral damage in full-term new-born. Aetiological factors, neonatal status and long-term follow-up. *Biol Neonat* 1969;14(3):234-50.

8. Lawn JE, Lee AC, Kinney M, Sibley L, Carlo WA, Paul VK, et al. Two million intrapartum-related stillbirths and neonatal deaths: Where, why, and what can be done? *Int J Gynaecol Obstet* 2009;107(S):S5-S19.

9. Robertson NJ, Iwata O. Bench to bedside strategies for optimizing neuroprotection following perinatal hypoxia-ischaemia in high and low resource settings. *Early Hum Dev* 2007;83(12):801-11.

10. The World Bank. South Africa: Upper Middle Income [Internet]. 2013 [cited 2013 March 30]. Available from: http://data.worldbank.org/country/south-africa.

11. Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. *Pediatr Res* 1999;46(3):274-80.

12. Human I, Kroon M, Bergman N, Fawcus S, Ntwana B. Patient population movement in a Cape Town obstetric service. *S Afr Med J* 2003;93(9):634.

13. Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 1999;81(1):F19-23.

14. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460): 663-70.

15. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361(14):1349-58.

16. Simbruner G, Mittal RA, Rohlmann F, Muche R, neon.EURO.network Trial Participants. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics* 2010;126(4):e771-8.

17. Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics* 2010;126(1):e131-9.

18. Lee AC, Cousens S, Darmstadt GL, Blencowe H, Pattinson R, Moran NF, et al. Care during labor and birth for the prevention of intrapartum-related neonatal deaths: a systematic review and Delphi estimation of mortality effect. *BMC Public Health* 2011;11(Suppl 3):S10.

19. Lawn JE, Kerber K, Enweronu-Laryea C, Massee Bateman O. Newborn survival in low resource settings-are we delivering? *BJOG* 2009; 116(Suppl 1):49-59.

20. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic–ischaemic encephalopathy. *Early Hum Dev* 2010;86(6):329-38.

21. Smith J, Wells L, Dodd K. The continuing fall in incidence of hypoxic-ischaemic encephalopathy in term infants. *BJOG* 2000;107(4):461-6.

22. Thornberg E, Thiringer K, Odeback A, Milsom I. Birth asphyxia: incidence, clinical course and outcome in a Swedish population. *Acta paediatrica* 1995;84(8):927-32.

23. Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, O'Sullivan F, Burton PR, et al. Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ* 1998;317(7172):1554-8.

24. MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* 1999;319(7216):1054-9.

25. Committee on Obstetric Practice. ACOG Committee Opinion. Number 326, December 2005. Inappropriate use of the terms fetal distress and birth asphyxia. *Obstet Gynecol* 2005;106(6):1469-70.

26. Fawcus S, Linley LL, Thompson CM, Greenfield D. Obstetric risk factors for neonatal encephalopathy. In: Proceedings of the 23rd Conference on Priorities in Perinatal Care in Southern Africa; 2004 March 9-12; Limpopo, South Africa. P.141-3.

27. De Knijf A, Delport S, Pattinson R. Confidential Enquiries into hypoxic ischaemic encephalopathy as a marker for assessing the quality of care of women in labour. In: Proceedings of the 30th Conference on Priorities in Perinatal Care in Southern Africa; 2011 March 8-11; Limpopo, South Africa. P.224.

28. Linley LL, Thompson CM, Fawcus S. Incidence, morbidity and mortality of hypoxic ischaemic encephalopathy in the PMNS. In: Proceedings of the 23rd Conference on Priorities in Perinatal Care in Southern Africa; 2004 March 9-12; Limpopo, South Africa. P.144-6.

29. Harrison V, Fawcus S, Jordaan E. Magnesium supplementation and perinatal hypoxia: outcome of a parallel group randomised trial in pregnancy. *BJOG* 2007;114(8):994-1002.

30. StatsSA. Census 2011 Statistical release P0301.4. statssagovza [Internet]. 2013 [updated 2013 February 8; cited 2013 April 3] ; Available from: http://www.statssa.gov.za/Publications/P03014/P030142011.pdf.

31. Day C, Barron P, Padarath A, English R. District health barometer 2010/11: Health Systems Trust [Internet]. 2011[cited 2013 April 3]. Available from: http://www.hst.org.za/publications/district-health-barometer-201011.

32. Health Systems Trust SA. Neonatal death rate (NNDR) (deaths <28 days old per 1 000 live births) [Internet]. 2011 [cited 2011 September 5]. Available from: http://indicators.hst.org.za/healthstats/152/data/geo.

33. Sanders D, Chopra M. Key challenges to achieving health for all in an inequitable society: the case of South Africa. *Am J Public Health* 2006;96(1):73-8.

34. van Coeverden de Groot HA, Davey DA, Howland RC. The Peninsula Maternity and Neonatal Service. An urban community perinatal programme. *S Afr Med J* 1982;61(2):35-6.

35. A randomised controlled trial of the effect of whole body cooling on the outcome of term infants with hypoxic ischaemic encephalopathy (ICE:Infant Cooling Evaluation trial). Australian New Zealand Clinical Trials Registry [database on the Internet]. 2001 [cited 2013 January 23]. Available from: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=1025.

36. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353(15):1574-84.

37. World Medical Association. World Medical Association Declaration of Helsinki. *World Med J* 2008;54(4):122-4.

Chapter 2 Background Literature review

2.1 Defining Hypoxic Ischaemic Encephalopathy

The process of defining hypoxic ischaemic encephalopathy (HIE), requires an understanding of the clinical and laboratory indicators of intrapartum hypoxia, in addition to the clinical signs of the associated encephalopathy. An understanding of the pathophysiology of cerebral injury is also important when interpreting the clinical signs.

2.1.1 Defining intrapartum hypoxia: early clinical indicators vs acid-base and lactate analysis

Intrapartum fetal hypoxia may occur secondary to maternal hypoxaemia or hypotension; other causes include insufficient perfusion secondary to umbilical cord compression, prolonged utero-placental vessel compression or placental separation. The fetus initially responds with a reduced heart rate, decreased movement, a redistribution of blood flow to vital organs (brain and heart) and reduced oxygen utilization [1, 2]. Persistent hypoxia and anaerobic metabolism cause lactic acidosis, cardiac decompensation and ultimately ischaemic injury to vital organs, including the brain [3]. Infants with HIE and/or metabolic acidosis at birth usually have intrapartum fetal cardiac abnormalities on the cardiotocograph (CTG). However, the CTG may be abnormal in the absence of these conditions and is not strongly predictive of fetal acidosis or subsequent brain injury [4] [5].

The Apgar score

In 1953, Virginia Apgar developed a clinical score derived from assessment after birth of: respiratory effort, colour, responses, tone and heart rate. Although infants born following intrapartum hypoxia typically have low scores, the score was not developed as an indicator of intrapartum hypoxia but was rather intended to identify infants who required ongoing resuscitation and to document their response to the intervention [6].

A recent population study of 1 028 705 term newborns in Sweden, found that low Apgar scores were documented in 7 787 infants and that they were associated with a significantly increased risk for cerebral palsy (CP): the odds ratio (OR) for CP progressively increased from 25 (95% Confidence Interval (CI) 20–32) to 498 (95% CI 458–452) as Apgar scores progressively decreased from 7 to 3 at age

5 minutes [7]. In a case-control study of 183 term infants with a 5-minute Apgar score of < 7, most infants (88%) with a score of < 4 and 69% of infants with a score of 4–6 had an abnormal CTG documented before delivery; a cord pH of < 7.15 was present in more than half of the infants in both categories [8]. HIE was documented in 59% and 13% respectively. Harrington et al. performed a systematic review of studies comparing an Apgar score of 0 at 1, 5 and 10 minutes to outcome. The composite score of 0 – 0 – 0 was associated with death or severe disability in 94% of cases [9].

In a follow-up study of 122 infants with HIE, Levene et al. found that a 10-minute Apgar score of ≤ 5 had a sensitivity of 43% and a specificity of 95% for adverse outcome [10]. In a matched case-control study of 35 acidaemic infants, King et al. found that neonatal encephalopathy (NE) did not occur if the 5-minute Apgar score was ≥ 7 [11]. Similarly, in a prospective case-control study of 131 infants with NE in Katmandu [12], none of the infants with NE had a 5-minute Apgar score > 7 and 98% of the encephalopathic infants had required some form of resuscitation vs. 11% of the non-encephalopathic control group.

In summary, a normal 5-minute Apgar score is very unusual in infants with HIE and the association between a low Apgar score at 5-minutes and subsequent HIE and/or CP is stronger at lower scores. However, abnormal neurological outcomes in infants born with low Apgar scores are only invariable at exceptionally low scores (0 – 0 – 0) and abnormal outcomes can occur after higher scores.

Acid-base analysis in the first hour of life

The accumulation of lactate and H^+ during anaerobic metabolism results in a lowering of the pH and base deficit. This process suggests that a threshold level of the pH, base deficit or lactate may indicate the presence of intrapartum hypoxia more specifically and objectively than a low Apgar score or an abnormal CTG.

In 1999, the International Cerebral Palsy Task Force (ICPTF) published a consensus statement [13] stating that a cord or early infant blood gas with pH of < 7 or a base deficit of ≥ 12 mmol/l is one of several criteria required to infer the presence of intrapartum hypoxia of sufficient severity to cause brain damage and these were subsequently modified (Box 2.1) by the American College of Obstetricians and Gynaecologists (ACOG) [14]. These statements might lead to the assumption that these thresholds define the presence or absence of *any* fetal hypoxia. However the upper limit of the base deficit for a term singleton vaginal delivery is 8.3 mmol/l [15]; the severity of metabolic acidosis at birth is less after delivery by caesarean section

vs. vaginal delivery [16]. Moreover, several epidemiological studies [17-19] have found that mild or moderate NE, presumed to be secondary to intrapartum hypoxia, was associated with a base deficit in the range 8–12 mmol/l during the first hour of life. The ICPTF and the ACOG cite a case-control study by Low et al. as evidence for defining the threshold base deficit as 12 mmol/l [17]. Low et al. compared short-term outcomes in four groups of neonates with an increasing extent of metabolic acidosis in arterial cord blood in each group. Although the majority of moderate-severe encephalopathy occurred in infants with a base deficit of > 12mmol/l, 6% of infants with moderate-severe encephalopathy had a base deficit in the range of 4–12 mmol/l. Da Silva [18] and Wayenberg [19] measured arterial base deficit at age 30–45 minutes: there were no infants with moderate or severe NE with a base deficit of < 10 mmol/l. A recent systematic review has examined the association of pH at birth with neonatal morbidity: a pH of < 7.24 in the first hour of life is associated with increased neonatal morbidity, although the association is strongest at a pH threshold of 7 [20].

Box 2.1
The ACOG criteria to define an acute intrapartum hypoxic event as sufficient to cause cerebral palsy [14]

Essential Criteria (must meet all four)

1. Evidence of a metabolic acidosis in fetal umbilical cord arterial blood obtained at delivery (PH <7 and base deficit ≥ 12mmol/l) [a]
2. Early onset of severe or moderate neonatal encephalopathy in infants born at 34 or more weeks gestation
3. Spastic quadraplegic or dyskinetic cerebral palsy
4. Exclusion of other identifiable aetiologies

Criteria that collectively suggest an intrapartum timing (within 48 hours of delivery) but are nonspecific to asphyxial insults

1. A sentinal hypoxic event occurring immediately before or during labour
2. A sudden sustained fetal bradycardia or the absence of fetal heart rate variability in the presence of persistent, late or variable decelerations, after the pattern was previously normal
3. Apgar scores of 0–3 beyond 5 minutes [b]
4. Onset of multisystem involvement within 72 hours of birth
5. Early imaging study showing evidence of acute non-focal cerebral abnormality

a, ICPTF accept "very early neonatal blood samples" in the absence of cord blood;
b, ICPTF accept scores of 0–6;
BD, base deficit

Lactate concentration in the first hour of life

The lactate concentration in cord or infant blood soon after birth might be a more appropriate indication of intrapartum hypoxia than base deficit because it is a measured parameter, unlike the base deficit, which is a calculated parameter. In addition, lactate is not significantly influenced by utero-placental lactate production [21]. However, several authors have studied the correlation between umbilical cord blood lactate and pH or base deficit: results vary from no correlation [22], weak correlation [23-26], to strong correlation [27, 28]. Several of these studies derived a threshold value of lactate, corresponding to fetal acidosis or a 5-minute Apgar score of < 7: the cut-off values ranged from 3.7 to 8 mmol/l [23, 26, 27]. Shah et al. studied plasma lactate at 1 hour after birth in 61 term newborns who were born following fetal distress and were depressed at birth with abnormal vital signs and an umbilical arterial pH of < 7. A lactate of > 7.5 mmol/l was associated with moderate-severe HIE with a sensitivity of 94% and specificity of 67% [29]. Da Silva et al. studied blood gases at age 30 minutes in 115 newborns born after fetal distress and/or with low Apgar scores at 1 or 5 minutes. Neurological complications did not occur if lactate was < 5 mmol/l while a lactate of > 9 mmol/l predicted moderate or severe encephalopathy with a sensitivity of 84% and a specificity of 67% [18].

Multiple criteria for intrapartum hypoxia

In clinical practice, early blood gas assessment is not always available, particularly in resource-limited settings. The ICPTF and the ACOG guidelines suggest that a sentinel hypoxic event, an abnormal fetal heart rate, a low Apgar score beyond 5 minutes, the onset of multisystem involvement within 72 hours of birth and early imaging showing acute cerebral abnormality are *all* required (Box 2.1) as evidence of intrapartum hypoxia [13, 14]. Dijxhoorn et al. reported poor predictive values for Apgar scores, acidaemia at birth and neonatal neurological morbidity in a cohort of 805 term infants born vaginally [30]. However, in a brain magnetic resonance imaging (MRI) study, Cowan et al. [31] recruited 351 term infants with NE plus three of seven possible criteria suggesting intrapartum hypoxia [26]. The criteria were: late fetal heart decelerations, an arterial cord pH of < 7.1, a 5-minute Apgar score of < 7, meconium-stained liquor, the delayed onset of respiration and multi-organ failure. Acute brain injury on MRI was present in 80% of infants meeting three of these criteria.

Interim comments

The threshold values for Apgar score, metabolic acidosis and lactic acidaemia that reliably identify the presence or absence of intrapartum hypoxia are yet to be adequately defined. HIE can occur in infants with pH and base deficit values that do not reach the thresholds recognized by the ACOG and ICPTF. The wide variation in the threshold levels and/or predictive power of base deficit, pH and lactate to identify the presence of intrapartum hypoxia may be explained, in part, by the variation that occurs physiologically with gestational age [32] and also by the variation that occurs between different devices and methods of measurement [33]. The fact that 22 % of infants with a 5-minute Apgar score of < 4 do not have evidence of abnormal fetal heart rate or significant acidosis at birth [8], indicates that these parameters should not be used to validate each other, and can only be used as crude predictors.

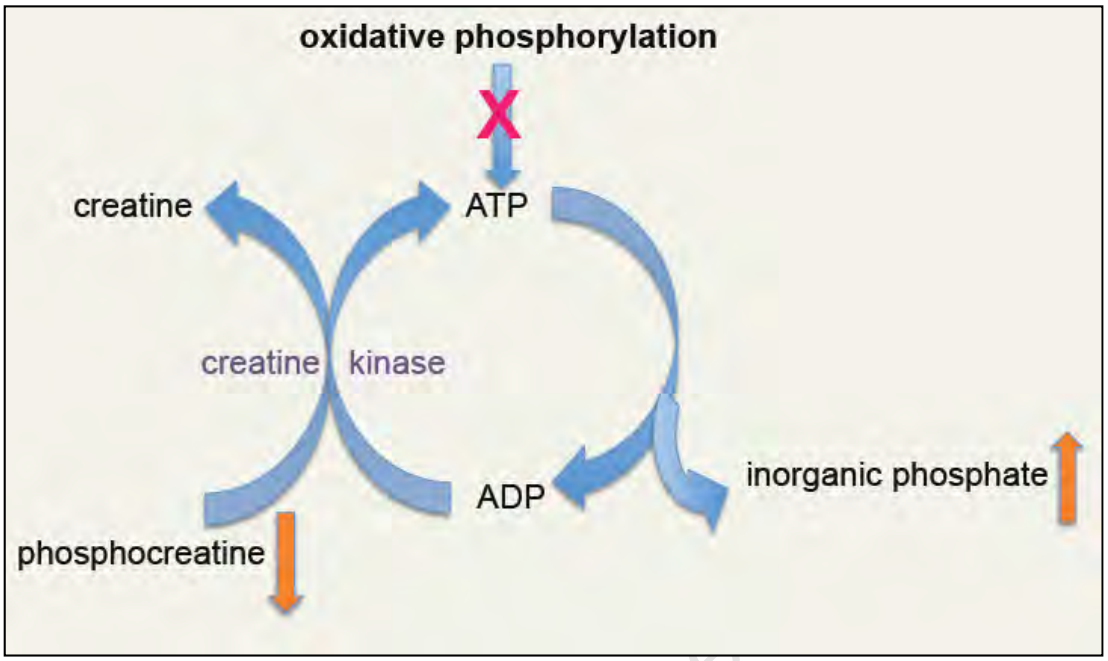
In the presence of indicators with low specificity there is a danger of inappropriately attributing the encephalopathy to intrapartum hypoxia when other pathologies may be responsible, particularly in a setting where intrapartum hypoxia is not common. However, when criteria other than blood gas criteria are accepted as proxies for intrapartum hypoxia, the majority of infants with associated encephalopathy have MRI evidence of acute hypoxic brain injury [31]. If criteria to define intrapartum hypoxia, other than blood gas alone, are not considered in settings where intrapartum monitoring is suboptimal, the incidence of both intrapartum hypoxia and HIE will probably be underestimated.

2.1.2 The pathophysiology of cerebral injury following transient hypoxia-ischemia

Energy metabolism and phases of energy failure

During cerebral hypoxia-ischaemia, the production of adenosine triphosphate (ATP) from oxidative phosphorylation is impaired. Inorganic phosphate (Pi) accumulates as a by-product from metabolism of ATP to adenosine diphosphate (ADP). The cell attempts to maintain normal levels of ATP via the creatine phosphokinase equilibrium reaction; phosphocreatine (PCr) is depleted in this process, as shown schematically in Figure 2.1 below [34].

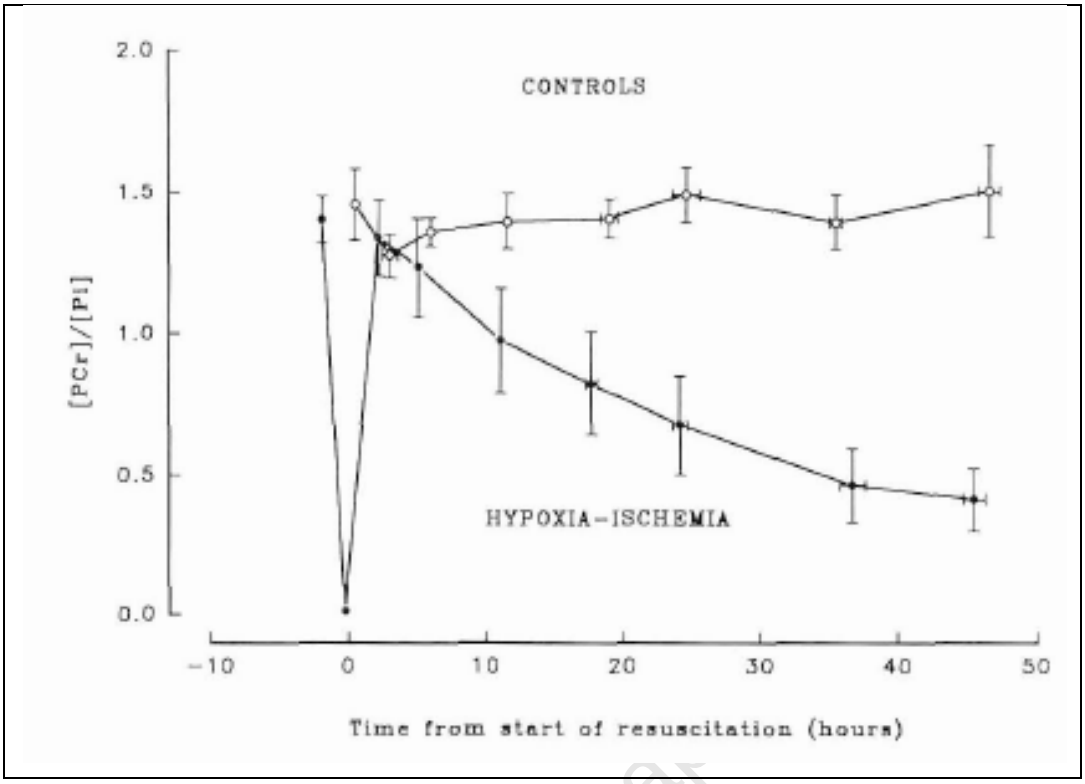
Figure 2.1 The creatine phosphokinase equilibrium reaction



ATP, adenosine triphosphate; ADP, adenosine diphosphate

Energy failure can therefore be seen on magnetic resonance spectroscopy (MRS) by a falling PCr/Pi fraction [35] or by a falling nucleotide triphosphate (NTP)/exchangeable phosphate pool (EPP) fraction [36]. Early Phosphorus (^{31}P) MRS studies of encephalopathic human infants successfully resuscitated after intrapartum hypoxia, showed a pattern of initial normal energy status after resuscitation, followed by delayed (secondary) energy failure [37, 38]. Subsequently, animal studies with MRS have clearly demonstrated a biphasic pattern of cerebral energy failure during and after resolution of hypoxia-ischaemia [39]. The PCr/Pi fraction decreases during experimental carotid artery occlusion in piglets (modelling the primary phase of energy failure during intrapartum hypoxia); after resuscitation energy status recovers at least partially (the latent phase) and subsequently falls again (the secondary phase of energy failure) [39]. The biphasic energy changes are depicted graphically in Figure 2.2 below.

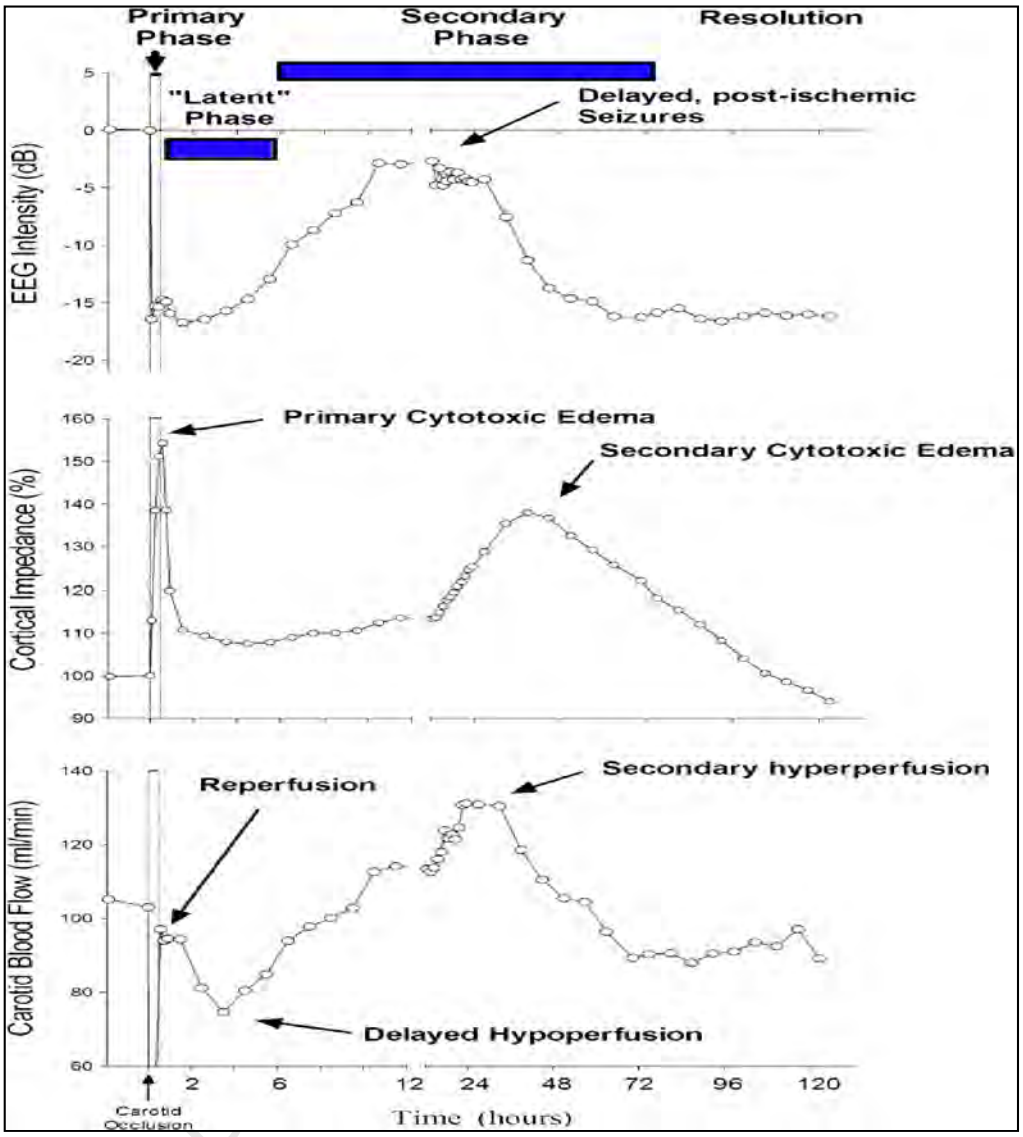
Figure 2.2 Biphasic energy failure in piglets [39]



[PCr]/[Pi] in the control group (○) and the experimental group (•) of piglets whose brains were subjected to acute hypoxia ischaemia. Values are means and standard error. (Reproduced with permission, Nature Publishing Group)

Studies of fetal sheep resuscitated after 30 minutes of global cerebral ischemia, demonstrate that during the latent phase of energy recovery, electroencephalogram (EEG) intensity and cortical impedance remain depressed, while carotid artery blood flow decreases [40]. The secondary phase of energy failure is characterized by cerebral hyp*er*perfusion, cytotoxic oedema and increased EEG intensity with the onset of seizures. The sequence of these events is shown in Figure 2.3 below. Clinical studies of human neonates suggest that the recovery time from primary energy failure to the onset of the latent phase is approximately 30–60 minutes [37, 41]. The latent phase of recovery of energy metabolism typically lasts from 2 to 8 hours but it can be very variable: a range of 1–24 hours has been reported [37, 38, 42-44].

Figure 2.3 Phases of cerebral injury after 30 minutes of global cerebral ischaemia in fetal sheep [40]

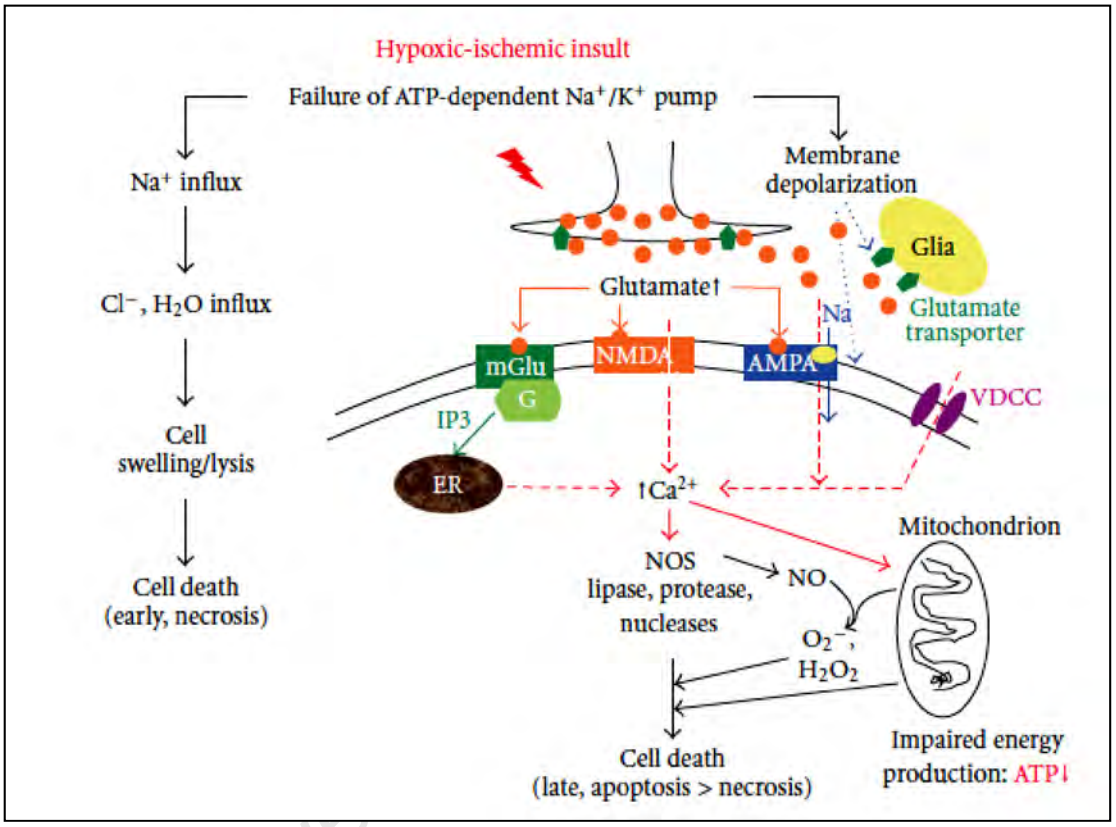


Cellular metabolism is restored and cell swelling decreases during the immediate reperfusion period (30 minutes). This is followed by a latent phase of normal energy metabolism, but with hypoperfusion and depressed EEG activity. Following the latent phase, there is secondary deterioration with delayed seizures, cytotoxic oedema and increased blood flow, culminating in failure of oxidative metabolism and damage. (Reproduced with permission, Elsevier)

Biochemical pathways

The biochemical pathways thought to be involved in the pathogenesis of HIE have recently been reviewed; a simplified, schematic summary of the events is shown in Figure 2.4 below [45].

Figure 2.4 Biochemical pathways involved in the pathogenesis of HIE [45]



In addition to early cell necrosis, the failure of the Na^+/K^+ pump causes membrane depolarisation, resulting in presynaptic glutamate release, reversal of glutamate transport in glia and neural terminals and activation of voltage-dependant calcium channels. This is followed by activation of NMDA, immature AMPA and metabotropic glutamate receptors culminating in excessive influx of Ca^{2+} into the cytosol. The cytosolic Ca^{2+} activates enzymes which culminate in delayed cell and or mitochondrial death. AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; ER, endoplasmic reticulum; mGlu, metabotropic glutamate; NMDA, N-methyl-D-aspartic acid; NOS, nitric oxide synthase; VDCC, voltage-dependent calcium channels. (Open access source. Reproduced under the Creative Commons Attribution License)

If the primary energy failure associated with the intrapartum hypoxic ischaemic event is severe, it may be associated with early cell necrosis. Energy failure is thought to be the initiating event that ultimately causes cell death associated with the primary phase (early cell necrosis): the failure of the ATP-dependant sodium–potassium pump, results in cytotoxic oedema (and cell lysis) due to intracellular accumulation of Na^+, Cl^-, Ca^{2+} and water. In addition to initiating early cell necrosis, the Na^+ influx causes presynaptic membrane depolarization, which results in excessive release of

excitatory neurotransmitters, particularly glutamate, and subsequently, post-synaptic intra-cellular accumulation of cytosolic Ca^{2+}. This initiates a chain of reactions, which are associated with delayed cell death (apoptosis) and after the latent phase, secondary energy failure. The increased cytosolic Ca^{2+} causes activation of phospholipases, nucleases and increased nitric oxide synthase (NOS) resulting in generation of free radicals and nitric oxide (NO), which is followed by damage to cellular components including the mitochondria. Apoptosis can be triggered by the above mechanisms but the mitochondria in particular play a pivotal role; free radical-mediated mitochondrial damage is associated with release of cytochrome C protein, which triggers the activation of a protease group known as caspases (in particular, caspase 3), which in turn lead to DNA fragmentation and apoptosis [46]. A continuum of both necrotic and apoptotic cell death ensues [47].

After successful resuscitation and the restoration of cerebral circulation and oxygenation (the reperfusion phase), the energy metabolism remains at least partially restored during the latent phase with progressive resolution of cytotoxic oedema [39, 48]. Although energy metabolism may seem normal during the latent phase, animal studies show histological evidence of considerable cell death at this time: Iwata et al. suggest that the latent phase represents normal energy metabolism which is limited to surviving tissue [36]. The same team of researchers also showed that a shorter latent phase was associated with more severe primary energy failure and increased neuronal death in both cortical and deep grey matter.

Interim comments

The accumulating biochemical, animal and human evidence, indicating a biphasic pattern of energy failure following transient hypoxia ischaemia, provides the rationale behind a therapeutic window of opportunity before and during the latent phase. Neuro-imaging after birth has confirmed that evolving cerebral damage occurs during the phase of secondary energy failure [49, 50], and there is a dose-response relationship between the degree of energy failure and the extent of cerebral damage that follows [44]. These features suggest that the clinical signs that follow transient hypoxia-schaemia are likely to follow a characteristic, and possibly diagnostic, course. The cascade of biochemical events lends itself to the potential for the use of intermediate by-products as potential biomarkers for disease presence and severity.

2.1.3 Clinical assessment of HIE

Newborns may be entirely asymptomatic following intrapartum hypoxia and the majority of infants who either require resuscitation or show metabolic acidosis on cord blood do not develop HIE [17]. However, newborns may display depressed Apgar scores at birth followed by a spectrum of clinical states varying from asymptomatic to degrees of HIE. In keeping with the pattern of associated energy failure, the clinical signs of HIE frequently show a biphasic pattern and evolve over the first few days. Infants with apparently mild HIE in the first hours of life can demonstrate signs of moderate HIE as the encephalopathy evolves later in the course [51-53]. Most grading systems describing the severity of HIE are based on three stages of encephalopathy, originally described by Sarnat and Sarnat and shown in Table 2.1 below. The description of the stages that follows is based primarily on their observations [51].

HIE grading according to Sarnat

Infants with stage 1 (mild) encephalopathy are irritable when examined. They tend to be hyperalert, difficult to console and feed poorly. Muscle tone is normal or increased and stretch reflexes are usually overactive: clonus lasting several seconds may occur. Their posture shows mild distal flexion in the form of an intermittently adducted (palmar) thumb position, also known as “fisting” or “cortical thumb”. Rhythmic segmental myoclonus may present as a course (4–7 Hz), apparently spontaneous, tremor in an extremity or in the facial muscles. The tremor may shift focus and is enhanced by sudden movement of the infant. The Moro reflex may be normal or exaggerated while the suck reflex is usually weak. The oculovestibular and tonic neck reflexes are usually normal. The autonomic system shows generalised sympathomimetic features: dilated pupils, tachycardia, sparse secretions and decreased gastrointestinal motility. Seizures are absent and the background EEG voltage is normal. In the series from Sarnat and Sarnat, the duration of stage 1 was variable, lasting from 1.5 to 18 hours, and was followed by resolution of signs or by progression to a more severe grade of encephalopathy [51]. Robertson and Finer reported that stage 1 could last for several days, until discharge from hospital [54].

Infants with stage 2 (moderate) encephalopathy have a depressed level of consciousness; they may be lethargic or obtunded. Muscle tone is decreased and there is strong distal flexion - persistent palmar thumb position, absent finger extension response to dorsal hand and finger stroking. Wrists may show a persistent flexed position. The distal flexion is enhanced by stimulation. Overactive stretch

reflexes, clonus and segmental myoclonus may also occur, as in stage 1 encephalopathy. The oculovestibular and tonic neck reflexes tend to be strong. The autonomic system shows generalised parasympathetic features: constricted pupils, bradycardia, profuse secretions and frequent stools.

Table 2.1 The Sarnat stages of hypoxic ischaemic encephalopathy [51]

Clinical data	Stage 1	Stage 2	Stage 3
Level of consciousness	Hyperalert	Lethargic or obtunded	Stuporous
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive	Decreased or absent
Segmental myoclonus	Present	Present	Absent
Suck	Weak	Weak or absent	Absent
Moro	Strong, low threshold	Weak, incomplete, high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or absent
Tonic neck	Slight	Strong	Absent
Autonomic function	Generalised sympathetic	Generalised parasympathetic	Both systems depressed
Pupils	Midriasis	Miosis	Variable, often unequal, poor light reflex
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial and salivary secretions	Sparse	Profuse	Variable
Seizures	None	Common (focal or multifocal)	Uncommon (excluding decerebration)
EEG findings	Normal (awake)	Early: low-voltage continuous delta and theta. Later: periodic pattern (awake) Seizures: focal 1–1.5 Hz spike and wave	Early: periodic pattern with isopotential phases. Later: totally isopotential
Duration	< 24 hours	2−14 days	Hours to weeks

Focal or generalised seizures commonly occur during stage 2 encephalopathy and the background EEG voltage shows moderate depression. The duration of stage 2 is

usually 4–6 days, but may be as long as 3 weeks, and is followed by resolution of signs or by progression to a more severe grade of encephalopathy [51].

Infants with stage 3 (severe) encephalopathy have a severely depressed level of consciousness, characterised by stupor or coma. They are usually flaccid and may have intermittent extensor posturing (decerebrate posturing). Stretch reflexes and primitive reflexes are usually absent or very weak. Both parasympathetic and sympathetic systems are depressed and the pupil response to light is usually very slow or absent. The EEG shows a periodic (burst-suppression) or isopotential pattern. Sarnat and Sarnat determined that seizures were uncommon based on intermittent EEG monitoring, but continuous EEG monitoring has shown that seizures are frequent, typically without visual clinical correlate, and they are often refractory to anticonvulsants [55].

In the series of 21 infants described by Sarnat and Sarnat, no infant had stage 1 HIE alone, but six of the seven infants with episodes of both stage 1 and stage 2 HIE, had a normal developmental outcome at 6–9 months (The highest stage attained in the first few days of life was the final stage assigned) [51]. Moreover, studies reporting 8-year outcomes of infants with mild HIE who have no evidence of transient hypotonia show that mild HIE defined in this way invariably carries a good prognosis [54, 56, 57]. Thirty-three percent of those with a maximum grade of moderate (stage 2) HIE, died or had severe disability, while all five infants who showed signs of severe HIE (stage 3) at any time either died or were severely disabled [51]. Other studies have shown a lower [56] or higher [58] incidence of death or disability (20–50%) after moderate HIE, and in normothermic infants, those with a longer duration of abnormal symptoms are more likely to be disabled [54, 58].

The Thompson HIE score

It is not clear from the series of Sarnat and Sarnat whether all the infants showed features of only one stage at a time, or if some infants showed signs of more than one stage at the same time. The Thompson HIE score addresses this issue [53]. The score is derived from nine aspects of the neurological examination of infants with HIE, shown in Table 2.2 below. Infants are assessed on a daily basis, starting at age 12–24 hours. The potential total score ranges from 0 to 22. The presence of signs associated with different grades occurring simultaneously, does not present problems because each sign is scored individually. This score allows a more precise description of infants by assigning a numeric score rather than “mild”, “moderate” or “severe”. Inter-rater reliability is very good with a kappa coefficient of 0.87.

In normothermic infants, a maximum score of > 10 during the first 7 days of life, predicts an abnormal outcome with 100% sensitivity and 61% specificity [53]. In comparison to the Sarnat stage system, a score of < 10 corresponds approximately to stage 1, a score of 11–14 corresponds to stage 2 and a score of ≥ 15 corresponds to stage 3. In the original series of 40 infants described by Thompson et al., cerebral palsy or death occurred in 57% of those infants with a maximum score of > 10 and in 82% of those with a maximum score of ≥ 15.

Both the Thompson score and the Sarnat stage provide prognostic information based on the maximum score or grade assigned in the first few days of life. The ability to prognosticate before the age of 5–6 hours became particularly important after the potential for neuroprotection with therapeutic hypothermia was established.

Table 2.2 The Thompson HIE score [53]

Score / Sign	1	2	3	Score
Limb tone	Generally hypertonic	Generally hypotonic	Flaccid	
Level of consciousness	Hyper-alert, staring, or excessive irritability	Lethargic	Comatose or stuporose	
Visible fits	Infrequent: < 3/day	Frequent: > 2/day		
Posture	Fisting and/or cycling	Strong distal flexion	Decerebrate	
Moro	Partial	Absent		
Grasp	Poor	Absent		
Suck	Poor	Absent and/or bites		
Respiratory effort	Hyperventilation	Transient apnoea	Apnoea + IPPV	
Fontanel	Full	Tense		
TOTAL				

The maximum score, based on the infant's clinical signs since the previous assessment, is recorded in each category and then totaled to obtain the final score.
IPPV, intermittent positive pressure ventilation

Modified Sarnat Encephalopathy Grading

Shalak et al. developed a modification of the Sarnat HIE grading system: the modified Sarnat encephalopathy grade (MSEG) made allowance for the simultaneous co-existence of signs associated with different stages of HIE and infants could be graded as mild, moderate or severe around or before age 6 hours [59]. Shalak's grading according to clinical signs is shown in Table 2.3 below. Encephalopathy was defined as the presence of one or more signs in at least three of the following six categories: level of consciousness, spontaneous activity, posture,

tone, primitive reflexes (suck or Moro), and autonomic nervous system (pupils, heart rate, or respiration). The number of mild, moderate or severe signs determined the final grade; if signs were equally distributed, the grade was based on the level of consciousness.

Table 2.3 Shalak's HIE grading system [59]

Clinical sign	Mild encephalopathy	Moderate encephalopathy	Severe encephalopathy
Level of consciousness	Hyperalert	Lethargic	Stupor or coma
Spontaneous activity	Normal	Decreased	No activity
Muscle tone	Normal	Hypotonia	Flaccid
Posture	Normal	Distal flexion	Decerebrate
Primitive reflexes			
Suck	Normal	Weak	Absent
Moro	Exaggerated	Weak; incomplete	Absent
Autonomic function			
Pupils	Normal	Constricted	Deviated, dilated or non-reactive to light
Heart rate	Normal	Bradycardia (< 100 BPM)	Variable
Respiration	Normal	Periodic breathing	Apnoea

BPM, beats per minute

Shalak et al. assessed 50 infants with suspected intrapartum hypoxia at age 5 ± 3 hours and 23% of infants without sufficient criteria for moderate-severe encephalopathy had an abnormal amplitude-integrated EEG within an hour of their assessment and later progressed to moderate encephalopathy which persisted at least until the fifth day [59]. The sensitivity and specificity of early moderate-severe encephalopathy to predict an abnormal outcome by discharge were both 78%. A combination of both mild and moderate encephalopathic clinical signs identified infants with an abnormal outcome at discharge with a sensitivity of 100%, but the specificity was only 50%. These data are in keeping with our knowledge of the evolving nature of HIE [51, 53]. They show that infants can subsequently develop moderate-severe encephalopathy following initial signs of mild encephalopathy.

Interim comments

While Shalak's MSEG provides a rapid and early method of assessment, the sensitivity was low unless features of mild and moderate MSEG were *both* present. A more detailed method of early clinical assessment such as the Thompson score may provide a more accurate method of identifying infants who have or will develop moderate-severe HIE.

2.2 Amplitude-integrated EEG

2.2.1 Technical background

The amplitude-integrated electroencephalogram (aEEG), was first described in the 1960s for use in adult intensive care. It displays a modified EEG that is considerably easier to set up and interpret than a conventional EEG [60]. An EEG signal is measured from fewer scalp electrodes than conventional EEG and a simpler configuration facilitates continuous monitoring for several days. The technical aspects of using aEEG in the newborn have been covered comprehensively in several texts [61-63]. The aEEG is derived by amplifying and then filtering the raw EEG. Filtering attenuates activity below 2 Hz and above 15 Hz. The filter minimizes (but does not completely exclude) artifacts from sweating, muscle activity, electrocardiographic or other electrical interference. The signal is then semi-logarithmically compressed to optimise visualization of activity between 0 and 10 μV. It is then rectified to positive output only, smoothed and time-compressed. The aEEG is conventionally written out at a slow speed of 6 cm/hour (compared to 1 cm/second conventional raw EEG speed). Variation in the amplitude produces easily recognizable patterns indicating changes in brain metabolism, arousal or seizures.

The standard configuration, is a cross-cerebral, single channel signal obtained from bi-parietal electrodes corresponding to positions P3 and P4 in the 10-20 EEG system: most prognostic data and classification systems were originally derived from this configuration. A bilateral fronto-parietal position (F3 and F4) has been used in some studies and some monitors also offer the option to monitor at additional sites in which case a bi-central position (C3 and C4) is usually used. If more than one site is monitored on each side, lateral localization and comparison of the activity in the cerebral hemispheres is possible. A neutral electrode is also usually positioned on the vertex (some monitors specify a different position) to measure and reduce the appearance of electrical interference. Most modern aEEG monitors also display the raw EEG at 1 cm/second at the paired electrode sites.

2.2.2 Classification and interpretation

Epileptic seizure activity in the aEEG is usually seen as an abrupt rise in the minimum amplitude and a simultaneous rise in the maximum amplitude, often followed by a short period of decreased amplitude [62].

Background activity of the aEEG can be described by two methods. A classification using voltage criteria alone was proposed by al Naqeeb et al. [64]:

Normal amplitude:	the upper margin of aEEG activity > 10 mV and the lower margin > 5 mV
Moderately abnormal amplitude:	the upper margin of aEEG activity > 10 mV and the lower margin < 5 mV
Suppressed amplitude:	the upper margin of aEEG activity < 10 mV and lower margin < 5 mV.

Al Naqeeb et al. studied 24 infants with encephalopathy within 12 hours of birth, at a median age of 5 hours. In this group the presence of an abnormal aEEG (either moderately abnormal or suppressed) predicted a poor neurological outcome with a sensitivity of 100% and specificity of 82%. The positive predictive value was 85% and negative predictive value, 100%. This method of classification was also used in the hypothermia trial of Gluckmann et al. [65]: 66% of the normothermic infants with an abnormal aEEG died or were severely disabled.

Hellström-Westas et al. [66] described a pattern-based recognition classification which has recently [67] been redefined to include reference to associated voltage changes as follows:

Continuous normal voltage (CNV):	Continuous background activity with minimum amplitude between 5 and 10 μV and maximum amplitude between 10–50 μV.
Discontinuous normal voltage (DNV):	Discontinuous background with minimum amplitude variable, but < 5 μV, and maximum amplitude > 10 μV.

Burst-suppression (BS):	Discontinuous background with minimum amplitude without variability at 0–2 μV, and bursts with amplitude > 25 μV.
Continuous low voltage (CLV):	Continuous background pattern of very low voltage (around or below 5 μV).
Inactive, flat trace (FT):	Mainly inactive (isoelectric tracing) background < 5 μV.

In this pattern-based classification system, CNV is considered normal, DNV is moderately abnormal while BS/CLV/FT are severely abnormal. The seminal work by Hellström-Westas et al. studying 47 term infants following intrapartum hypoxia, showed that BS/CLV/FT at age 6 hours had 95% sensitivity and 89% specificity for predicting poor long term outcome [66]. In a larger study including 68 infants after intra-partum hypoxia, Eken et al. showed that BS/CLV/FT at age 6 hours had 94% sensitivity and 79% specificity for predicting poor long-term outcome [68]. In another large study, Toet et al. found that BS/CLV/FT at 3 hours had a sensitivity of 85% and specificity of 77% for poor long-term outcome and at 6 hours these figures increased to 91% and 86% respectively [69]. The aEEG appearance at 3 hours was further related to the development of encephalopathy: 22 of the 26 infants with CNV remained Sarnat stage 1 HIE, three of five infants with DNV developed Sarnat stage 2 HIE, 24 of 26 infants with BS developed Sarnat stage 2 or 3 HIE and all seven infants with CLV/FT developed Sarnat grade 2 or 3 HIE.

The prognostic implications of an aEEG background activity showing DNV are not clear and data interpretation is hampered by the small numbers of infants studied with this aEEG pattern. In the series from Toet et al., five of the six infants with DNV at 3 hours and the only infant with DNV at 6 hours had a normal outcome [69]. The aEEG in four of the five infants with a normal outcome converted to CNV by 6 hours and the aEEG in the infant with an abnormal outcome converted to BS by 6 hours. In the presence of signs of moderate-severe encephalopathy, DNV was considered sufficient criteria for cooling in those studies that utilised abnormal aEEG as an entry criteria [65, 70, 71]. In a retrospective study of an aEEG in cooled vs. normothermic infants, Thoresen et al. observed that three of the nine normothermic infants with DNV had a severely abnormal outcome vs. none of the eight cooled infants with DNV, and they suggested that DNV may represent infants who are sufficiently abnormal to benefit from cooling [72].

2.2.3 Implications of persistently abnormal background activity

The available evidence suggests that an infant with HIE who has not been cooled and has an aEEG that remains severely abnormal (BS/CLV/FT) for 24 hours, will invariably have a very poor outcome; Van Rooij et al. followed a cohort of 160 normothermic infants with HIE. All 19 infants who had BS/CLV/FT persisting until 24 hours, either died or were severely disabled [73]. Another prognostic indicator on the aEEG is the onset of sleep-wake cycling (SWC). Osredkar et al. studied a cohort of 171 normothermic infants with HIE and found that the onset of SWC after 36 hours was significantly associated with a poor outcome; the negative predictive value was 48%, while 82% of those infants who developed SWC before 36 hours had a normal outcome [74].

The predictive value of aEEG is altered when infants are treated with therapeutic hypothermia [72, 75]. Hallberg et al. reported on a small cohort of term infants presenting with clinical signs of moderate HIE within the first 6 hours, who were cooled to 33.5 °C for 72 hours and followed up to age 12 months; all of the 14 infants whose aEEG had normalised (CNV/DNV background and no seizures) by 24 hours had a normal outcome, four of the ten infants whose aEEG had normalised between 36 and 48 hours had a normal outcome, but three of the four infants whose aEEG normalised *at* 48 hours and all four infants whose aEEG did not normalise *by* 48 hours had a poor outcome [75]. Thoresen et al. followed a cohort of 72 infants who had clinical signs *and* aEEG evidence of moderate-severe HIE in the first 6 hours after birth [72]. Forty-three infants were cooled to 33.5 °C with whole body cooling or 34.5 °C with selective head cooling. Five of the seven infants with BS/CLV/FT persisting until 36–47 hours had a normal developmental outcome at 18 months. All of the 15 infants with a persistently severely abnormal aEEG *at* 48 hours either died or were severely disabled, while all of the cooled infants whose aEEG corrected to CNV or DNV before 24 hours had normal developmental outcomes at 18 months, as did two of the three cooled infants whose aEEG corrected to CNV or DNV by 24–36 hours. Thoresen et al. also investigated the value of SWC in cooled infants; the median time of onset of SWC in cooled infants with a normal outcome was 36 hours and all cooled infants who developed SWC before 60 hours had a normal outcome at 18 months [72].

Interpretation of the data in the reports by Hallberg et al. and Thoresen et al. is limited by the small number of cooled infants with an abnormal outcome at follow-up (a total of 22 of 66 cooled infants) and the lack of provision of confidence intervals

around predictive values for abnormal aEEG at specified time intervals for abnormal outcome. Hallberg et al. also did not report on the association between the delayed onset of SWC and hypothermia. These limitations may leave room for some circumspection regarding applicability of absolute cut-off times when prognosticating based on the aEEG appearance, however the strong association of death or disability in infants with a severely abnormal aEEG persisting ≥ 48 hours was consistent across both studies.

Alternative neurological monitoring modalities to predict outcome

Several imaging and monitoring techniques other than aEEG have been extensively evaluated as potential tools to predict outcome in normothermic newborn infants with HIE [76]. In 2012, Van Laerhoven et al. published a systematic review of prognostic tests predicting outcome of term neonates with HIE at 18 months. The most predictive tests were aEEG, EEG, visual evoked potentials, MRI and MRS; MRI was more predictive than MRS [76]. Their findings differed from the earlier systematic review of Thayyil et al. who concluded that MRS was a more accurate quantitative biomarker than MRI [77]. Rutherford et al. showed that the predictive value of MRI is not affected by therapeutic hypothermia [78]. The value of the above predictive tests, other than aEEG and MRI, have yet to be adequately validated in infants treated with hypothermia, they require highly specialised resources, and they are generally not feasible to perform within the first 6 hours of life. Near infra-red spectroscopy is another modality that is being increasingly studied, but in normothermic infants with HIE, aEEG shows a closer relationship with outcome [79].

Cerebral ultrasound is a highly accessible imaging modality. However, Eken et al. found that abnormal outcome occurred in over 50% of infants with HIE and a normal cerebral ultrasound [68]; Boo et al. similarly reported poor prediction of adverse outcome using cerebral ultrasound [80]. A more recent study in 2006 found that cerebral ultrasound can correlate well with MRI when performed by radiologists using modern high-specification ultrasound machines [81] – this modality is likely to become increasingly useful as the technology involved advances, but it will remain highly operator-dependent. A Pourcelot's resistance index ≤ 0.55, measured with cerebral doppler ultrasound can assist in predicting outcome in normothermic infants, but a systematic review showed variable accuracy across several studies and it is less predictive in cooled infants [82].

Interim comments

With regard to the above data, the aEEG is currently the most accurate bedside prognostic tool available to predict normality or moderate-severe HIE at age 6 hours or less, and it has been used as an essential criterion in determining whether neuroprotective therapy is indicated. Similarly, in cooled infants at or beyond age 48 hours, a persistently severely abnormal aEEG predicts death or severe disability and could provide a proxy for a poor long-term outcome – this is particularly relevant in the resource-limited setting of the Southern Cape Peninsula.

2.3 Neuroprotective hypothermia for newborn infants

The latent phase of transient recovery of energy metabolism in infants with HIE, provides a potential window of opportunity to institute neuroprotective treatment that may prevent or delay the secondary energy failure and the associated cerebral damage [83]. Following several decades of pre-clinical work and several clinical trials of both selective head cooling and whole body cooling in neonatal intensive care units [84], therapeutic hypothermia has been established as a safe and effective therapy for babies with moderate to severe neonatal encephalopathy if performed under similar conditions as in the original clinical trials [85, 86]. Cooling trials have required treatment to start by 6 hours after birth for inclusion into the study but there is experimental evidence suggesting that cooling should be started as early as possible to optimise benefits [36, 87].

2.3.1 Animal research

The history of therapeutic hypothermia and the translation of animal evidence to bedside practice has been extensively reviewed [84, 88-91]. Animal research on the effects of hypothermia began as long ago as 1949 with Miller's study on newborn guinea pigs [92], but it was the seminal works by Thoresen et al. in 1995 [93], Gunn et al. in 1998 [94] and Shanakaran et al. in 2002 [95], that informed the basis of subsequent human research protocols. Thoresen et al. [93] applied the piglet model of asphyxia followed by MRS analysis showing falling PCr/Pi and NTP/EPP fractions as evidence of secondary energy failure [39]; they showed unequivocally that a reduction in core temperature by 3.5 °C for 12 hours prevented the secondary energy failure that occurred in asphyxiated sham-operated, normothermic control piglets.

Gunn et al. compared brain histology in cooled vs, sham-cooled fetal sheep; they demonstrated significant neuroprotection in instrumented post-hypoxic near-term fetal sheep, when core temperature was lowered by 1–3 °C and extradural temperature was lowered by 4–5 °C before the onset of seizures, (by 5.5 hours after reperfusion) for a period of 72 hours [94]. Using the same model, if re-warming was started before 72 hours, “rebound” seizures occurred in some fetuses [83]. When hypothermia was delayed until after seizures had started (8.5 hours after reperfusion) there was no evidence of neuroprotection [87]. Shankaran et al. showed the feasability of prolonged whole body cooling to a core temperature of 33.5 °C in newborn piglets, and developed a clinical model for use in human infants using a servo-controlled cooling mat [95].

Although the data from Gunn and Shanakaran’s early animal work informed the human trials that followed, animal research to further explore the mechanisms and optimal application of therapeutic hypothermia is ongoing. The lack of neuroprotection following delayed hypothermia after seizures in fetal sheep [87] and the finding that a shorter latent phase is associated with more severe insults in piglets [36], suggests that cooling should be started as early as possible after hypoxic-ischaemic insults to obtain maximum benefit. The mechanisms by which hypothermia provides neuroprotection following a hypoxic ischaemic insult are multi-factorial and are not precisely defined. Two recent detailed reviews have discussed the potential mechanisms based predominantly on animal and in vitro studies [90, 96]; the mechanisms pertaining to processes are summarized in Table 2.4 below.

Table 2.4 Potential mechanisms of neuroprotection with hypothermia [90,96]

Mechanism	Detail
Decreases cerebral metabolism	Decreased cerebral metabolism is associated with delayed delayed onset of depolarisation. This mechanism cannot be the primary mechanism of protection because cooling still improves outcome when the duration of the depolarisation is controlled.
Reduces cytotoxic oedema	Cooling can prevent intracellular ion and water entry. Reduction of cytotoxic oedema is not independently protective.
Reduces epileptiform transient activity and seizure amplitude	This may be due to reduced metabolic demand of the seizures. Reduced epileptiform transient activity and reduced seizure amplitude is associated with neural protection, but reduced numbers of delayed seizures are not.
Extends the phase of secondary hypoperfusion and prevents the phase of secondary hyperperfusion	An extended phase of secondary hypoperfusion in the latent phase is associated with neural protection, whereas post-insult hyperperfusion is strongly associated with neural injury.
Preserves cerebral high-energy metabolite production in vivo, suggesting preservation of mitochondrial function	In vitro, mitochondrial preservation is observed in cellular studies, with neural protection commencing in the latent phase.
Suppresses apoptosis (delayed cell death)	Hypothermia suppresses apoptotic pathways, particularly the suppression of activated caspase-3 in the latent phase, which is associated with neural protection.
Inhibits the inflammatory cascade	Cooling inhibits post-depolarisation release of excitatory amino acids, production of superoxide and NO. It can suppress the oxygen free radical burst and subsequent peroxidation of cell membrane lipids. It suppresses both microglial activation and the accumulation of polymorphonuclear leukocytes. The neuroprotective processes commencing in the latent phase are associated with most neural protection.
Enhances the activity of glutamate receptor antagonists	This process commences in the latent phase and is associated with neural protection.

NO, nitric oxide

2.3.2 Human research

Publication of research into the potential benefit of hypothermia for human newborns after intrapartum hypoxia began with the case series of Westin and Miller in 1959 [97]. They cooled Infants who had failed to breath after birth in a cold water bath until breathing resumed spontaneously; the infants re-warmed passively and neurological outcomes were reported as normal in five of the six infants. In 1964, Westin and Miller reported 80% “intact survival” in a larger series of 65 infants cooled with a similar method [98]. However, further research on human infants was abandoned for many years after Silverman [99] and Day [100] showed, in randomized controlled trials, that a cold environment was associated with poor outcomes in preterm infants. Non-randomised studies of cooling human infants emerged in the Russian literature in the 1980s [101], but randomised controlled therapeutic trials only began in the 1990s after sufficient data from animal studies had accumulated to inform appropriate methodology in humans [85]. Data from these trials has recently been extensively analysed in three meta-analyses published between 2010 and 2013 [85, 86, 102].

A meta-analysis in 2012, by Tagin et al., included trials comparing therapeutic hypothermia to normothermia for newborns with HIE, including 1214 newborns in seven trials reporting mortality and neurodevelopmental outcome at 18 months [85]. The duration of cooling in all the trials was 72 hours and infants were re-warmed at approximately 0.5 °C per hour or slower. Four of the trials cooled to a target core temperature of 33–34 °C using whole body hypothermia and three trials used selective cerebral hypothermia; the two largest selective hypothermia trials cooled to a target core temperature of 34–35 °C while a further smaller trial cooled to core temperatures varying from 34.5 to 36.5 °C. Hypothermia reduced the risk of the composite outcome of death or major neurodevelopmental disability at age 18 months (Risk ratio (RR) 0.76, 95% CI 0.69–0.84; and number needed to treat (NNT) 7, 95% CI 5–10). Moreover, therapeutic hypothermia increased survival with normal neurological function (RR 1.63, 95% CI 1.36–1.95). Hypothermia reduced the risk of death or major disability in newborns with moderate HIE (RR 0.67, 95% CI 0.56–0.81; NNT 6, 95% CI 4–11) as well as in newborns with severe HIE (RR 0.83, 95% CI 0.74–0.92; NNT 7, 95% CI 5–16). When whole body cooling was compared to selective head cooling, there were no significant differences in the reduction of death or major disability [85].

Early in 2013, Jacobs et al. published an updated Cochrane review and meta-analysis including 1 505 term and late preterm infants in 11 randomised and quasi-randomised controlled trials comparing the outcome of term and late preterm infants with moderate-severe HIE with and without therapeutic hypothermia [86]. They included the same trials as did Tagin et al., but also included four trials that were excluded by Tagin et al. because those trials did not report outcomes to 18 months. Jacobs et al. confirmed the findings of Tagin et al. and also reported a significant reduction in mortality from all 11 studies (RR 0.75, 95% CI 0.64–0.88; NNT 11, 95% CI 8–25) as well as a significant reduction in neurodevelopmental disability in survivors from the eight studies that reported outcomes to 12 months (RR 0.77, 95% CI 0.63–0.94; NNT 8, 95% CI 5–14).

An earlier meta-analysis in 2010 by Shah et al., included 13 randomized or quasi-randomized clinical trials of therapeutic hypothermia that reported mortality [102]. Although they did not include the most recently published neo.nEURO.network trial [71], they found a similar reduction in the risk of mortality or neurodevelopmental disability in survivors after either whole body or selective cerebral cooling as was reported in the later analyses. Importantly, Shah et al. also found that the reduction in adverse outcome was only significant if the *target* core temperature was ≤ 34 °C, irrespective of which cooling method was used [102].

2.3.3 International recommendations and cooling methods in the Southern Cape Peninsula

In 2006, the National Institute of Child Health and Human Development (NICHD) [103] and the American Academy of Pediatrics' Committee on the Fetus and Newborn [104] issued statements recommending that therapeutic hypothermia for newborns with HIE should not yet be a standard of care for all institutions, but advised that it would appropriate for those institutions already offering hypothermia as a standard of care to continue, as long as entry criteria and methodology was in line with existing studies.

A basic method of cooling was developed at Mowbray Maternity Hospital (MMH) and short-term outcomes were already published in 1999 [105]. However, following the published outcomes of the two large randomized controlled cooling trials by Gluckman [65] and Shankaran [106], the interim presentations of the basic method used in the Infant Cooling Evaluation (ICE) study [107, 108], and the findings of four systematic reviews suggesting benefit from hypothermia [109-112], the cooling method used at MMH was modified early in 2008 to ensure that entry criteria and

methodology were appropriate; this cooling method was also used at Groote Schuur Hospital (GSH) and New Somerset Hospital (NSH). In the same month that the modifications were formalised, an international statement was published, urging the use of therapeutic hypothermia as a standard of care in settings where intensive care was available [113].

The modified cooling method in use at GSH, NSH and MMH involved cooling to a target core temperature of 34 °C for 72 hours and then re-warming slowly by 0.2 °C per hour. Core temperature was measured and controlled inserting a temperature probe between the infant and the mattress; the probe was secured to the skin of the infant with a reflective adhesive patch, and connected to a servo-controlled radiant warmer (Servocrib, Servocare Medical Industries cc, Cape Town) set at a target infant temperature of 34 °C (The lowest target infant temperature that the warmer was capable of at the time). Hypothermia was induced by applying one or two refrigerated soft, 12 x 12 cm, 250 g gel-packs (Penguin Manufacturers, International Health Care Distributors) around the head. Before use, the packs were stored in refrigerator at 7–10 °C: this gel-pack cooling method was similar to that used in the ICE trial [108], but instead of manual adjustments to the radiant warmer as done in the ICE trial, the Cape Town method utilised the servo-control function of the radiant warmer. A reflective Perspex shield was placed over the infant's head to prevent facial heating by the warmer, as was done in the CoolCap trial by Gluckman et al. [65]. An insulated skin temperature measured between the infant and the mattress was used a proxy for core temperature, because this was technically easier and less costly, and a good correlation between the sites had previously been established in a similar setting [114]. The incremental re-warming at 0.2 °C per hour was chosen, to ensure that re-warming did not occur too rapidly: prior experience of the clinicians had shown that a target increment of 0.5°C per hour tended to be associated with inadvertent excessively rapid re-warming when using a radiant warmer. Cerebral function (aEEG) was monitored during cooling with the BrainZ BRM2 monitor (BrainZ Instruments Ltd, Auckland, NZ).

Although the cooling method described above was similar to those used in the ICE trial, the ability of method to achieve appropriate core temperatures had not been evaluated. An evaluation was necessary before research studying cooling outcomes in this context could commence. This evaluation formed one of the objectives of this thesis.

In 2010, subsequent to the data obtained for this thesis and based on the three largest cooling trails that had already been reported by that time [65, 70, 106], the International Liason Committee on Resuscitation (ILCOR) made the following recommendation [115], “Newly born infants born at or near-term with evolving moderate to severe hypoxic-ischemic encephalopathy should be offered therapeutic hypothermia. Whole body cooling and selective head cooling are both appropriate strategies. Cooling should be initiated and conducted under clearly defined protocols with treatment in neonatal intensive care facilities and with the capability for multidisciplinary care. Treatment should be consistent with the protocols used in the randomized clinical trials (ie, begin within 6 hours of birth, continue for 72 hours after birth, and re-warm over at least 4 hours).” The cooling of infants with methods and recruitment protocols similar to the ICE protocol is in keeping with this recommendation.

2.4 References

1. Peeters LL, Sheldon RE, Jones MD, Makowski EL, Meschia G. Blood flow to fetal organs as a function of arterial oxygen content. *Am J Obstet Gynecol* 1979;135(5):637-46.

2. Wassink G, Bennet L, Booth LC, Jensen EC, Wibbens B, Dean JM, et al. The ontogeny of hemodynamic responses to prolonged umbilical cord occlusion in fetal sheep. *Journal of applied physiology* 2007;103(4):1311-7.

3. Dawes G. Birth Asphyxia, Resuscitation, and Brain Damage. In: Dawes G, editor: Foetal and Neonatal Physiology. Chicago: Year Book Medical Publishers, Inc; 1969. p.141-59.

4. Larma JD, Silva AM, Holcroft CJ, Thompson RE, Donohue PK, Graham EM. Intrapartum electronic fetal heart rate monitoring and the identification of metabolic acidosis and hypoxic-ischemic encephalopathy. *Am J Obstet Gynecol* 2007;197(3):301.e1-.e8.

5. Spencer JA, Badawi N, Burton P, Keogh J, Pemberton P, Stanley F. The intrapartum CTG prior to neonatal encephalopathy at term: a case-control study. *Br J Obstet Gynaecol* 1997;104(1):25-8.

6. Apgar V. A proposal for a new method of evaluation of the newborn infant. *Curr Res Anesth Analg* 1953;32(4):260-7.

7. Thorngren-Jerneck K, Herbst A. Perinatal factors associated with cerebral palsy in children born in Sweden. *Obstet Gynecol* 2006;108(6):1499-505.

8. Hogan L, Ingemarsson I, Thorngren-Jerneck K, Herbst A. How often is a low 5-min Apgar score in term newborns due to asphyxia? *Eur J Obstet Gynecol Reprod Biol* 2007;130(2):169-75.

9. Harrington DJ, Redman CW, Moulden M, Greenwood CE. The long-term outcome in surviving infants with Apgar zero at 10 minutes: a systematic review of the literature and hospital-based cohort. *Am J Obstet Gynecol* 2007;196(5):463.e1-5.

10. Levene MI, Sands C, Grindulis H, Moore JR. Comparison of two methods of predicting outcome in perinatal asphyxia. *Lancet* 1986;1(8472):67-9.

11. King TA, Jackson GL, Josey AS, Vedro DA, Hawkins H, Burton KM, et al. The effect of profound umbilical artery acidemia in term neonates admitted to a newborn nursery. *J Pediatr* 1998;132(4):624-9.

12. Ellis M, Manandhar N, Manandhar DS, Costello AMD. Risk factors for neonatal encephalopathy in Kathmandu, Nepal, a developing country: unmatched case-control study. *BMJ* 2000;320(7244):1229-36.

13. MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* 1999;319(7216):1054-9.

14. Committee on Obstetric Practice. ACOG Committee Opinion. Number 326, December 2005. Inappropriate use of the terms fetal distress and birth asphyxia. *Obstet Gynecol* 2005;106(6):1469-70.

15. Riley RJ, Johnson JW. Collecting and analyzing cord blood gases. *Clin Obstet Gynecol* 1993;36(1):13-23.

16. Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. *BMJ* 1988;297(6640):24-7.

17. Low JA, Lindsay BG, Derrick EJ. Threshold of metabolic acidosis associated with newborn complications. *Am J Obstet Gynecol* 1997;177(6):1391-4.

18. Da Silva S, Hennebert N, Denis R, Wayenberg JL. Clinical value of a single postnatal lactate measurement after intrapartum asphyxia. *Acta Paediatr* 2000;89(3):320-3.

19. Wayenberg JL. Threshold of metabolic acidosis associated with neonatal encephalopathy in the term newborn. *J Matern Fetal Neonatal Med* 2005;18(6):381-5.

20. Malin GL, Morris RK, Khan KS. Strength of association between umbilical cord pH and perinatal and long term outcomes: systematic review and meta-analysis. *BMJ* 2010;340:c1471.

21. Nordström L, Achanna S, Naka K, Arulkumaran S. Fetal and maternal lactate increase during active second stage of labour. *BJOG* 2001;108(3):263-8.

22. Chanrachakul B, Chua S, Nordström L, Yam J, Arulkumaran S. Umbilical artery blood gas and lactate in healthy newborns. *J Med Assoc Thai* 1999;82(4):388-93.

23. Westgren M, Divon M, Horal M, Ingemarsson I, Kublickas M, Shimojo N, et al. Routine measurements of umbilical artery lactate levels in the prediction of perinatal outcome. *Am J Obstet Gynecol* 1995;173(5):1416-22.

24. Kruger K, Kublickas M, Westgren M. Lactate in scalp and cord blood from fetuses with ominous fetal heart rate patterns. *Obstet Gynecol* 1998;92(6):918-22.

25. Nordström L, Malcus P, Chua S, Shimojo N, Arulkumaran S. Lactate and acid-base balance at delivery in relation to cardiotocography and T/QRS ratios in the second stage of labour. *Eur J Obstet Gynecol Reprod Biol* 1998;76(2):157-60.

26. Suidan JS, Young BK. Outcome of fetuses with lactic acidemia. *Am J Obstet Gynecol* 1984;150(1):33-7.

27. Shirey T, St Pierre J, Winkelman J. Cord lactate, pH, and blood gases from healthy neonates. *Gynecol Obstet Invest* 1996;41(1):15-9.

28. Gjerris AC, Stær-Jensen J, Jørgensen JS, Bergholt T, Nickelsen C. Umbilical cord blood lactate: A valuable tool in the assessment of fetal metabolic acidosis. *Eur J Obstet Gynecol Reprod Biol* 2008;139(1):16-20.

29. Shah S, Tracy M, Smyth J. Postnatal lactate as an early predictor of short-term outcome after intrapartum asphyxia. *J Perinatol* 2004;24(1):16-20.

30. Dijxhoorn MJ, Visser GH, Fidler VJ, Touwen BC, Huisjes HJ. Apgar score, meconium and acidaemia at birth in relation to neonatal neurological morbidity in term infants. *Br J Obstet Gynaecol* 1986;93(3):217-22.

31. Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 2003;361(9359):736-42.

32. Wiberg N, Kallen K, Herbst A, Aberg A, Olofsson P. Lactate concentration in umbilical cord blood is gestational age-dependent: a population-based study of 17 867 newborns. *BJOG* 2008;115(6):704-9.

33. Nordström L. Lactate measurements in scalp and cord arterial blood. *Curr Opin Obstet Gynecol* 2001;13(2):141-5.

34. Atkinson D. The energy change of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. *Biochemistry (Mosc)* 1968;7:4030-4.

35. Delpy DT, Gordon RE, Hope PL, Parker D, Reynolds EO, Shaw D, et al. Noninvasive investigation of cerebral ischemia by phosphorus nuclear magnetic resonance. *Pediatrics* 1982;70(2):310-3.

36. Iwata O, Iwata S, Thornton JS, De Vita E, Bainbridge A, Herbert L, et al. "Therapeutic time window" duration decreases with increasing severity of cerebral hypoxia-ischaemia under normothermia and delayed hypothermia in newborn piglets. *Brain Res* 2007;1154:173-80.

37. Azzopardi D, Wyatt JS, Cady EB, Delpy DT, Baudin J, Stewart AL, et al. Prognosis of newborn infants with hypoxic-ischemic brain injury assessed by phosphorus magnetic resonance spectroscopy. *Pediatr Res* 1989;25(5): 445-51.

38. Hope PL, Cady EB, Tofts PS, Hamilton PA, Costello AMD, Delpy DT, et al. Cerebral energy metabolism studied with phosphorous NMR spectroscopy in normal and birth asphyxiated infants. *The Lancet* 1984;324(8399):366-70.

39. Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, et al. Delayed ("secondary") cerebral energy failure after acute hypoxia- ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatr Res* 1994;36(6):699-706.

40. Bennet L, Booth L, Gunn AJ. Potential biomarkers for hypoxic-ischemic encephalopathy. *Semin Fetal Neonatal Med* 2010;15(5):253-60.

41. Robertson NJ, Iwata O. Bench to bedside strategies for optimizing neuroprotection following perinatal hypoxia-ischaemia in high and low resource settings. *Early Hum Dev* 2007;83(12):801-11.

42. Wyatt J. Applied physiology: brain metabolism following perinatal asphyxia. *Current Paediatrics* 2002;12(3):227-31.

43. Younkin DP, Papadopoulos MD, Leonard JC, Subramanian VH, Eleff S, Leigh JS, et al. Unique aspects of human newborn cerebral metabolism evaluated with phosphorus nuclear magnetic resonance spectroscopy. *Annals of Neurology* 1984;16(5):581-6.

44. Roth SC, Baudin J, Cady E, Johal K, Townsend JP, Wyatt JS, et al. Relation of deranged neonatal cerebral oxidative metabolism with neurodevelopmental outcome and head circumference at 4 years. *Dev Med Child Neurol* 1997;39(11):718-25.

45. Lai M-C, Yang S-N. Perinatal Hypoxic-Ischemic Encephalopathy. *J Biomed Biotechnol* 2011;2011(6):1-6.

46. Johnston MV, Trescher WH, Ishida A, Nakajima W. Neurobiology of hypoxic-ischemic injury in the developing brain. *Pediatr Res* 2001;49(6):735-41.

47. Northington FJ, Zelaya ME, Riordan DP, Blomgren K, Flock DL, Hagberg H, et al. Failure to complete apoptosis following neonatal hypoxia-ischemia manifests as "continuum" phenotype of cell death and occurs with multiple manifestations of mitochondrial dysfunction in rodent forebrain. *Neuroscience* 2007;149(4):822-33.

48. Williams CE, Gunn A, Gluckman PD. Time course of intracellular edema and epileptiform activity following prenatal cerebral ischemia in sheep. *Stroke* 1991;22(4):516-21.

49. McKinstry RC, Miller JH, Snyder AZ, Mathur A, Schefft GL, Almli CR, et al. A prospective, longitudinal diffusion tensor imaging study of brain injury in newborns. *Neurology* 2002;59(6):824-33.

50. Nakajima W, Ishida A, Lange MS, Gabrielson KL, Wilson MA, Martin LJ, et al. Apoptosis Has a Prolonged Role in the Neurodegeneration after Hypoxic Ischemia in the Newborn Rat. *J Neurosci* 2000;20(21):7994-8004.

51. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;33(10):696-705.

52. Fenichel GM. Hypoxic-ischemic encephalopathy in the newborn. *Arch Neurol* 1983;40(5):261-6.

53. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

54. Robertson C, Finer N. Term infants with hypoxic-ischemic encephalopathy: outcome at 3.5 years. *Dev Med Child Neurol* 1985;27(4):473-84.

55. van Rooij L, de Vries L, Handryastuti S, Hawani D, Groenendaal F, van Huffelen A, et al. Neurodevelopmental outcome in term infants with status epilepticus detected with amplitude-integrated electroencephalography. *Pediatrics* 2007;120(2):e354.

56. Robertson CM, Finer NN, Grace MG. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. *J Pediatr* 1989;114(5):753-60.

57. Robertson CM, Finer NN. Educational readiness of survivors of neonatal encephalopathy associated with birth asphyxia at term. *J Dev Behav Pediatr* 1988;9(5):298-306.

58. Shankaran S, Laptook AR, Tyson JE, Ehrenkranz RA, Bann CM, Das A, et al. Evolution of Encephalopathy during Whole Body Hypothermia for Neonatal Hypoxic-Ischemic Encephalopathy. *The Journal of pediatrics* 2012;160(4):567-72.

59. Shalak LF, Laptook AR, Velaphi SC, Perlman JM. Amplitude-integrated electroencephalography coupled with an early neurologic examination enhances prediction of term infants at risk for persistent encephalopathy. *Pediatrics* 2003;111(2):351-7.

60. Maynard DE. EEG analysis using an analogue frequency analyser and a digital computer. *Electroencephalogr Clin Neurophysiol* 1967;23(5):487.

61. De Vries LS, Hellstrom-Westas L. Role of cerebral function monitoring in the newborn. *Arch Dis Child Fetal Neonatal Ed* 2005;90(3):F201-7.

62. De Vries LS, Toet MC. Amplitude integrated electroencephalography in the full-term newborn. *Clin Perinatol* 2006;33(3):619-32.

63. Hellström-Westas L, de Vries LS, Rosén I. An Atlas of Amplitude-integrated EEGs in the Newborn. First ed. New York and London: The Parthenon Publishing group; 2003.

64. Al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D. Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. *Pediatrics* 1999;103(6 Pt 1):1263-71.

65. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460): 663-70.

66. Hellstrom-Westas L, Rosen I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. *Arch Dis Child Fetal Neonatal Ed* 1995;72(1):F34-8.

67. Hellstrom-Westas L, Rosen I, de Vries LS, Greisen G. Amplitude-integrated EEG Classification and Interpretation in Preterm and Term Infants. *Neoreviews* 2006;7(2):e76-87.

68. Eken P, Toet MC, Groenendaal F, de Vries LS. Predictive value of early neuroimaging, pulsed Doppler and neurophysiology in full term infants with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 1995;73(2):F75-80.

69. Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 1999;81(1):F19-23.

70. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361(14):1349-58.

71. Simbruner G, Mittal RA, Rohlmann F, Muche R, neon.EURO.network Trial Participants. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics* 2010;126(4):e771-8.

72. Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics* 2010;126(1):e131-9.

73. Van Rooij LG, Toet MC, Osredkar D, van Huffelen AC, Groenendaal F, de Vries LS. Recovery of amplitude integrated electroencephalographic background patterns within 24 hours of perinatal asphyxia. *Arch Dis Child Fetal Neonatal Ed* 2005;90(3):F245-51.

74. Osredkar D, Toet MC, van Rooij LG, van Huffelen AC, Groenendaal F, de Vries LS. Sleep-wake cycling on amplitude-integrated electroencephalography in term newborns with hypoxic-ischemic encephalopathy. *Pediatrics* 2005;115(2):327-32.

75. Hallberg B, Grossmann K, Bartocci M, Blennow M. The prognostic value of early aEEG in asphyxiated infants undergoing systemic hypothermia treatment. *Acta Paediatr* 2010;99(4):531-6.

76. Van Laerhoven H, de Haan TR, Offringa M, Post B, van der Lee JH. Prognostic tests in term neonates with hypoxic-ischemic encephalopathy: a systematic review. *Pediatrics* 2013;131(1):88-98.

77. Thayyil S, Chandrasekaran M, Taylor A, Bainbridge A, Cady EB, Chong WKK, et al. Cerebral Magnetic Resonance Biomarkers in Neonatal Encephalopathy: A Meta-analysis. *Pediatrics* 2010;125(2):e382-e95.

78. Rutherford M, Ramenghi LA, Edwards AD, Brocklehurst P, Halliday H, Levene M, et al. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled trial. *Lancet Neurol* 2010;9(1):39-45.

79. Toet MC, Lemmers PM, van Schelven LJ, van Bel F. Cerebral oxygenation and electrical activity after birth asphyxia: their relation to outcome. *Pediatrics* 2006;117(2):333-9.

80. Boo NY, Chandran V, Zulfiqar MA, Zamratol SM, Nyein MK, Haliza MS, et al. Early cranial ultrasound changes as predictors of outcome during first year of life in term infants with perinatal asphyxia. *J Paediatr Child Health* 2000;36(4):363-9.

81. Daneman A, Epelman M, Blaser S, Jarrin JR. Imaging of the brain in full-term neonates: does sonography still play a role? *Pediatr Radiol* 2006;36(7): 636-46.

82. Elstad M, Whitelaw A, Thoresen M. Cerebral Resistance Index is less predictive in hypothermic encephalopathic newborns. *Acta Paediatr* 2011;100(10):1344-9.

83. Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest* 1997;99(2):248-56.

84. Edwards AD. The Discovery of Hypothermic Neural Rescue Therapy for Perinatal Hypoxic-Ischemic Encephalopathy. *Semin Pediatr Neurol* 2009;16(4):200-6.

85. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for Neonatal Hypoxic Ischemic Encephalopathy: An Updated Systematic Review and Meta-analysis. *Arch Pediatr Adolesc Med* 2012;166(6):558-66.

86. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane database of systematic reviews* 2013;1:CD003311.

87. Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. *Pediatr Res* 1999;46(3):274-80.

88. Thoresen M. Cooling the newborn after asphyxia — physiological and experimental background and its clinical use. *Semin Neonatol* 2000;5(1): 61-73.

89. Thoresen M, Whitelaw A. Therapeutic hypothermia for hypoxic-ischaemic encephalopathy in the newborn infant. *Curr Opin Neurol* 2005;18(2):111-6.

90. Gunn AJ, Thoresen M. Hypothermic neuroprotection. *NeuroRx* 2006;3(2):154-69.

91. Azzopardi D, Edwards AD. Hypothermia. *Semin Fetal Neonatal Med* 2007;12(4):303-10.

92. Miller JA. Factors in Neonatal Resistance to Anoxia. I. Temperature and Survival of Newborn Guinea Pigs Under Anoxia. *Science* 1949;110(2848):113-4.

93. Thoresen M, Penrice J, Lorek A, Cady EB, Wylezinska M, Kirkbride V, et al. Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatr Res* 1995;37(5):667-70.

94. Gunn AJ, Gunn TR, Gunning MI, Williams CE, Gluckman PD. Neuroprotection with prolonged head cooling started before postischemic seizures in fetal sheep. *Pediatrics* 1998;102(5):1098-106.

95. Shankaran S, Laptook A, Wright LL, Ehrenkranz RA, Donovan EF, Fanaroff AA, et al. Whole-Body Hypothermia for Neonatal Encephalopathy: Animal Observations as a Basis for a Randomized, Controlled Pilot Study in Term Infants. *Pediatrics* 2002;110(2):377-85.

96. Drury PP, Bennet L, Gunn AJ. Mechanisms of hypothermic neuroprotection. *Semin Fetal Neonatal Med* 2010;15(5):287-92.

97. Westin B, Enhorning G. An experimental study of the human fetus with special reference to asphyxia neonatorum. *Acta Paediatr* 1955;44(Suppl 103):79-81.

98. Miller JA, Jr., Miller FS, Westin B. Hypothermia in the Treatment of Asphyxia Neonatorum. *Biol Neonat* 1964;6:148-63.

99. Silverman WA, Fertig JW, Berger AP. The influence of the thermal environment upon the survival of newly born premature infants. *Pediatrics* 1958;22(5):876-86.

100. Day RL, Caliguiri L, Kamenski C, Ehrlich F. Body temperature and survival of premature infants. *Pediatrics* 1964;34:171-81.

101. Kopchev S. Cranio-cerebral hypothermia in obstetrics. *Moscow:Medicina* 1985:1-112.

102. Shah PS. Hypothermia: a systematic review and meta-analysis of clinical trials. *Semin Fetal Neonatal Med* 2010;15(5):238-46.

103. Higgins RD, Raju TN, Perlman J, Azzopardi DV, Blackmon LR, Clark RH, et al. Hypothermia and perinatal asphyxia: executive summary of the National Institute of Child Health and Human Development workshop. *J Pediatr* 2006;148(2):170-5.

104. Blackmon LR, Stark AR. Hypothermia: a neuroprotective therapy for neonatal hypoxic-ischemic encephalopathy. *Pediatrics* 2006;117(3):942-8.

105. Simbruner G, Haberl C, Harrison V, Linley L, Willeitner AE. Induced brain hypothermia in asphyxiated human newborn infants: a retrospective chart analysis of physiological and adverse effects. *Intensive Care Med* 1999;25(10):1111-7.

106. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353(15):1574-84.

107. Jacobs SE, Stewart M, Inder TE, Doyle L, Morley C, editors. Feasability of a pragmatic randomised controlled trial of whole body cooling for term newborns with hypoxic-ischaemic encephalopathy. Paper presented at: Hot topics in Neonatology; 2002 December 9 -10; Washington, D.C.

108. Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch Pediatr Adolesc Med* 2011;165(8):692-700.

109. Edwards AD, Azzopardi DV. Therapeutic hypothermia following perinatal asphyxia. *Arch Dis Child Fetal Neonatal Ed* 2006;91(2):F127-31.

110. Schulzke SM, Rao S, Patole SK. A systematic review of cooling for neuroprotection in neonates with hypoxic ischemic encephalopathy - are we there yet? *BMC Pediatr* 2007;7:30.

111. Shah PS, Ohlsson A, Perlman M. Hypothermia to treat neonatal hypoxic ischemic encephalopathy: systematic review. *Arch Pediatr Adolesc Med* 2007;161(10):951-8.

112. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2007(4):CD003311.

113. Hoehn T, Hansmann G, Bührer C, Simbruner G, Gunn AJ, Yager J, et al. Therapeutic hypothermia in neonates. Review of current clinical data, ILCOR recommendations and suggestions for implementation in neonatal intensive care units. *Resuscitation* 2008;78(1):7-12.

114. Horn AR, Woods DL, Thompson C, Els I, Kroon M. Selective cerebral hypothermia for post-hypoxic neuroprotection in neonates using a solid ice cap. *S Afr Med J* 2006;96(9 Pt 2):976-81.

115. Perlman JM, Wyllie J, Kattwinkel J, Atkins DL, Chameides L, Goldsmith JP, et al. Part 11: Neonatal Resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. *Circulation* 2010;122(16_suppl_2):S516-S38.

Chapter 3

Early clinical and serum predictors of moderate-severe hypoxic ischaemic encephalopathy: a systematic review

3.1 Introduction

The benefit of therapeutic hypothermia for moderate-severe hypoxic ischaemic encephalopathy (HIE) is likely to be optimal if cooling is commenced as soon as possible after birth [1, 2]. However, the evolving clinical signs of encephalopathy [3-5] may be associated with a delayed diagnosis of moderate-severe HIE. If referring hospitals wait until clinical signs of moderate-severe HIE are obvious, subsequent transport delays and/or difficulty in providing effective cooling during transport may delay the initiation of cooling. There is therefore a need for an early predictor to select infants for further assessment or referral.

The amplitude-integrated electroencephalogram (aEEG) is a sensitive and specific early predictor of abnormal long-term outcome in infants with HIE [6-8]; an abnormal aEEG background at or before 6 hours was used to indicate moderate-severe encephalopathy and the need for cooling in several trials of therapeutic hypothermia [9-11]. However, the equipment and expertise required for aEEG are not available in all centres, especially in resource-limited settings. A simpler bedside biomarker that can identify infants who have or will develop moderate-severe HIE is needed.

In 2009, a systematic review and limited meta-analysis of biomarkers of brain injury in HIE identified 22 publications with adequate data and outcome reporting beyond 12 months [12]. The authors identified urine lactate, urine protein S100, cord blood interleukin-6, serum non-protein-bound iron, serum CD14 cell NFκB activation, serum interleukin-8 and serum ionised calcium as potential predictors of death or abnormal outcome. However, the study concluded that there was insufficient evidence to recommend routine clinical use of the reviewed biomarkers. Biomarkers associated with the short-term outcome of moderate-severe HIE may be easier to define as the outcome of moderate-severe HIE should be available in every infant. A meta-analysis in 2010 examined the association between umbilical cord pH and perinatal outcomes including HIE, but moderate-severe HIE was not studied as a discrete outcome. Moreover, the review did not limit itself to measurements in the first 6 hours of life [13], and the authors highlighted the lack of high-quality prognostic studies examining the ability of cord pH and base deficit to predict neonatal morbidity.

The aim of this chapter is to systematically review published studies of early (< 6 hours) clinical assessments or serological biomarkers (including blood gas parameters) and their prediction of moderate-severe HIE in the first week of life of term newborn infants.

3.2 Methods

The methods are based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) [14] and the revised quality assessment tool for diagnostic accuracy studies (QUADAS-2) [15].

3.2.1 Eligibility criteria

Papers describing studies of term newborn infants with or at risk of HIE, that met the following criteria were included:

i) The study cohort (the population) consisted of term infants with suspected intrapartum hypoxia.

ii) The study compared a clinical assessment or serological tests obtained before age 6 hours (the index test) between infants with moderate-severe HIE (the reference standard/outcome) and infants with mild or no HIE (the comparator) during the first week of life; index tests using an aEEG were not included.

iii) The study stated threshold values for the relevant tests/assessments that were either pre-specified or derived during the study.

iv) The study provided sufficient data to calculate sensitivity and specificity.

3.2.2 Data source and search strategy

Pubmed was used to search dates between 1946 and September 2012 on Medline, using combinations of terms in the title or abstract that defined the population (term infants with intrapartum hypoxia), the outcome (encephalopathy) and the predictive or prognostic nature of the study. The search was limited to English language publications. The specific search string used is shown in Box 3.1.

Box 3.1 Search string used in Pubmed

((((neonate OR neonatal OR newborn OR newborns OR "term infant" OR full term[Title/Abstract])) AND (acidosis OR asphyxia OR asphyxiated OR hypoxic[Title/Abstract])) AND (encephalopathy[Title/Abstract])) AND (predictive OR predictors OR predictor OR threshold OR indicator OR indicators OR prognosis[Title/Abstract]) AND ("humans"[MeSH Terms] AND English[lang])

3.2.3 Study identification and data extraction

The titles of publications identified by the initial search string were reviewed. Abstracts of all potentially relevant titles were reviewed and full-text articles were obtained for all potentially eligible publications. Data were extracted and entered into a pre-defined spreadsheet. Corroborative raw data were requested from authors of all included papers via postal and/or email correspondence.

3.2.4 Data items and critical appraisal

The following quantitative data were collected from each index test studied: the threshold level of the index test and either the sensitivity and specificity of each test or raw data to populate 2x2 tables. Qualitative data were extracted to allow the comparison, critical appraisal and assessment of risk of bias according to the four domains defined by the QUADAS-2 criteria [15]: patient selection, the index test, the reference standard, and flow and timing. The following qualitative data were extracted: the source population, the method of cohort selection, the inclusion criteria, the exclusion criteria, the number excluded, the cohort size, the number with moderate-severe HIE, the method of index test measurement, the method of derivation of the threshold value, the method and timing of the reference standard, and blinding between the reference standard and the index test. These data informed answers to signalling questions (answered as "yes", "no" or "unclear") that were the basis for the assignment of risk of bias or lack of applicability in the QUADAS-2 domains. Two clinicians (AR Horn and MC Harrison) agreed on consensus answers to these questions and assigned consensus risk and applicability levels ("low", "high" or "unclear") to each index test study.

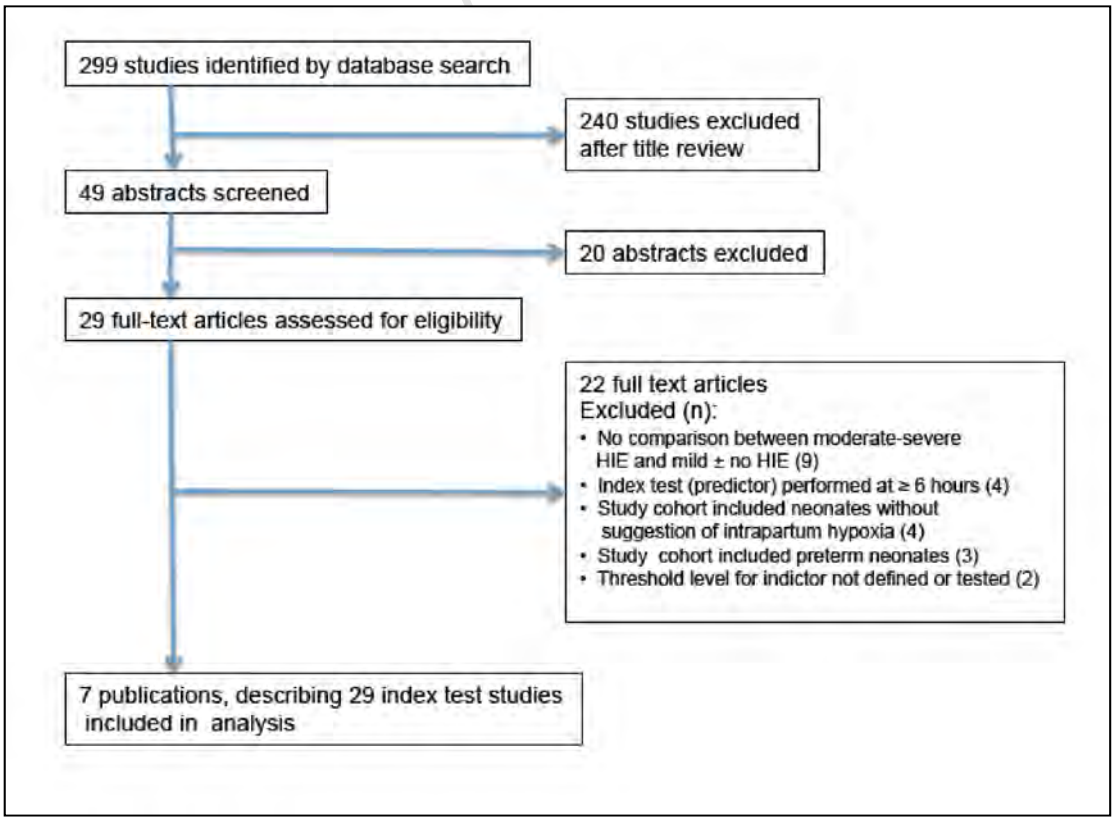
3.2.5 Data presentation

Both quantitative and qualitative data are presented for each study in tabular and graphical format. Statistical and graphical analysis was performed using Stata version 12 (Stata Corp, Texas, USA). The midas command was used to derive confidence intervals for sensitivity, specificity and the diagnostic odds ratio (DOR) for each test and to present these data on a forest plot, as well as on a receiver operating characteristic (ROC) curve plane. Meta-analysis was not performed due to the heterogeneous nature of the type and timing of the tests; although similar tests such as a blood gas analysis in the first hour were used on more than occasion, meta-analysis is still inappropriate because of the use of different cut-off thresholds in each test.

3.3 Results

The initial search strategy identified 299 papers. Forty-nine potentially relevant titles were identified and following review of the abstracts, 29 full-text papers were assessed. Seven publications [16-22] contained studies of index tests that met eligibility criteria (Figure 3.1): these papers collectively described 29 index tests.

Figure 3.1 Flow chart showing the process of article selection



HIE, hypoxic ischaemic encephalopathy

3.3.1 Quality assessment

The individual index tests and reference standards are summarised and numbered in Tables 3.1–3.3 below. The tests are grouped as follows: tests that are derived from arterial blood gas sampling in the first hour of life (tests 1a−15a); tests that are derived from clinical assessment before age 6 hours (tests 1c−3c); and tests derived from assessment of serum biomarkers other than arterial blood gas assessment alone (tests 1s−11s). None of the studies stated whether the interpretation of the reference standard was blinded or not, but bias was deemed inevitable in those in which the index test included a subjective clinical assessment, or in which the timing of the reference test was not clearly stated. The timing of the reference standard was not clear in 35% of tests. Although the biomarkers studied by Nagdyman (5a, 6a, 1s–10s) [20] and Qian (11s) [22] were included, the threshold values were difficult to interpret as they did not state whether infants with moderate-severe HIE had levels greater than or equal to the threshold values.

Table 3.1 Index test vs. reference standard: arterial blood gas in the first hour of life

Test no.	Author	Year	Index test	Threshold	Reference standard
1a	Wayenberg	1994	BD30 after colloid	> 7.9 mmol/l	Moderate or severe HIE (worst grade in first 5 days of life, defined by Finer)
2a	Da Silva	2000	BD30	> 12.5 mmol/l	Moderate or severe HIE (worst grade in at least the first 3 days of life, defined by Finer)
3a	Da Silva	2000	Lactate at 30 min	> 9 mmol/l	
4a	Da Silva	2000	Lactate and BD at 30 min	> 9 & > 12.5 mmol/l	
5a	Nagdyman	2001	Cord blood BD	> 17 mmol/l	Moderate or severe HIE (worst grade in the first 3 days of life, defined by Sarnat)
6a	Nagdyman	2001	Cord blood pH	< 6.9	
7a	Shah	2004	BD within 1 h	> 8 mmol/l	Moderate or severe HIE (worst grade in the first 3 days of life, defined by Sarnat)
8a	Shah	2004	pH within 1 h	< 7.1	
9a	Shah	2004	Lactate within 1 h	> 7.5 mmol/l	
10a	Wayenberg	2005	BD30	> 10 mmol/l	Moderate or severe HIE (worst grade in the “first days of life”, defined by Finer)
11a	Wayenberg	2005	BD30	> 12 mmol/l	
12a	Wayenberg	2005	BD30	> 14 mmol/l	
13a	Wayenberg	2005	BD30	> 16 mmol/l	
14a	Wayenberg	2005	BD30	> 18 mmol/l	
15a	Wayenberg	2005	BD30	> 20 mmol/l	

BD, base deficit; BD30, base deficit at age 30 minutes; HIE, hypoxic ischaemic encephalopathy; h, hour; min, minute

Table 3.2 Index test vs. reference standard: clinical signs before age 6 hours

Test no.	Author	Year	Index test	Threshold	Reference standard
1c	Wayenberg	1994	ENS at 30 min	< 5	Moderate or severe HIE (worst grade in first 5 days of life, defined by Finer)
2c	Nagdyman	2001	AS at 1 min	< 3	Moderate or severe HIE (worst grade in the first 3 days of life, defined by Sarnat)
3c	Wayenberg	2010	LS at 30 min	> 1	Moderate or severe HIE (worst grade in the "first days of life", defined by Finer)

AS, apgar score; ENS, early neurological score; HIE, hypoxic ischaemic encephalopathy; LS, logistic core; min, minute

Table 3.3 Index test vs. reference standard: serum biomarkers other than arterial blood gas alone before age 6 hours

Test no.	Author	Year	Index test	Threshold	Reference standard
1s	Nagdyman	2001	BSCK at 2 h	18.8 U/l	Moderate or severe HIE (worst grade in the first 3 days of life, defined by Sarnat)
2s	Nagdyman	2001	PS100 at 2 h	8.5 mcg/l	
3s	Nagdyman	2001	BSCK & PS100 at 2 h	18.8 U/l 8.5 mcg/l	
4s	Nagdyman	2001	NSE at 2 h	44 mcg/l	
5s	Nagdyman	2001	BSCK at 2 h & Cord arterial pH	18.8 U/l < 6.9	
6s	Nagdyman	2001	BSCK at 2 h & Cord arterial BD	18.8 U/l > 17 mmol/l	
7s	Nagdyman	2001	BSCK at 2 h & 1 min AS	18.8 U/l < 3	
8s	Nagdyman	2001	PS100 at 2 h & Cord arterial pH	8.5 mcg/l < 6.9	
9s	Nagdyman	2001	PS100 at 2 h & Cord arterial BD	8.5 mcg/l > 17 mmol/l	
10s	Nagdyman	2001	PS100 at 2 h & 1 min AS3	8.5 mcg/l < 3	
11s	Qian	2009	Cord Blood: PS100	2.02 mcg/l	Moderate or severe HIE (worst grade in the first 7 days of life, defined by Levine)

AS, apgar score; BD, base deficit; BSCK, brain-specific creatine kinase; h, hour; HIE, hypoxic ischaemic encephalopathy; PS100, protein S 100

Data describing patient-selection methods, exclusion criteria and cohort size are presented in Table 3.4 below. Data describing index test measurement and threshold derivation are shown in Table 3.5. These data informed the responses to the signalling questions summarised in Figure 3.2. The signalling questions informed the consensus risk and applicability assessments for each test, as shown in Table 3.6.

Applicability was "unclear" in one test (1a) in which the predictor was a base deficit of > 7.9mmol/l measured after the infant received colloid at age 30 minutes, because colloid administration after birth is not routinely recommended [16]. Furthermore, it was not clear how many infants received colloid. All the other tests were assessed as applicable in every domain, but each publication used slightly different criteria to define asphyxia or risk of HIE. Blood gas criteria were particularly variable: some studies [20, 21] required an abnormal cord or infant blood gas, while others [16], required either an abnormal blood gas or the need for resuscitation. One study [22] did not require blood gas criteria at all and several others [17-19] did not require a specific threshold, but rather the *availability* of a blood gas at 30 minutes.

The risk of bias was deemed to be "high" in at least one domain of every test and an "unclear" risk of bias was recorded in at least one other domain of every test. There was a "high" risk of bias in the domain of patient selection in three tests described in one paper [17], because it was not clear if patients were consecutively selected and the number of infants meeting exclusion criteria were not listed: the risk for all the remaining tests was rated as "unclear". Only one paper [21] describing three tests clearly stated that patients were consecutively selected. However, the data was collected retrospectively and the numbers of infants meeting exclusion criteria were not listed. Two of the papers [20, 22], including 14 tests, were secondary analyses (sub-studies) within larger studies. The risk of bias in the index test domain was rated as "high" for all these tests. All of the tests derived a threshold for the test rather than utilising a pre-specified threshold: one paper [16] did not describe the method of deriving the threshold for the tests and three others [16, 19, 20] did not describe all test methods adequately.

The most common omissions from the test methods were the sample collection details and the details of the analyser used. There was no discussion on blinding of index test interpretation in the three studies [16, 19, 20] in which a subjective clinical assessment formed the basis of the test. The risk of bias in the domain of reference standard was rated as "high" in 41% and "unclear" in the other studies

Table 3.4 Patient selection methods and criteria for inclusion/ exclusion

Author Year Test No.	Source population and method of cohort selection	Cohort Inclusion criteria	Exclusion criteria (number excluded)	Reasons for exclusions (n)	No. in final cohort: No. with moderate-severe HIE
Wayenberg 1994 1a 1c	Prospective study. Full-term neonates born in Hospital Francais or admitted to the NICU of Erasmus Hospital from July 89–December 91. Method of selection (consecutive, random, or other) not stated.	Inclusion decision at age 30 min. Full-term with two or more of: 1. Fetal distress (abnormal FHR or MSL) 2. AS at 1 or 5 min < 7 or time to establish spontaneous regular respiration > 1 min 3. Arterial BD30 > 10 mmol/l *and* either abnormal perfusion or respiratory distress	Causes of neurological abnormalities other than asphyxia, other neurological abnormalities, metabolic disease, infectious disease, maternal drug use (12)	Neonatal intoxication or withdrawal due to maternal drug use (8), subarachnoid haemorrhage (1), congenital rubella (1), marfan syndrome (1), metabolic disease (1)	60: 13
Da Silva 2000 2a 3a 4a	Prospective study. Full-term neonates born at a University Hospital and an urban regional hospital (specific sites not named nor geographically identified), from January 1993–December 1997. Method of selection not stated.	Inclusion decision at age 30 min. Full-term with: 1. Haemodynamic, respiratory, or neurological abnormalities persisting 30 min after birth 2. Fetal distress suggested by abnormal FHR or fresh MSL and/or AS at 1 or 5 min < 7 or time to establish spontaneous regular respiration > 1 min 3. Arterial BD and lactate measured at 30 min	Congenital malformations, severe birth trauma, birth weight < 10th centile, septicaemia, pulmonary diseases, causes of impairment other than asphyxia	158 infants met first two screening criteria, but only 115 met all three criteria.	115: 20
Nagdyman 2001 5a 6a 2c 1s – 10s	Prospective study. Neonates ≥ 37 wks gestation born between June 1998 and December 1990. Thirty infants meeting inclusion criteria were selected. Method of selection not stated. *This was a sub-study of a cohort that formed part of a case-control study comparing asphyxia to no asphyxia.*	Inclusion decision at birth for asphyxiated cohort; ≥ 37 wks gestation with: 1. Arterial cord pH < 7, or 2. Arterial cord pH 7.01–7.1 and AS "after" 5 min < 7	Congenital anomalies, tumours, maternal drug addiction, severe infections, congenital metabolic disorders (0)	None excluded	30: 7
Shah 2004 7a 8a 9a	Retrospective study. Inborn neonates at ≥ 37 wks at birth at the level 3 NICU of Nepean Hospital (a tertiary referral centre for high-risk neonates in Western Sydney). All neonates born between January 1997 and December 2001, meeting entry criteria were selected consecutively.	Inclusion based on data at age 30 min. ≥ 37 wks gestation with: 1. Fetal distress (abnormal FHR or MSL) 2. AS at 5 min < 5 3. Haemodynamic, respiratory, or neurological abnormalities persisting 30 min after birth 4. Arterial cord pH < 7	Life-threatening congenital malformations, chromosomal abnormalities, intracranial haemorrhage, septicaemia, pulmonary disorders, causes of impairment other than asphyxia	Infants with exclusion criteria were not accounted for but, causes of neurological abnormalities other than asphyxia were ruled out by appropriate investigations.	61: 19

Author Year Test No.	**Source population and method of cohort selection**	**Cohort inclusion criteria**	**Exclusion criteria** (number excluded)	**Reasons for exclusions (n)**	**No. in final cohort: No. with moderate-severe HIE**
Wayenberg 2005 10a 11a 12a 13a 14a 15a	Prospective study. Term neonates born at a University Hospital and an urban regional hospital (specific sites not named nor geographically identified), from January 1990 to December 1997. Method of selection not stated.	Inclusion decision at age 30 min. Term neonates with: 1. Haemodynamic (decreased perfusion or hypotension), respiratory (distress or depression), or neurological (hypotonia, loss of reactivity or seizures) abnormalities persisting 30 min after birth 2. Arterial BD measured at age 30–45 min	Congenital malformations, severe birth trauma, weight < 10th centile, drug-induced depression, culture positive infection, pulmonary diseases, or causes of impairment other than asphyxia (60)	Arterial BD not measured at 30–45 min. 42 neonates excluded; 15 with mild HIE, 7 with moderate or severe HIE: Maternal drug intoxication (9), subarachnoid haemorrhage (3), fetal alcohol syndrome (3), prenatal cerebral damage (2), septicaemia (2), metabolic disease (2), and curarisation for respiratory reasons (3)	237: 41
Qian 2009 11s	Prospective study. Neonates ≥ 37 wks gestation born between June 2003 and December 2006. Forty infants meeting inclusion criteria were selected. Method of selection not stated. *This was a sub-study of a cohort that formed part of a case-control study comparing asphyxia to no asphyxia.*	Inclusion decision at birth: ≥ 37 wks gestation with at least three of: 1. Fetal bradycardia < 100 bpm or late decelerations or absent variability 2. Thick MSL 3. AS at 5 min < 7 4. Resuscitation with positive pressure ventilation and oxygen >1 min immediately after birth	Maternal drug addiction, congenital infection, perinatal infection, or chorioamnionitis	Infants with exclusion criteria were not accounted for.	40: 15
Wayenberg 2010 3c	Prospective study. Full-term neonates born at a University Hospital and an urban regional hospital (specific sites not named nor geographically identified), from January 1998 to December 2005. Method of selection not stated.	Inclusion decision at age 30 min. Term neonates with: 1. Haemodynamic (decreased perfusion or hypotension), respiratory (distress or depression), or neurological (hypotonia, loss of reactivity or seizures) abnormalities persisting 30 min after birth 2. Arterial BD at 30 (25–60) min > 10 mmol/l	Birth weight < 3rd centile, major congenital abnormalities, pulmonary diseases, severe birth trauma, sepsis, lack of ENS (19)	Lack of ENS (15, of whom 6 developed moderate or severe HIE) and ventilation and sedation (4)	75: 15

AS, apgar score; BD, base deficit; BD30, base deficit at age 30 minutes; bpm, beats per minute; ENS, early neurological score; FHR, fetal heart rate; HIE, hypoxic ischaemic encephalopathy; min, minute; MSL, meconium-stained liquor; NICU, neonatal intensive care unit; wks, weeks

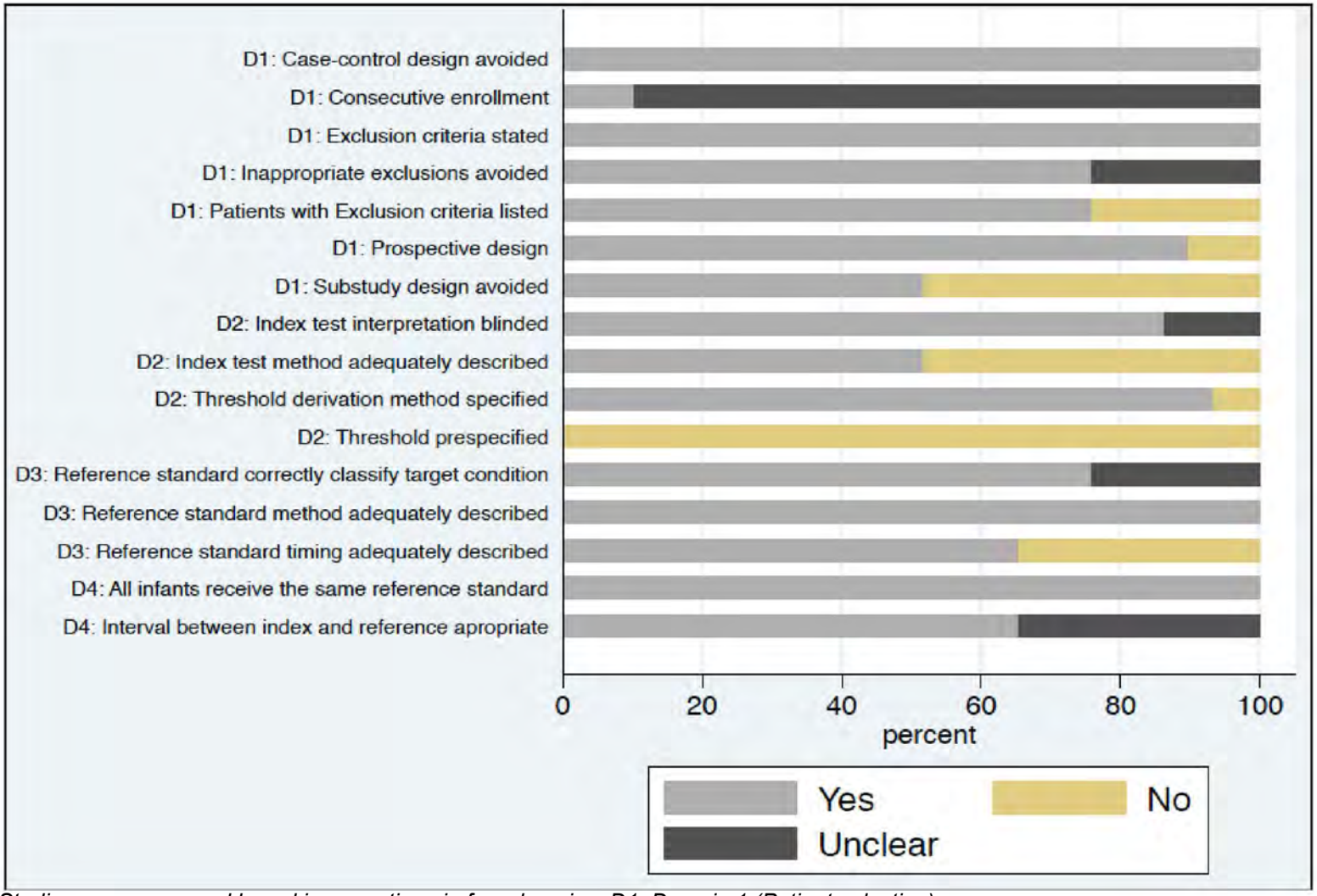
Table 3.5 Index test measurement and threshold derivation

Author year	**Index test or component** (proportion of cohort receiving index test)	**Method of measurement**	**Was a threshold derived or pre-stated?** If derived, what method was used to derive the threshold(s)?
Wayenburg 1994	ENS (100%)	All neonates assessed at age 30 min and scored according to the presence of the following neurological signs: Consciousness : normal/hyperalert = 2, lethargy = 1, stupor = 2 Respiratory pattern: regular = 2, irregular = 1, absent = 0 Moro/grasping: normal/increased = 2, decreased = 1, absent = 0	A threshold was derived. Method of deriving threshold not stated.
Wayenburg 1994	Arterial BD30 after colloid	Radial arterial BD was measured at age 30 min after infusion of 10 ml/kg of colloid, only in infants with "hypoperfusion". Specific handling details and analyser type not stated.	A threshold was derived. Method of deriving threshold not stated.
Da Silva 2000	Arterial BD and lactate at 30 min (100%)	BD and lactate were measured from radial artery blood at age 30 min. 0.3–0.5 ml blood collected in a heparinised syringe and 0.5 ml in fluoride. Stored on ice and rapidly sent to lab. Blood gas measured immediately on a standard analyser. Fluoride sample: serum separated from red cells and analysed within 15 min using an enzymatic method (Abbott TDx/FLx analyser).	Thresholds were derived from ROC curve analysis.
Nagdyman 2001	Serum BSCK at 2 h (100%)	Serum was collected at age 2 h. Initial sample handling was not described. BSCK was determined at 25 °C according to the optimised German standard method on Dax 72 or Modular PP random assessment clinical analysers. CK isoenzymes were fractionated electrophoretically and the area under the curve was used to calculate it's concentration. Measurement time: 0.5 h.	The optimal threshold was derived from ROC curve analysis.
Nagdyman 2001	Serum PS100 at 2 h (100%)	Serum was collected at age 2 h. Initial sample handling was not described. PS100 was measured manually with a sandwich type immuniluminometric assay kit that used MAb and an LB952 luminometer. The assay measured the β chains in both the $\beta\beta$ and $\alpha\beta$ dimers. Measurement time: 3.5 h. Low detection limit 0.02 μg/l.	The optimal threshold was derived from ROC curve analysis.
Nagdyman 2001	Cord arterial pH & BD (100%)	Base deficit and pH measured on cord blood. Specific handling details and analyser type not stated.	The optimal threshold was derived from ROC curve analysis.

Author year	**Index test or component** (proportion of cohort receiving index test)	**Method of measurement**	**Was a threshold derived or pre-stated?** If derived, what method was used to derive the threshold(s)?
Nagdyman 2001	AS at 1 min (100%)	AS assigned at age 1 min.	The optimal threshold was derived from ROC curve analysis.
Shah 2004	Arterial pH, BD and lactate at age ≤ 1 h (100%)	BD and lactate levels were measured from indwelling arterial catheters within 1 h after birth before administration of sodium bicarbonate. Blood gases and lactates were analysed immediately after collection using Radiometer ABL 624 analyser.	The optimal thresholds at 1 hr were derived from ROC curve analysis.
Wayenberg 2005	Arterial BD30 (100%)	BD was measured between 30 and 45 min after birth. 0.5 ml of radial arterial blood was taken into a heparinised syringe. Blood gas was immediately analysed in standard analyser.	The optimal threshold was derived from ROC curve analysis and predictive values were determined for the threshold as well as intervals above and below the threshold.
Qian 2009	Cord arterial PS100 (100%)	At delivery, the umbilical cord was clamped before any signs of breathing, and arterial blood was drawn into 5 ml plastic syringes flushed with a 1000 U/ml heparin solution. The samples left after blood gas analysis were centrifuged at 900 g for 10 min and the supernatants were stored at - 70°C. PS100 was measured by indirect enzyme-linked immunosorbent assay. Detailed methodology was provided. The sensitivity of the assay was 0.02 μg/l.	The optimal threshold was derived from ROC curve analysis and predictive values were determined for the threshold.
Wayenberg 2010	LS at 30 min (100%)	The LS was calculated from the ENS and the BD30 measured in radial arterial blood. (sample handling and measurement not described). LS = (0.33 x BD30) – ENS	The formula for LS was derived in an earlier study and was LS = (0.33 x BD30) – ENS. The optimal threshold was derived from ROC curve analysis and predictive values were determined for the threshold as well as levels above and below to optimise sensitivity and PPV.

AS,apgar score; BD, base deficit; BD30, base deficit at age 30 minutes; BDAC, base deficit after colloid; BSCK, brain-specific creatine kinase; ENS, early neurological score; h, hour; LS, logistic score; min, minute; NSE, neuron specific enolase; pHAC, pH after colloid; PPV, positive predictive value; PS 100, protien S 100; ROC, receiver operating characteristic

Figure 3.2 Study quality assessed by signalling questions recommended by QUADAS-2 guidelines



Studies were assessed by asking questions in four domains: D1, Domain 1 (Patient selection); D2, Domain 2 (Index test); D3, Domain 3 (Reference standard); D4, Domain 4 (Flow and timing)

Table 3.6 Risk of bias and applicability concerns in each index test study

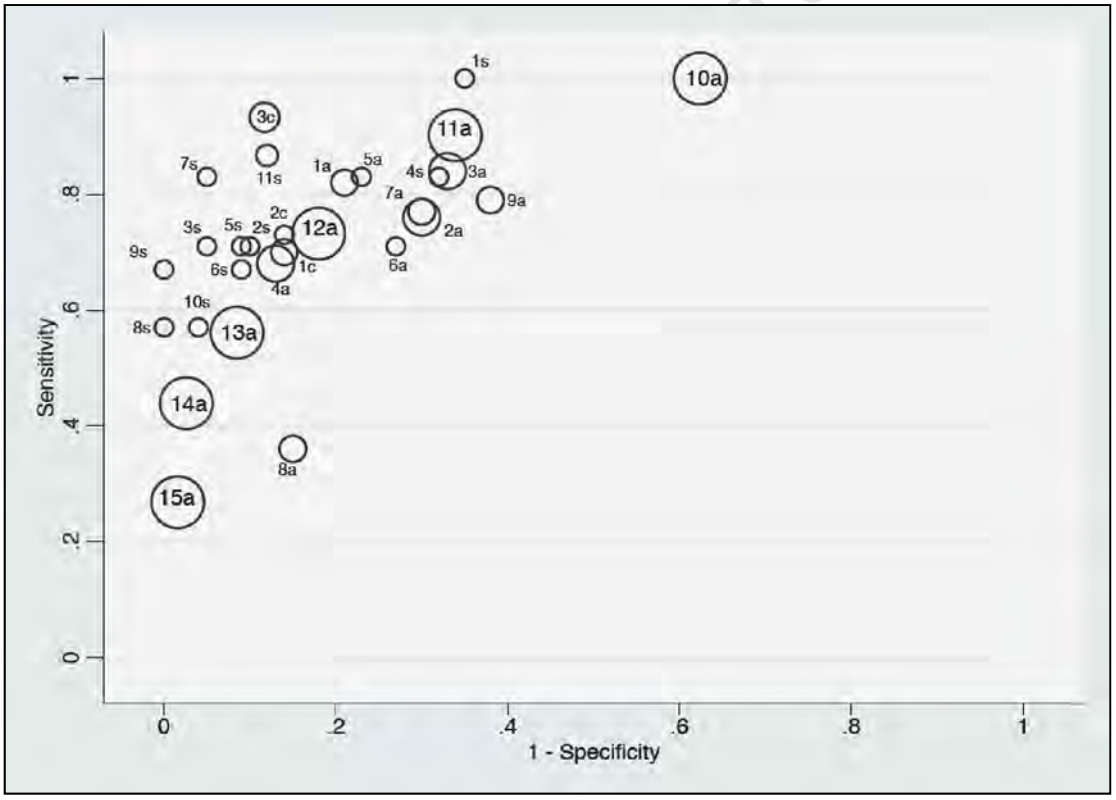
Test no.	Author	Year	Index test description	Threshold	Risk of bias				Applicability concerns		
					Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
1a	Wayenberg	1994	BD30 after colloid	> 7.9 mmol/l	?	H	H	L	L	?	L
2a	Da Silva	2000	BD30	> 12.5 mmol/l	H	H	H	?	L	L	L
3a	Da Silva	2000	Lactate at 30 min	> 9 mmol/l	H	H	H	?	L	L	L
4a	Da Silva	2000	Lactate & BD at 30 min	> 9 & > 12.5 mmol/l	H	H	H	?	L	L	L
5a	Nagdyman	2001	Cord blood BD	> 17 mmol/l	?	H	?	L	L	L	L
6a	Nagdyman	2001	Cord blood pH	< 6.9	?	H	?	L	L	L	L
7a	Shah	2004	BD within 1 h	> 8 mmol/l	?	H	?	L	L	L	L
8a	Shah	2004	pH within 1 h	< 7.1	?	H	?	L	L	L	L
9a	Shah	2004	Lactate within 1 h	> 7.5 mmol/l	?	H	?	L	L	L	L
10a	Wayenberg	2005	BD30	> 10 mmol/l	?	H	H	?	L	L	L
11a	Wayenberg	2005	BD30	> 12 mmol/l	?	H	H	?	L	L	L
12a	Wayenberg	2005	BD30	> 14 mmol/l	?	H	H	?	L	L	L
13a	Wayenberg	2005	BD30	> 16 mmol/l	?	H	H	?	L	L	L
14a	Wayenberg	2005	BD30	> 18 mmol/l	?	H	H	?	L	L	L
15a	Wayenberg	2005	BD30	> 20 mmol/l	?	H	H	?	L	L	L
1c	Wayenberg	1994	ENS at 30 min	< 5	?	H	H	L	L	L	L
2c	Nagdyman	2001	Apgar score at 1 minute	< 3	?	H	?	L	L	L	L
3c	Wayenberg	2010	LS at 30 min	> 1	?	H	H	?	L	L	L
1s	Nagdyman	2001	BSCK at 2 h	18.8 U/l	?	H	?	L	L	L	L
2s	Nagdyman	2001	PS100 at 2 h	8.5 mcg/l	?	H	?	L	L	L	L
3s	Nagdyman	2001	BSCK & PS100 at 2 h	18.8 U/l & 8.5 mcg/l	?	H	?	L	L	L	L
4s	Nagdyman	2001	NSE at 2 h	44 mcg/l	?	H	?	L	L	L	L
5s	Nagdyman	2001	BSCK at 2 h & Cord arterial pH	18.8 U/l & < 6.9	?	H	?	L	L	L	L
6s	Nagdyman	2001	BSCK at 2 h & Cord arterial BD	18.8 U/l & > 17 mmol/l	?	H	?	L	L	L	L
7s	Nagdyman	2001	BSCK at 2 h & 1 min Apgar score	18.8 U/l & < 3	?	H	?	L	L	L	L
8s	Nagdyman	2001	PS100 at 2 h & Cord arterial pH	8.5 mcg/l & < 6.9	?	H	?	L	L	L	L
9s	Nagdyman	2001	PS100 at 2 h & Cord arterial BD	8.5 mcg/l & > 17 mmol/l	?	H	?	L	L	L	L
10s	Nagdyman	2001	PS100 at 2 h & 1 min Apgar score	8.5 mcg/l & < 3	?	H	?	L	L	L	L
11s	Qian	2009	Cord Blood: PS100	2.02 mcg/l	?	H	?	L	L	L	L

AS, apgar score; BD, base deficit; BD30, base deficit at age 30 minutes; BSCK, brain-specific creatine kinase; ENS, early neurological score; h, hour; LS, logistic score; NSE, neuron specific enolase; PS100, protein S 100. L Low risk/concern; H High risk/concern; ? Unclear risk / concern

3.3.2 Predictors of moderate-severe HIE

The ROC plane plot of sensitivity and specificity of all the tests is shown in Figure 3.3 below. The three tests with the highest combined sensitivity and specificity are in the top-left corner of the ROC plane plot: study 3c (a logistic score > 1), study 11s (cord blood protein S100 threshold of 2.02 μg/l) and study 7s (a brain-specific creatine kinase threshold of 18.8 U/l at 2 hours combined with a 1-minute Apgar score < 3). The logistic score described by Wayenberg et al. was derived from a combination of a numeric score based on a neurological assessment and the base deficit, both measured at 30 minutes (logistic score = [0.33xbase deficit at 30 minutes] – early neurological score) [19].

Figure 3.3 Sensitivity and specificity plotted in receiver operating characteristic plane

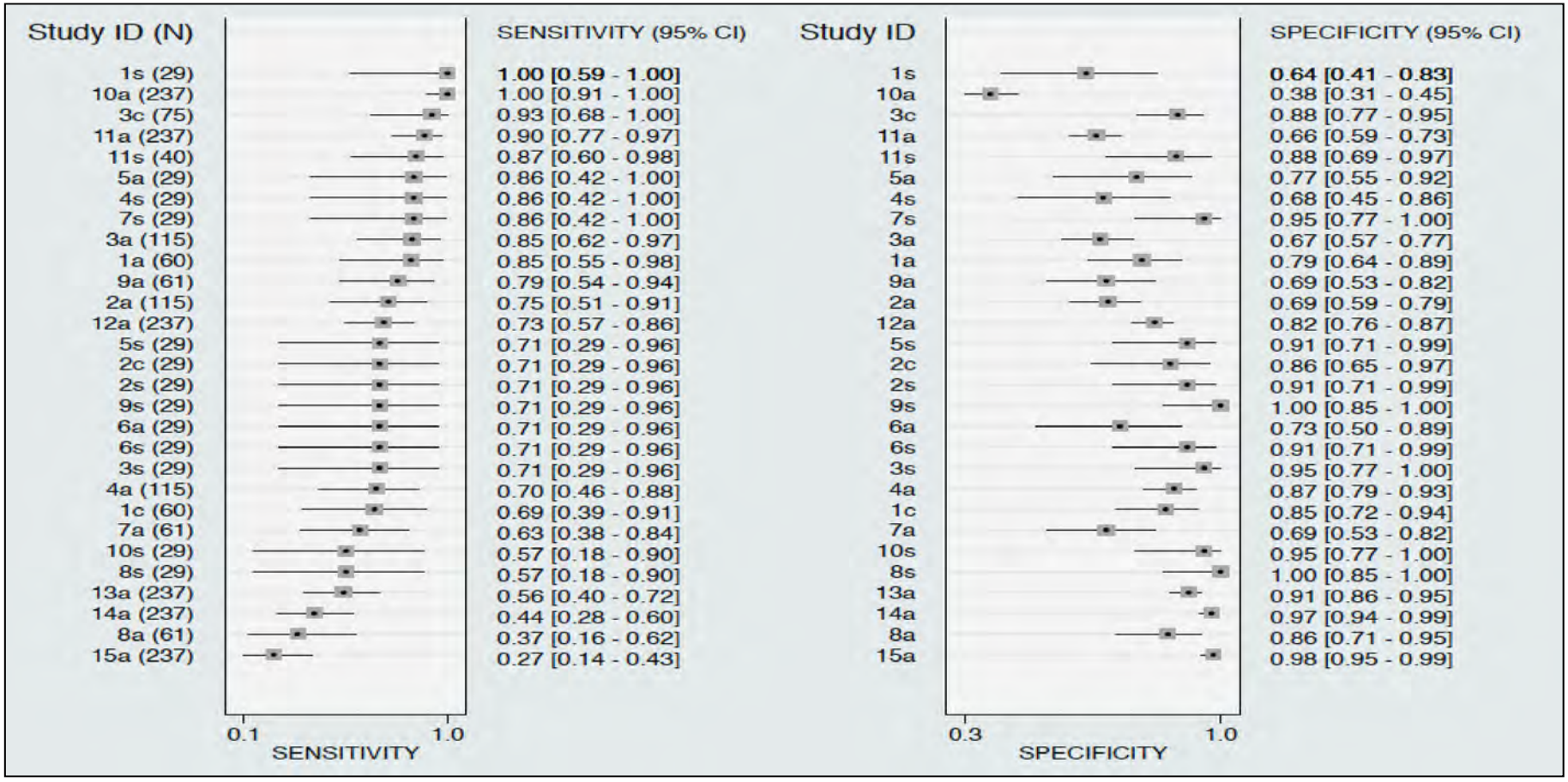


The receiver operating characteristic (ROC) plot shows the tests with the highest sensitivity at the top, and those with the highest specificity (lowest value for 1 – specificity) at the left. In this setting, Sensitivity = the number of infants who have moderate-severe HIE, who also have a positive test (true positives) as a proportion of all infants who have moderate-HIE = True Positive Rate; Specificity = the number of infants who do not have moderate-severe HIE, who also have a negative test (true negatives) as proportion of all infants who do not have moderate-severe HIE. 1 – Specificity = False Positive Rate. The ROC plot facilitates the identification of the test(s) that minimises both false positives and false negatives. The sensitivity and specificity of an ideal test would both be 1, but sensitivity is most important in a screening test. The size of each plot is weighted according to the size of cohort.

The forest plot of sensitivity and specificity is shown in Figure 3.4 below. Studies 3c, 11s and 7s had similar sensitivity (ranging from 86% to 93%), but confidence intervals were wide and overlapped between these tests and the majority of the other tests. The three studies also had similar specificities (ranging from 88% to 95%). The confidence intervals for specificity overlapped between the three tests, but tests 7s and 3c had significantly higher specificities than other tests with similar sensitivity (based on overlap of the means and confidence intervals). The forest plot of likelihood ratios is shown in Figure 3.5 below. Only seven tests had an LR- below 0.2 but the confidence intervals of two of these tests extended to 1 (suggesting non-significance). Tests 3c and 7s had the highest LR+ of the remaining five tests. The forest plot of the diagnostic odds ratios is shown in Figure 3.6 below. The odds ratios for studies 3c and 7s were the highest values of all the studies: 126 and 106 respectively. However, the overlapping confidence intervals of the sensitivity, specificity and DOR beyond the means of these tests, as well as the majority of the other tests, do not suggest significant differences.

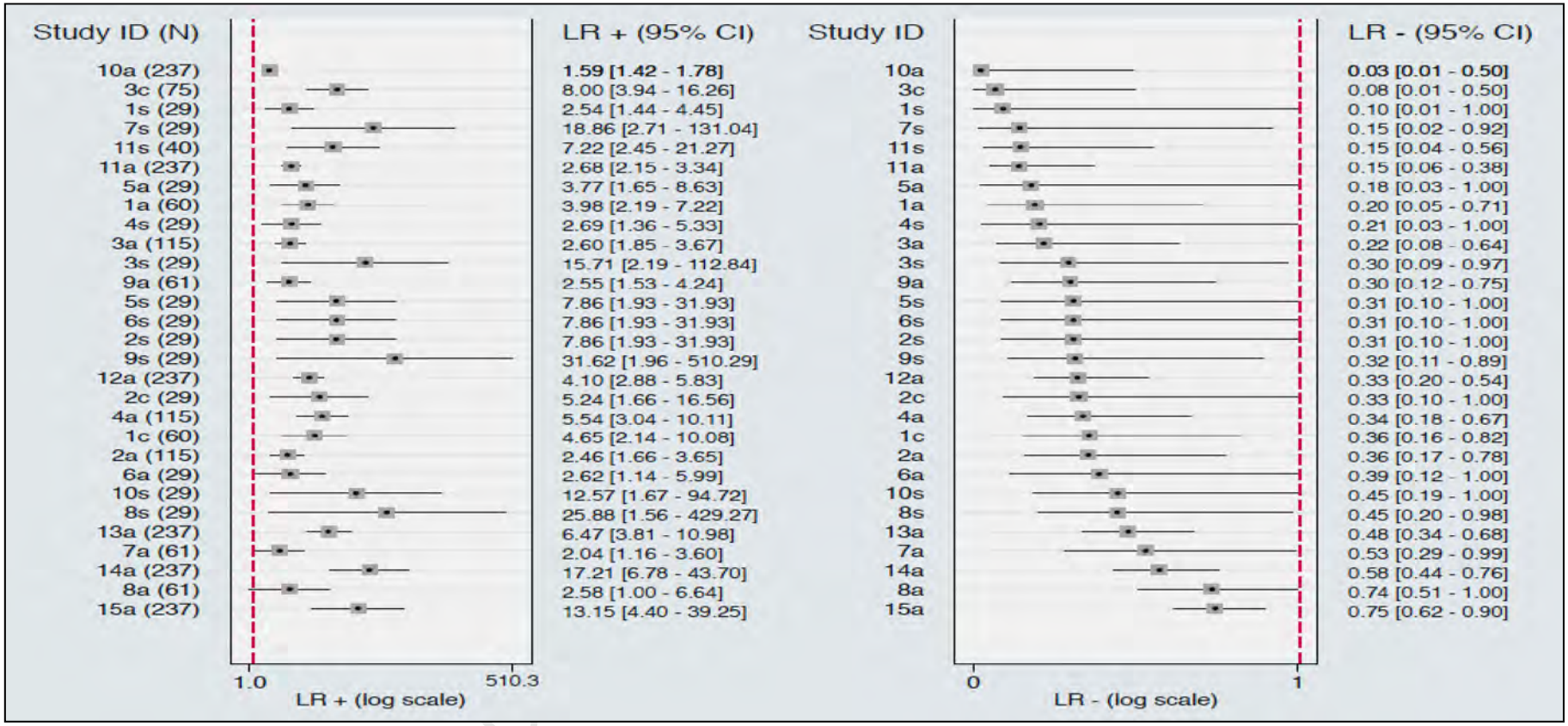
Only two tests had a sensitivity of 100%: tests 1s (a brain-specific creatine kinase threshold of 18.8 U/l at 2 hours) and 10a (an arterial base deficit at 30 minutes > 10 mmol/l). The LR- confidence interval of test 1s extended to 1 while test 10a had a very low specificity (38%). There were significant differences in the predictive values of two tests utilising the arterial base deficit within the first hour of life: test 10a (a base deficit ≥ 10mmol/l at age 30 minutes) had a sensitivity of 100% (95% CI 91 – 100) and specificity of 38% (95% CI 31 – 45) vs. a sensitivity of only 63% (95% CI 38 – 84) and specificity of 69% (95% CI 53 – 82) in test 7a (a base deficit threshold of > 8mmol/l measured in the first hour after birth). However, the LR- of test 7a was very wide (0.29–0.99). BD thresholds of > 12mmol/l were associated with progressively decreasing sensitivity.

Figure 3.4 Forest plot of sensitivity and specificity



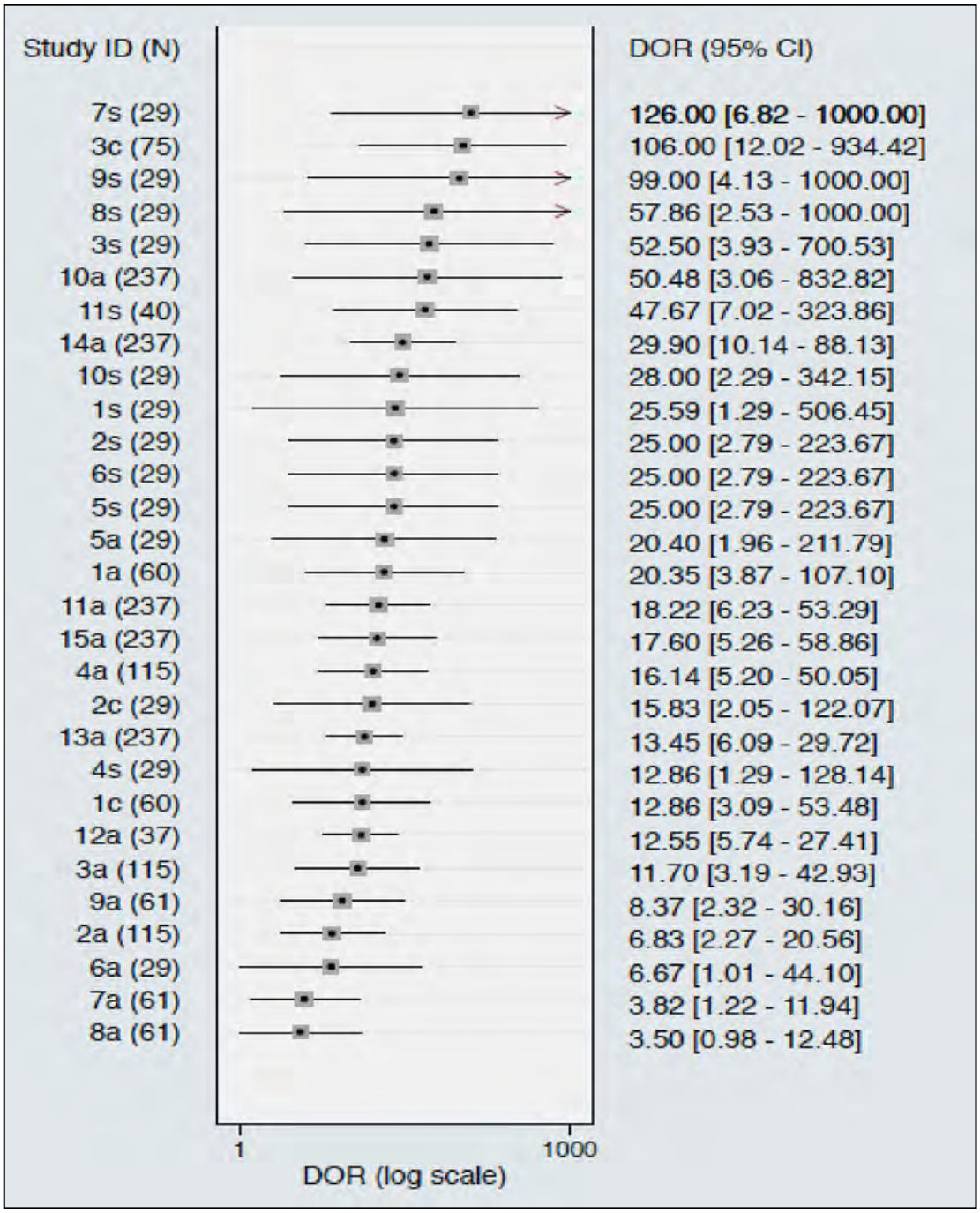
The forest plot shows tests in order of highest sensitivity. The mean (grey square with a black dot) and the 95% CI is shown. The plotting of sensitivity and specificity in this way allows visual comparison between test statistics. The wider Tests are considered significantly different if the 95 % CI of one test does not over lap the mean of another test. The wider the CI, the less certainty there is of the true mean.
ID, identity; CI, confidence interval

Figure 3.5 Forest plot of likelihood ratios



The positive LR (LR+) = true positive rate/false positive rate = sensitivity/ (1 – specificity) = the increase in likelihood of the patient having the disease if the test is positive; the perfect test has a LR+ = infinity. The negative LR (LR –) = false negative rate / true negative rate = (1 – sensitivity)/specificity = decrease in likelihood of the patient having the disease if the test is negative; the perfect test has a LR – = zero. Because the LR is based on a ratio of sensitivity and specificity, it does not vary with varying prevalence. A screening test for a disease where a negative test excludes the patient from lifesaving therapy, should have a low LR –. The forest plot format shows tests in order of ascending values for negative LR. The mean (grey square with a black dot) and the 95% CI is shown. A forest plot of LR in this way allows visual comparison between test statistics. Tests are considered significantly different if the 95 % CI of one test does not over lap the mean of another test. The wider the CI, the less certainty there is of the true mean; the closer the mean or the CI is to 1, the less discriminatory is the test. CI, confidence interval; ID, Identity; LR, likelihood ratio; log, logarithmic

Figure 3.6 Forest plot of diagnostic odds ratios



The DOR = LR+ / LR – ; It is an overall indicator of the diagnostic accuracy of a test. The DOR describes how many times higher the odds are of obtaining a positive test in a diseased (affected) person, than a non-diseased person. The forest plot format shows tests in order of descending values for DOR. The mean (grey square with a black dot) and the 95% CI is shown. A forest plot of DOR in this way allows visual comparison between test statistics. Tests are considered significantly different if the 95 % CI of one test does not over lap the mean of another test. The wider the CI, the less certainty there is of the true mean; the closer the mean or the CI is to 1, the less discriminatory is the test.
CI, confidence interval; DOR, diagnostic odds ratio; ID, Identity; log, logarithmic

3.4 Discussion

This systematic review identified 29 index test studies described in seven papers studying thresholds of clinical assessments or serum biomarkers measured in the first 6 hours of life as predictors of moderate-severe HIE. The quality assessment showed a high risk of bias in all seven papers and quantitative analysis revealed very wide confidence intervals in predictive values for the majority of the tests. The most predictive index test that maintained a high sensitivity was the Logistic Score at age 30 minutes with a threshold of > 1, described by Wayenberg et al. [19].

3.4.1 Quality issues

The main sources of bias during patient selection were the variable inclusion criteria, the lack of detail on the method of patient selection (e.g. consecutive or random or other) and the lack of detail of excluded patients. The studies that only included infants with severely abnormal blood gases may have inappropriately excluded some infants. The most common overall reason for a high risk of bias was the use of a derived threshold rather than a pre-specified threshold for the index test. While it must be acknowledged that the aim of most studies was to determine a threshold, data-driven selection of optimal cut-off levels has been shown to be associated with better test performance than independent samples for which the same threshold is used [23]. Blinding of both index and reference test interpretation was poorly reported. Although blinded interpretation of a clinical index test and reference standard (moderate-severe encephalopathy) is not possible in those cases in which the reference standard is already present at the time the index test is performed, different assessors could be used for the index test vs. the reference standard: this approach was not discussed in any of the studies. All studies described the method of performing the reference standard, but several studies did not state the precise time period within which the reference standard was performed.

3.4.2 Clinical implications

Although the Logistic Score was the most predictive test, the high risk of bias limits immediate clinical application. Furthermore, the benefit of this test beyond age 30 minutes is not known and it requires validation in blinded studies in which the threshold is pre-specified. Although the brain-specific creatine kinase level at 2 hours in the presence of a low 1-minute Apgar score and cord blood protein S 100 also had high predictive values, the timing of the tests is crucial and a bedside kit would need to be developed before they could be used in clinical practice.

Three studies in this review (1a, 7a and 10a) reported moderate-severe HIE occurring at a threshold base deficit below 12 mmol/l [16, 18, 21]. One study (10a) reported 100% sensitivity at a threshold of 10 mmol/l [18]. Importantly, this study did not require a blood gas threshold for recruitment. In the other two studies [16, 21], a lower threshold of 8 mmol/l was not 100% sensitive; the difference was significant in one of these studies despite recruitment being limited to infants with a pH of < 7 [21]. These data show that HIE can occur at a higher threshold blood gas values than those suggested by the International Cerebral Palsy Task Force (i.e. that a pH < 7 and a base deficit ≥ 12mmol/l are required in order to assign a diagnosis of pathological fetal academia) [24]. The use of higher threshold values to define potential intrapartum hypoxia, and hence risk of moderate-severe HIE, is in keeping with the finding that the umbilical arterial base deficit two standard deviations below normal following singleton normal vaginal delivery is 8.3 mmol/l [25]. Moreover, a meta-analysis examining the association between cord pH and perinatal outcome determined a dose-response rather than an absolute threshold [13].

Varying definitions of pathological fetal acidaemia can confound outcomes in epidemiological studies of HIE [26] and may be important to consider when deciding whether to administer neuroprotective therapy. Several cooling studies required a base deficit of > 16 mmol/l as evidence of intrapartum hypoxia [9-11], but others suggested a higher threshold of > 10mmol/l [27] or ≥ 12 mmol/l [28]. The data from this study suggests that lower thresholds of > 10mmol/l will not predict moderate-severe HIE inappropriately if it is combined with an early clinical assessment. In this review, a lactate of > 9mmol/l at 30 minutes had a sensitivity and specificity of 85% and 67% respectively. Da Silva et al. [17] found that serum lactate was > 5 mmol/l at 30 minutes in all infants who subsequently developed moderate-severe HIE, but the specificity was not stated and is likely to be low. However, this low threshold may be useful when combined with an early clinical evaluation.

The role of early clinical assessment as the final arbiter is emphasised by the recent findings of a population-based, prospective matched control study of 78 infants born with metabolic acidosis (umbilical artery pH < 7.05 and base deficit > 12mmol/l) [29]: the infants with acidosis who appeared well and did not have early signs of encephalopathy (base deficit range: 12.2–16.6 mmol/l) were not at increased risk of developing neurological or psychological problems compared to non-acidotic controls (base deficit range: -3.9 to 10.2 mmol/l).

3.4.3 Conclusions

This review has limitations. The literature search was limited to English language and publications outside of Medline were not reviewed. The strengths of the review are the clearly defined eligibility criteria and the process of rigorous qualitative evaluation according to published guidelines.

In conclusion, our review identified a small number of studies identifying predictors of moderate-severe HIE and all studies showed a high risk of bias. The Logistic Score at 30 minutes was the strongest predictor. A base deficit of > 10 mmol/l at 30 minutes alone, was the most sensitive predictor but the specificity is too low to allow use as a predictor on its own. Sequential base deficit thresholds of > 12 mmol/l were associated with progressively decreasing sensitivity and will miss infants with moderate-severe HIE if used as an essential criteria for the diagnosis of moderate-severe HIE. All the thresholds identified in this review require validation in the form of pre-specified thresholds in prospective and appropriately blinded studies. There is a need to re-examine predictors of moderate-severe HIE other than by means of aEEG in high-quality prospective studies. In particular, early clinical assessment, combined with intrapartum history or blood gas data, should be evaluated further as it is the most accessible and cost-effective test available.

3.5 References

1. National Institute for Health and Clinical Excellence: Therapeutic hypothermia with intracorporeal temperature monitoring for hypoxic perinatal brain injury [internet]. 2010 [updated 2010 May 26; cited 2012 August]. Available from: http://www.nice.org.uk/nicemedia/live/11315/48809/48809.pdf.

2. Perlman M, Shah PS. Hypoxic-Ischemic Encephalopathy: Challenges in Outcome and Prediction. *J Pediatr* 2011;158(2):e51-e4.

3. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;33(10): 696-705.

4. Fenichel GM. Hypoxic-ischemic encephalopathy in the newborn. *Arch Neurol* 1983;40(5):261-6.

5. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

6. Hellström-Westas L, Rosén I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. *Arch Dis Child Fetal Neonatal Ed* 1995;72(1):F34-8.

7. Spitzmiller RE, Phillips T, Meinzen-Derr J, Hoath SB. Amplitude-integrated EEG is useful in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischemic encephalopathy: a meta-analysis. *J Child Neurol* 2007;22(9):1069-78.

8. Toet MC, Hellström-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 1999;81(1):F19-23.

9. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460): 663-70.

10. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361(14):1349-58.

11. Simbruner G, Mittal RA, Rohlmann F, Muche R, neon.EURO.network Trial Participants. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics* 2010;126(4):e771-8.

12. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. *Pediatr Neurol* 2009;40(3):215-26.

13. Malin GL, Morris RK, Khan KS. Strength of association between umbilical cord pH and perinatal and long term outcomes: systematic review and meta-analysis. *BMJ* 2010;340:c1471.

14. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6(7):e1000097.

15. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155(8):529-36.

16. Wayenberg JL, Vermeylen D, Bormans J, Magrez P, Muller MF, Pardou A. Diagnosis of severe birth asphyxia and early prediction of neonatal neurological outcome in term asphyxiated newborns. *J Perinat Med* 1994;22(2):129-36.

17. Da Silva S, Hennebert N, Denis R, Wayenberg JL. Clinical value of a single postnatal lactate measurement after intrapartum asphyxia. *Acta Paediatr* 2000;89(3):320-3.

18. Wayenberg JL. Threshold of metabolic acidosis associated with neonatal encephalopathy in the term newborn. *J Matern Fetal Neonatal Med* 2005;18(6):381-5.

19. Wayenberg JL. The logistic score: a criterion for hypothermia after perinatal asphyxia? *J Matern Fetal Neonatal Med* 2010;23(5):448-54.

20. Nagdyman N, Kömen W, Ko HK, Müller C, Obladen M. Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. *Pediatr Res* 2001;49(4):502-6.

21. Shah S, Tracy M, Smyth J. Postnatal lactate as an early predictor of short-term outcome after intrapartum asphyxia. *J Perinatol* 2004;24(1):16-20.

22. Qian J, Zhou D, Wang Y-W. Umbilical artery blood S100beta protein: a tool for the early identification of neonatal hypoxic-ischemic encephalopathy. *Eur J Pediatr* 2009;168(1):71-7.

23. Leeflang MMG, Moons KGM, Reitsma JB, Zwinderman AH. Bias in sensitivity and specificity caused by data-driven selection of optimal cutoff values: mechanisms, magnitude, and solutions. *Clin Chem* 2008;54(4):729-37.

24. MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* 1999;319(7216):1054-9.

25. Riley RJ, Johnson JW. Collecting and analyzing cord blood gases. *Clin Obstet Gynecol* 1993;36(1):13-23.

26. Horn AR, Swingler GH, Myer L, Harrison MC, Linley LL, Nelson C, et al. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. *J Perinat Med* 2013;41(2):211-217.

27. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353(15):1574-84.

28. Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch Pediatr Adolesc Med* 2011;165(8):692-700.

29. Hafström M, Ehnberg S, Blad S, Norén H, Renman C, Rosén KG, et al. Developmental outcome at 6.5 years after acidosis in term newborns: a population-based study. *Pediatrics* 2012;129(6):e1501-7.

Chapter 4

Defining hypoxic ischaemic encephalopathy in newborn infants: benchmarking in a South African population

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Author contributions

I was the principal investigator. I conceptualised the study, wrote the research proposal, supervised the data collection and analysed the data. I wrote all versions of the manuscript and submitted it to the journal. GHS, LM and NJR contributed to discussions around the design of the study and reviewed the paper critically for important intellectual content. MCH, LLL, CN, LT and NRR contributed to data acquisition. All authors reviewed and approved the final version of the paper.

Notes

Minor amendments recommended by the examiners of the thesis have been included.

The data-collection sheet is included at the end of the thesis as Appendix A.

Abstract

Objectives

There are few population-based studies of hypoxic ischaemic encephalopathy (HIE) in Sub-Saharan Africa and the published criteria that are used to define and grade HIE are too variable for meaningful comparisons between studies and populations. Our objectives were:

(i) to investigate how the incidence of HIE in our region varies with different criteria for intrapartum hypoxia; and

(ii) to determine how encephalopathy severity varies with different grading systems.

Method

We reviewed the records of infants with a diagnosis of HIE, born between September 2008 and March 2009 in public facilities in the Southern Cape Peninsula, South Africa. The incidence of HIE was calculated according to four different definitions of intrapartum hypoxia and graded according to three different methods.

Results

Depending on which defining criteria were applied, the incidence of HIE varied from 2.3 to 4.3 per 1 000 live births. The incidence of mild HIE ranged from 0.4 to 1.3 per 1 000 live births. The incidence of moderate-severe HIE ranged from 1.5 to 3.7 per 1 000 live births. Ninety-seven of the 110 infants (88%) reviewed had at least one intrapartum-related abnormality. Only 62 infants (56%) had a blood gas performed in the first hour of life.

Conclusion

The incidence and grade of HIE can vary more than two-fold in the same population, depending on which defining criteria are used. Consensus definitions are needed for benchmarking.

Introduction

The proportion of child deaths occurring throughout the world during the neonatal period, increased from 38% [1] to over 41% [2] in the past decade. Neonatal deaths in the first week of life have been identified as the group that has made the least progress towards achieving Millennium Development Goal 4 of reducing child mortality, and an audit of adverse outcomes related to acute intrapartum events is proposed as one of the top research priorities [3]. Data collection has been directed at intrapartum stillbirths and early neonatal deaths, but there is increasing recognition of the need to determine the incidence of neonatal encephalopathy (NE), particularly hypoxic ischaemic encephalopathy (HIE), as a major source of morbidity and mortality [4, 5].

NE is the clinical manifestation of disordered neonatal brain function in the term infant in the early neonatal period [6]. The etiology of NE is varied, with genetic, metabolic and infective conditions presenting with similar clinical signs. The disorder is termed HIE if there is evidence that intrapartum hypoxia is the cause of the encephalopathy [7].

A recent international review [8] of three population studies [9-11] in the United Kingdom, Australia and Sweden between 1991 and 1996 derived an HIE incidence of 1.5 per 1 000 live births. Interpretation and direct comparison of these studies was limited because different definitions of HIE were used. A standardised definition and classification of HIE is needed for comparison between studies of populations and benchmarking throughout the world.

The actual incidence of HIE may be under-reported when applying stringent medico-legal criteria, which require strong evidence of obstetric causality, including blood gas values or multiple intrapartum anomalies as evidence of intrapartum hypoxia [12]. Lawn et al. [4] used the term “intrapartum-related neonatal mortality rate” to describe deaths in infants ≥ 2 kg where intrapartum abnormalities were present and intrapartum hypoxia was the suspected cause of death. The same authors referred to “intrapartum-related impairment” to describe impaired survivors following birth asphyxia. Applying this principle and terminology to NE may allow the identification of infants with probable HIE who do not meet medico-legal criteria.

The objectives of this study were:

(i) to investigate how the incidence of HIE in the Southern Cape Peninsula of South Africa varies with different criteria for intrapartum hypoxia; and

(ii) to determine how encephalopathy severity varies with the grading system used.

Methods

The study was approved by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town, and the study conforms to the principles of the Declaration of Helsinki, version 2008 [13].

Setting

This study was performed at three referral hospitals in Cape Town serving the Southern Cape Peninsula of the Western Cape in South Africa: Groote Schuur Hospital (GSH), a tertiary referral hospital with 75 neonatal beds and 5 760 deliveries per annum; New Somerset Hospital (NSH), a secondary referral hospital with 42 neonatal beds and 7 100 deliveries per annum; and Mowbray Maternity Hospital (MMH), a secondary referral hospital with 73 beds and 9 200 deliveries per annum. Delivery figures are for 2009.

The Southern Cape Peninsula has a well-defined health service within the Western Cape Province. The service comprises primary healthcare clinics, primary healthcare hospitals, midwife-run primary healthcare obstetric units and three referral hospitals. Women frequently present in active labour, with limited antenatal or intrapartum data. Many primary care facilities do not have facilities for performing blood gas analysis. If home births occur, the infants are taken to one of the healthcare facilities after delivery. All infants with HIE in the region are referred to the secondary or tertiary hospitals.

Study sample

The hospital records of infants born between September 1, 2008 and March 31, 2009 were retrospectively reviewed. Inclusion criteria were: a gestational age of ≥ 36 weeks at birth, a birth weight of ≥ 2 kg and a diagnosis of HIE in the hospital record.

Exclusion criteria were any of the following: congenital infection confirmed on culture or serology and/or typical external signs such as hepatosplenomegaly or skin rash, chromosomal syndromes, cerebral malformations, lethal congenital malformations, neonatal abstinence syndrome due to maternal substance abuse, or metabolic encephalopathy.

Regional birth data

The number of live births in the Southern Cape Peninsula during the study period was obtained from the Perinatal Problem Identification Programme database maintained at MMH. This database contains birth data from all the clinics and hospitals within the service and it includes home births. The incidence of HIE in the Southern Cape Peninsula was calculated using this data as a denominator. The neonatal mortality rate (NMR) for the Southern Cape Peninsula was not available.

Data collection and analysis

The following maternal and neonatal data were collected from the infants' admission records: maternal antenatal booking data; infant weight, gender and gestation; mode of delivery; Apgar scores at 1, 5 and 10 minutes; delivery room interventions; blood gas findings in the first hour of life; peripartum complications; infant neurological assessments during the first three days of life; survival; and length of stay.

Evidence of potential intrapartum hypoxia was defined according to three different published criteria and a less restricted classification; "intrapartum-related abnormality" was also defined (Table 4.1). The published criteria included those recommended by the International Cerebral Palsy Task Force (ICPTF) [12] and criteria recommended by several therapeutic hypothermia trials, including the National Institute of Child Health and Human Development (NICHD) trial [14], the Coolcap trial [15] and the TOBY trial [16]. Classification in one group did not exclude classification in another because the criteria for each group overlapped. After infants were classified according to evidence for intrapartum hypoxia or intrapartum-related abnormalities, the encephalopathy was graded according to documented clinical signs indicating moderate-severe NE defined by Sarnat and Sarnat [17], Thompson et al. [18], and Shankaran et al. [14] and the incidence of clinical seizures was documented. The clinical data were derived from neurological assessments that were recorded daily as a standard of care using the numeric HIE score described by Thompson et al. [18]. Moderate-severe NE was defined as a maximum score of 11 or more because this threshold was previously correlated with moderate-severe

NE defined by Sarnat et al. [18]. Moderate-severe NE according to Shankaran et al. was defined by the presence of one or more signs in at least three of six possible categories [14], but pupil response and heart rate were not considered as these signs were not uniformly recorded in the hospital record. Infants with a depressed level of consciousness and/or seizures were graded as moderate-severe NE, according to Sarnat and Sarnat [17].

Table 4.1 Classifying criteria indicating potential intrapartum hypoxia or an intrapartum-related abnormality

Classification	Criteria
ICPTF Blood gas criteria [12]	A first-hour blood gas with base deficit ≥ 12 mmol/l or pH < 7
NICHD criteria required for therapeutic hypothermia [14]	A first-hour blood gas with base deficit > 16 mmol/l or pH < 7, OR If base deficit is 10–15.9 mmol/l or pH is 7.01–7.15 or blood gas is not available, then a history of an acute intrapartum event is required; *plus, either* an Apgar score at 10 minutes ≤ 5 *or* respiratory support continued for 10 minutes after birth An "intrapartum event" was defined by the presence of one or more of the following abnormalities: abruptio placentae, intrapartum haemorrhage other than abruption, uterine rupture, prolapsed cord, maternal seizures or arrest, abnormal fetal heart rate (bradycardia, delayed decelerations or decreased beat-to-beat variability), shoulder dystocia or head entrapment
CoolCap [15] / TOBY [16] criteria required for therapeutic hypothermia	A first-hour blood gas with base deficit ≥ 16 mmol/l or pH < 7, OR respiratory support continued for 10 minutes after birth, OR an Apgar score at 10 minutes ≤ 5
Intrapartum-related abnormality	A first-hour blood gas with base deficit ≥ 10 mmol/l or pH < 7, OR an acute intrapartum event as defined above, OR an Apgar score at 5 minutes < 7, OR MSL, OR a prolonged second stage of labour, OR respiratory support continued for 10 minutes after birth

ACOG, American College of Obstetricians and Gynecologists;
ICPTF, International Cerebral Palsy Task Force; MSL, meconium-stained liquor
NICHD; National Institute of Child and Human Development

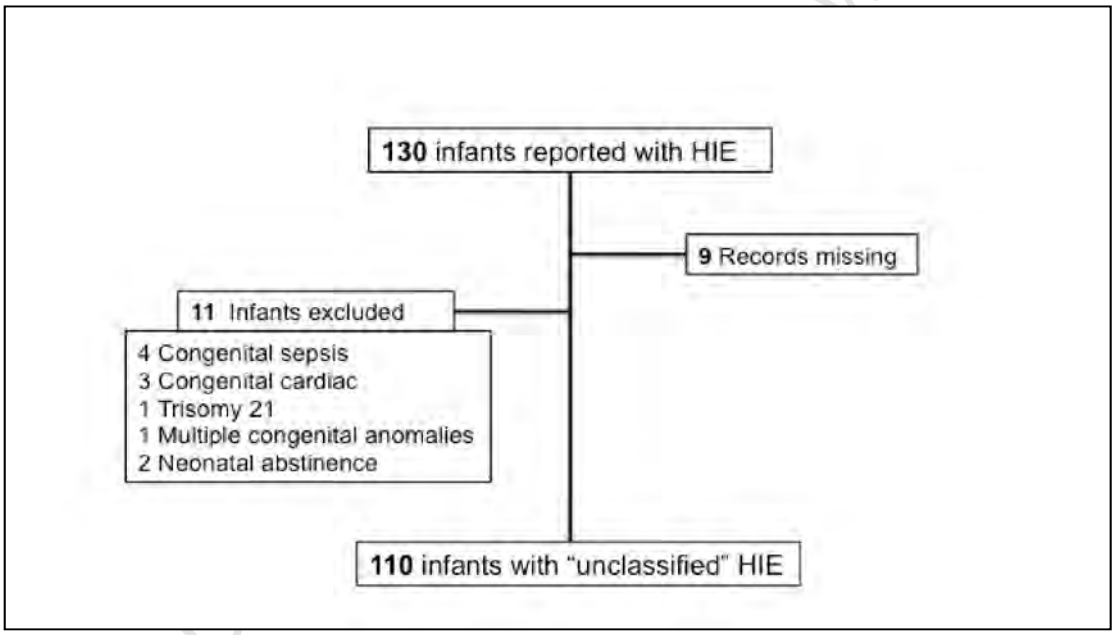
Data were analysed with Stata 11 (Stata Corporation, Texas, USA). Non-parametric data were described using medians and interquartile ranges (IQR). Proportions within subgroups were compared using Fisher's exact test. Because of the dependent nature of the data, a one-sample test method was used: the differences between HIE incidences according to different definitions were accepted as significant if the confidence intervals of one incidence did not overlap with the observed value of the other incidence. In addition, the McNemar exact test was used

to derive p-values by comparing the standard error of the means (incidences). All statistical tests are two-sided at alpha = 0.05.

Results

There were a total of 22 674 live births in the Southern Cape Peninsula during the period studied. Inclusion criteria were met in 130 infants, but infant records were not available for nine of these infants and 11 infants were excluded. The infant records of the remaining 110 infants were reviewed (Figure 4.1).

Figure 4.1 Newborns with a diagnosis of HIE during the 7-month study period



HIE, hypoxic ischaemic encephalopathy

Perinatal data and short-term neonatal outcomes are shown in Table 4.2. Most mothers were younger than 28 years, and almost a quarter were HIV positive. Twenty per cent of the infants were born in a primary health care facility and 90% of the infants had a birth weight of more than 2500 g. Of the 16% of infants who died, most did so by three days after birth.

The 5-minute Apgar score was documented in 107 of the 110 infants (97%), and in 70 infants, the 5-minute Apgar score was < 7. Only 62 infants (56%) had a blood gas performed in the first hour of life. The proportions of infants with abnormal blood gases and low 5-minute Apgar scores are shown in Table 4.3. A first-hour blood gas

was available in only 46 of the 70 infants (66%) with a 5-minute Apgar score of < 7, and 43 of these 46 infants (93%) had a first-hour pH of < 7.0 or a base deficit of ≥ 12 mmol/l.

Table 4.2 Perinatal characteristics, complications and short-term neonatal outcome (N = 110)

Characteristics, complications and outcomes	n (%) / median (IQR)
Maternal Baseline Characteristics	
Age (years)	24 (20–27)
HIV negative	80 (73%)
Antenatal syphillis, but adequately treated	2 (2%)
Infant baseline characteristics	
Birth weight (grams)	3 165 (2 915–3 520)
Male gender	59 (54%)
Gestation (weeks)	40 (39–40)
Born in secondary or tertiary hospital	88 (80%)
Delivery mode	
Caesar	34 (31%)
NVD	56 (51%)
Forceps/ventouse	19 (17%)
Breech	1 (1%)
Apgar score	
1 minute (n = 107)	3 (2–5)
5 minute (n = 107)	6 (4–7)
10 minute (n = 104)	7 (6–8)
Resuscitation and blood gas assessment at birth	
Chest compressions given at birth (unknown in 1)	27 (25%)
Adrenaline given at birth (unknown in 1)	10 (9%)
Cord blood gas done	30 (27%)
Blood gas in 1st hour or cord gas done	62 (56%)
Worst pH 1st hour (n = 62)	7.06 (6.93–7.17)
Worst base deficit 1st hour (n = 62)	17.5 (20.5–14)
Peripartum complications	
Abruptio placentae	7 (6%)
Ante partum haemorrhage other than abruption	1 (1%)
Uterine rupture	0
Prolapsed cord	5 (5%)
MSL	43 (39%)
Maternal seizures	2 (2%)
Abnormal fetal heart rate	40 (36%)
Prolonged second stage	22 (20%)
Shoulder dystocia	3 (3%)
Head entrapment	1 (1%)
Maternal arrest	0
Prolonged rupture of membranes (Two had chorioamnionitis)	4 (4%)
Chorioamnionitis	3 (3%)
Maternal sepsis	2 (2%)
Short-term neonatal outcome	
Encephalopathy at age < 6 hours (unknown in 4)	95 (87%)
Death or Thompson Score ≥ 15	35 (32%)
Death	17 (16%)
Age of death in days (n = 17)	1 (1–3)
Length of stay if survived (n = 93)	8 (5–11)

HIV, human immunodeficiency virus; IQR, interquartile range; NVD, normal vertex delivery

Table 4.3 5-minute Apgar scores in infants with blood gas data in the first hour of life (N = 62)

Blood gas parameter (N = 62)		n	5-minute Apgar score < 7 n (%)	P
Base deficit ≥ 10 mmol/l	Yes	54	43 (80)	0.022
	No	8	3 (38)	
Base deficit ≥ 12 mmol/l or pH < 7	Yes	53	43 (81)	0.007
	No	9	3 (33)	
Base deficit ≥ 16 mmol/l or pH < 7	Yes	43	33 (72)	0.538
	No	19	13 (68)	

Intrapartum events (defined in Table 4.1) were present in 45 of 110 infants (41%). When meconium-stained liquor and a prolonged second stage of labour were added as alternative criteria to intrapartum events, a further 27 (24%) and 12 (11%) infants were classified respectively. The addition of a 5-minute Apgar score of < 7, a first-hour base deficit of ≥ 10 mmol/l and continued respiratory support at 10 minutes as further alternative individual criteria enabled the classification of 97 of the 110 infants reviewed (88%) and defined the category, "intrapartum-related abnormalities". Twelve of the 13 infants who did not have a documented intrapartum-related abnormality also did not have a blood gas assessment in the first hour of life.

The incidence of intrapartum-related abnormalities and three different incidences of intrapartum hypoxia associated with HIE or clinical seizures within the same population of infants are shown in Table 4.4. When the different methods of grading HIE were applied to these groupings, the incidence of moderate-severe HIE varied from 1.5 to 3.7 per 1 000 live births and the incidence of mild HIE ranged from 0.4 to 1.3 per 1 000 live births.

Table 4.4 HIE incidence per 1 000 live births according to various classification criteria for intrapartum hypoxia and HIE grade

	ICPTF [12] (95% CI)	NICHD [14] (95% CI)	Coolcap / TOBY [15] [16] criteria (95% CI)	Intrapartum-related abnormality (95% CI)
All grades of HIE	2.3 (1.7–3)	2.3 (1.8–3)	2.9 † (2.3–3.8)	4.3 ‡ (3.5–5.2)
Moderate-severe HIE defined by Shankaran [14]	1.9 (1.4–2.6)	1.9 (1.4–2.6)	2.5 † (1.9–3.3)	3.7 ‡ (2.9–4.5)
Moderate-severe HIE defined by Sarnat [17]	1.8 (1.3–2.4)	1.8 (1.3–2.5)	2.3 † (1.7–3.1)	3.4 ‡ (2.7–4.2)
Moderate-severe HIE defined by Thompson [18] Score > 10	1.5 (1–2.1)	1.5 (1.1–2.1)	2.0 † (1.4–2.7)	3.0 ‡ (2.4–3.8)
Clinical seizures present	1.3 (0.9–1.9)	1.4 (0.9–1.9)	1.7 † (1.2–2.4)	2.8 ‡ (2.1–3.6)

‡, p < 0.00001 compared to similar grade defined by Coolcap/TOBY criteria for intrapartum hypoxia;
†, p < 0.005 compared to similar grade defined by NICHD/ICPTF criteria for intrapartum hypoxia;
CI, confidence interval; ICPTF, International Cerebral Palsy Task Force blood gas criteria to define intrapartum hypoxia; NICH, National Institute of Child and Human Development cooling study criteria to define intrapartum hypoxia

Discussion

If the presence of one or more intrapartum-related abnormalities, in association with NE without other obvious cause, is accepted as an indication of intrapartum hypoxia, then our data suggest that the incidence of HIE in the public health sector of the Southern Cape Peninsula during the study period was 4.3 per 1 000 live births. If more stringent evidence of intrapartum hypoxia, defined by a blood gas pH of < 7 or a base deficit of ≥ 12 mmol/l in the first hour of life is required, then the incidence was only 2.3 per 1 000 live births: with a large proportion of infants lacking blood gas data, this would represent a significant under-reporting of HIE in our setting. The incidence of mild HIE varied from 0.4 to 1.3 per 1 000 live births and the incidence of moderate-severe HIE varied from 1.5 to 3.7 per 1 000 live births, depending on which criteria for both intrapartum hypoxia and HIE grading were used.

A recent global health review of worldwide NMR, intrapartum-related NMR and NE reported an increasing incidence of NE and intrapartum-related NMR with increasing NMR, although the ranges were wide [4]. In settings with a NMR of 6–15 per 1 000 live births, the NE incidence was 4.7–8.7 per 1 000 live births [4]. The NMR for the

Southern Cape Peninsula was not available, but the Western Cape Provincial NMR was 6 per 1 000 live births for 2009 [19]: an HIE incidence of 4.3 per 1 000 live births is lower than expected for this NMR, but within the NE incidence of 3.6–10.2 per 1 000 live births recorded for regions with a NMR of 16–30 per 1 000 live births. These discrepancies may relate to the lack of uniform diagnostic criteria for NE and HIE.

Many NE grading classifications are category-based modifications of Sarnat and Sarnat's original description [17], and infants may have characteristics in more than one category, resulting in diagnostic uncertainty. The amplitude-integrated electroencephalogram (aEEG) is probably the most objective and accurate method of grading NE [20, 21], but it is not uniformly available. The clinical classification systems proposed by Thompson et al. [18] and Shankaran et al. [14] allow grading when signs are present in different categories. The Shankaran et al. method is intended for use before age 6 hours [14]. In our study, this method classified more infants with moderate-severe encephalopathy than did any other method, although the incidence using this method was not significantly different in comparison to either the Sarnat or Thompson grading systems.

There are few published data on the incidence of HIE in Sub-Saharan Africa. A hospital-based study from South Africa during 2009 showed an incidence of 8.3 per 1 000 live births [22]. A population-based study in the Southern Cape Peninsula reported HIE in 3.6 per 1 000 live births and moderate-severe HIE in 1.7 per 1 000 live births in 2002 [23]. A more recent clinic-based study, within the same region, determined an incidence of 9 per 1 000 live births [24]. All three studies used different criteria for HIE. In the Southern Cape Peninsula study, the selection criteria were; a history suggesting intrapartum hypoxia, a birthweight of > 2 500 g and NE. Infants with encephalopathy presumed to be due to causes other than intrapartum hypoxia were excluded. Moderate-severe NE was defined as a Thompson encephalopathy score of > 10 [18]. Our data are comparable to the earlier study in the region if we define intrapartum hypoxia as intrapartum-related abnormalities and restrict our sample to infants with a birth weight of > 2 500 g. In our study, the incidence of HIE with these criteria would be 3.8 per 1 000 live births and that of moderate-severe HIE, 2.7 per 1 000 live births. These figures suggest an apparent increase in the incidence of HIE between 2002 and 2009. The increase in HIE morbidity might be explained, in part, by the large increase in live births per month for the region, from 2 292 in 2002 to 3 239 in 2009. Because of potentially different criteria for intrapartum hypoxia, these figures must be interpreted with caution.

The ICPTF and the American College of Obstetricians and Gynecologists (ACOG) state that a cord or early infant blood gas with a pH of < 7 or a base deficit of ≥ 12 mmol/l represents significant fetal acidaemia and intrapartum hypoxia [12, 25] and indicates potential permanent brain damage when associated with early onset NE. There was no claim that these thresholds define the level of fetal acidaemia that indicates fetal hypoxia per se. The normal range of umbilical arterial base excess varies according to the mode of delivery. The upper limit of the base deficit for a term singleton vaginal delivery is reportedly 8.3 mmol/l [26], but the severity of metabolic acidosis is less if delivery is by caesarean section [27]. Several epidemiological studies [28-30] have found that mild or moderate NE, presumed to be secondary to intrapartum events, occurred with a base deficit in the range 8–12 mmol/l during the first hour of life. Da Silva et al. [29] and Wayenberg et al. [30] applied Finer's definition of NE [31] and measured arterial base excess at age 30–45 minutes. There were no infants with moderate or severe NE with a base deficit of < 10 mmol/l. Several therapeutic hypothermia trials [14-16] required a base deficit of ≥ 16 mmol/l as a blood gas criterion for intrapartum hypoxia. This threshold was presumably an attempt to ensure that very few mildly affected infants were included. Despite data suggesting that umbilical arterial acidosis is only associated with HIE when it is primarily metabolic in origin [32], the use of low pH as a criterion is perhaps vindicated by a recent meta-analysis concluding that a pH of < 7.24 in the first hour of life is associated with increased neonatal morbidity and the association is strongest at a pH threshold of 7 [33].

Indicators other than fetal or early neonatal acidosis for evidence of intrapartum hypoxia have also been used. The ICPTF and the ACOG guidelines suggest that a sentinel hypoxic event, an abnormal fetal heart rate, a low Apgar score beyond 5 minutes, onset of multisystem involvement within 72 hours of birth and early imaging showing acute cerebral abnormality are *all* required if intrapartum hypoxia is to be inferred [12, 25].

Other studies support the use of alternative criteria. In a brain magnetic resonance imaging (MRI) study, Cowan et al. [34] recruited term infants with NE and three criteria suggesting intrapartum hypoxia. The criteria were: late fetal heart decelerations, an arterial cord pH of < 7.1, an Apgar score of < 7 at 5 minutes, meconium-stained liquor, delayed onset of respiration, and multi-organ failure. Acute brain injury on MRI was present in 80% of infants meeting three of these criteria. Meconium-stained liquor was the only documented indication of an intrapartum abnormality in 24% of the infants in our study. Buchmann and Velaphi proposed that

a 5-minute Apgar score of < 7 and/or intrapartum fetal distress on cardiotocograph could be used as indicators of fetal hypoxia in the absence of arterial cord blood analysis [35]. In our study, 93% of the infants with an Apgar score of < 7 at 5 minutes, who also had a first-hour blood gas done, had a pH of < 7.0 or a base deficit of ≥ 12 mmol/l. These data suggest that a 5-minute Apgar score may be a viable proxy for fetal acidosis (However, in our cohort such a strategy would still fail to correctly identify the remaining 7% of the infants as having HIE). If criteria of a low 5-minute Apgar score or an abnormal fetal heart rate are applied to our population, 79 of the 110 infants are identified as being exposed to intra-uterine hypoxia. A case-control study of obstetric risk factors associated with NE in Cape Town [36] determined that the greatest obstetric risk factor was that of a prolonged second stage of labour (odds ratio 11.96, 95% confidence interval 2.93–56.4), but inadequate or absent monitoring of the fetal heart was noted in 51% of the infants with NE. In our study, a prolonged second stage of labour was the only indication of intrapartum abnormality in 11% of the infants and only 40 of the 110 infants (36%) had a *documented* abnormal fetal heart rate, although this may have been due to inadequate fetal heart monitoring or inadequate documentation.

A potential limitation in this study, is that all cases in the cohort had already been diagnosed with HIE by attending clinicians and cases were identified based on the review of hospital records; It is possible that some cases of HIE, particularly atypical cases may have been missed. However, our data show that HIE was diagnosed using much less restrictive criteria than are generally recommended, so the impact of this limitation is likely to be small.

In conclusion, our data show that there is wide variation in the incidence and grade of HIE, depending on which criteria are used. The use of less restrictive criteria resulted in a significant increase in the incidence of HIE from 2.3 to 4.3 per 1 000 live births. The term “intrapartum-related neonatal encephalopathy” may be useful to describe infants with suspected HIE, on the basis of the presence of NE with one or more intrapartum-related events in the absence of other causes of NE. This terminology may be more appropriate medico-legally and epidemiologically than the term “HIE”. The measurement of moderate-severe intrapartum-related NE rather than all encephalopathy would facilitate standardised comparisons. Further research should focus on establishing consensus definitions that can be used for meaningful population studies and benchmarking of NE, intrapartum-related NE, HIE, and the severity or grade of encephalopathy.

References

1. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? *The Lancet* 2005;365(9462):891-900.

2. Lawn JE, Kerber K, Enweronu-Laryea C, Massee Bateman O. Newborn survival in low resource settings-are we delivering? *BJOG* 2009;116:49-59.

3. Lawn JE, Bahl R, Bergstrom S, Bhutta ZA, Darmstadt GL, Ellis M, et al. Setting research priorities to reduce almost one million deaths from birth asphyxia by 2015. *PLoS Med* 2011;8(1):e1000389.

4. Lawn JE, Lee AC, Kinney M, Sibley L, Carlo WA, Paul VK, et al. Two million intrapartum-related stillbirths and neonatal deaths: Where, why, and what can be done? *Int J Gynaecol Obstet* 2009;107(S):S5-S19.

5. Lee AC, Cousens S, Darmstadt GL, Blencowe H, Pattinson R, Moran NF, et al. Care during labor and birth for the prevention of intrapartum-related neonatal deaths: a systematic review and Delphi estimation of mortality effect. *BMC Public Health* 2011;11(Suppl 3):S10.

6. Nelson KB, Leviton A. How much of neonatal encephalopathy is due to birth asphyxia? *Am J Dis Child* 1991;145(11):1325-31.

7. Hankins GD, Speer M. Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. *Obstet Gynecol* 2003;102(3):628-36.

8. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic–ischaemic encephalopathy. *Early Hum Dev* 2010;86(6):329-38.

9. Smith J, Wells L, Dodd K. The continuing fall in incidence of hypoxic-ischaemic encephalopathy in term infants. *BJOG* 2000;107(4):461-6.

10. Thornberg E, Thiringer K, Odeback A, Milsom I. Birth asphyxia: incidence, clinical course and outcome in a Swedish population. *Acta paediatrica* 1995;84(8):927-32.

11. Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, O'Sullivan F, Burton PR, et al. Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ* 1998;317(7172):1554-8.

12. MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* 1999;319(7216):1054-9.

13. World Medical Association. World Medical Association Declaration of Helsinki. *World Med J* 2008;54(4):122-4.

14. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353(15):1574-84.

15. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460): 663-70.

16. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361(14):1349-58.

17. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;33(10): 696-705.

18. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

19. Health Systems Trust SA. Neonatal death rate (NNDR) (deaths <28 days old per 1 000 live births) [Internet]. 2011 [Cited 2011 September 5]; Available from: http://indicators.hst.org.za/healthstats/152/data/geo.

20. Shalak LF, Laptook AR, Velaphi SC, Perlman JM. Amplitude-integrated electroencephalography coupled with an early neurologic examination enhances prediction of term infants at risk for persistent encephalopathy. *Pediatrics* 2003;111(2):351-7.

21. Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 1999;81(1):F19-23.

22. De Knijf A, Delport S, Pattinson R. Confidential Enquiries into hypoxic ischaemic encephalopathy as a marker for assessing the quality of care of women in labour. Proceedings of the 30th Conference on Priorities in Perinatal Care in Southern Africa; 2011 March 8-11; Limpopo, South Africa. P.224.

23. Linley LL, Thompson CM, Fawcus S. Incidence, morbidity and mortality of hypoxic ischaemic encephalopathy in the PMNS. Proceedings of the 23rd Conference on Priorities in Perinatal Care in Southern Africa; 2004 March 9-12; Limpopo, South Africa. P.144-6.

24. Harrison V, Fawcus S, Jordaan E. Magnesium supplementation and perinatal hypoxia: outcome of a parallel group randomised trial in pregnancy. *BJOG* 2007;114(8):994-1002.

25. Committee on Obstetric Practice. ACOG Committee Opinion. Number 326, December 2005. Inappropriate use of the terms fetal distress and birth asphyxia. *Obstet Gynecol* 2005;106(6):1469-70.

26. Riley RJ, Johnson JW. Collecting and analyzing cord blood gases. *Clin Obstet Gynecol* 1993;36(1):13-23.

27. Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. *BMJ* 1988;297(6640):24-7.

28. Low JA, Lindsay BG, Derrick EJ. Threshold of metabolic acidosis associated with newborn complications. *Am J Obstet Gynecol* 1997;177(6):1391-4.

29. Da Silva S, Hennebert N, Denis R, Wayenberg JL. Clinical value of a single postnatal lactate measurement after intrapartum asphyxia. *Acta Paediatr* 2000;89(3):320-3.

30. Wayenberg JL. Threshold of metabolic acidosis associated with neonatal encephalopathy in the term newborn. *J Matern Fetal Neonatal Med* 2005;18(6):381-5.

31. Finer NN, Robertson CM, Richards RT, Pinnell LE, Peters KL. Hypoxic-ischemic encephalopathy in term neonates: perinatal factors and outcome. *J Pediatr* 1981;98(1):112-7.

32. Low JA, Panagiotopoulos C, Derrick EJ. Newborn complications after intrapartum asphyxia with metabolic acidosis in the term fetus. *Am J Obstet Gynecol* 1994;170(4):1081-7.

33. Malin GL, Morris RK, Khan KS. Strength of association between umbilical cord pH and perinatal and long term outcomes: systematic review and meta-analysis. *BMJ* 2010;340:c1471.

34. Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 2003;361(9359):736-42.

35. Buchmann EJ, Velaphi SC. Confidential enquiries into hypoxic ischaemic encephalopathy. *Best Pract Res Clin Obst Gynaecol* 2009;23(3):357-68.

36. Fawcus S, Linley LL, Thompson CM, Greenfield D. Obstetric risk factors for neonatal encephalopathy. Proceedings of the 23rd Conference on Priorities in Perinatal Care in Southern Africa; 2004 March 9-12; Limpopo, South Africa. P.141-3.

Chapter 5

Evaluating a simple method of neuroprotective hypothermia for newborn infants in South Africa

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Author contributions

I was the principal investigator. I conceptualised the study, wrote the research proposal, supervised the data collection and analysed the data. I wrote all versions of the manuscript and submitted it to the journal. MCH and LLL contributed to data acquisition and reviewed the paper critically for important intellectual content.

All authors approved the final version of the paper.

Notes

Minor amendments recommended by the examiners of the thesis have been included.

The consent forms and data-collection sheets are included at the end of the thesis as Appendices B and C respectively.

Abstract

This study describes and evaluates a simple method of neuroprotective hypothermia for infants with hypoxic ischaemic encephalopathy (HIE). Five term infants with HIE were cooled by applying soft, cold gel-packs to the head. A radiant warmer, set to 34 ºC, servo-controlled the temperature measured by a probe between the infant's back and the mattress. The infants' heads were shielded from the warmer. After 72 hours, the infants were re-warmed at 0.2 ºC per hour, by adjusting the radiant warmer. A rectal temperature of 34 ºC was attained in a median time of 45 minutes. Mean rectal temperatures during cooling were 33.9 ± 0.3 ºC. There was good agreement between insulated back temperatures and deep rectal temperatures; the mean difference was – 0.01 ± 0.23 ºC and the limits of agreement were from – 0.46 to 0.45 ºC There were no major or irreversible adverse events during cooling. This method of cooling achieved rectal temperatures within the target range of 33–34 ºC and re-warming was effective.

Introduction

In well-resourced settings, therapeutic hypothermia is increasingly being recommended for newborn infants with moderate or severe hypoxic ischaemic encephalopathy (HIE) [1-3]. Two randomised controlled trials [4, 5] and three systematic reviews [6-8] concluded that whole-body hypothermia to a core body temperature of 33–34 °C, or selective head-cooling with a core body temperature of 34–35 °C commenced within 6 hours of birth, and maintained for 72 hours, resulted in a significant reduction of death or severe disability in this group of infants. International consensus statements [9, 10] suggest that therapeutic hypothermia for newborns with HIE should only be offered if the methodology follows the established protocols described in published studies, but the cooling equipment used in these studies has only recently become available in South Africa and is expensive and requires specialised training.

The use of therapeutic hypothermia in resource-limited settings is still considered experimental [11]. Although many hospitals in South Africa have neonatal intensive care facilities, the staffing shortages and budget limitations have prompted further research into simple, inexpensive cooling methods. Mowbray Maternity Hospital (MMH) is a level 2 regional maternity hospital where a basic method of neuroprotective hypothermia was described in 1999 [12]. The encouraging short-term outcomes from that study and the accumulating evidence of benefit from hypothermia [4, 5] led to the continued use of a modification of this method. Before the modified method could be considered in further prospective studies and similar resource-limited settings, the efficacy of the method had to be evaluated further.

We planned to evaluate the efficacy of the method in five term infants. We aimed to determine if target rectal temperature was achieved within an acceptable time, to determine if rectal temperatures were maintained in an acceptable range, and to determine if the method of monitoring the skin temperature between the skin and the mattress is a suitable alternative to direct rectal temperature monitoring.

Method

This study was done in the neonatal unit of MMH, a satellite teaching hospital of the University of Cape Town, South Africa. The study was approved by the Faculty of Health Sciences Human Research Ethics Committee of the University of Cape Town and conformed to the principles of the Declaration of Helsinki [13].

Temperature data were collected prospectively on five infants who were being cooled according to the existing standard of care at MMH. The only intervention additional to the existing standard of care in these infants was the monitoring and logging of rectal temperatures and two additional skin temperatures. Informed consent for data collection and the publication of images was obtained from the mothers of the infants.

At MMH, infants receiving neuroprotective hypothermia were required to meet the following criteria:

(i) a gestational age of ≥ 36 weeks and a birth weight of ≥ 2 000 g;

(ii) age < 6 hours at initiation of hypothermia;

(iii) signs of HIE *and* seizures or voltage suppression on the (amplitude-integrated electroencephalogram) aEEG as defined by al Naqeeb et al. [14], *or* if HIE was clinically severe or seizures were clinically evident, then aEEG criteria were not required; and

(iv) a base deficit of ≥ 16 in the first hour of life on cord or infant arterial blood, *or* a 10-minute Apgar score of < 7 or requiring some form of assisted ventilation at age 10 minutes plus an obstetric history suggestive of intrapartum hypoxia.

Infants were not cooled if any of the following were present: major congenital abnormalities, active bleeding, obvious sepsis, persistent pulmonary hypertension requiring a fractional inspired oxygen > 0.8 to maintain oxygen saturation of 94%, or if there was severe hypoglycaemia or electrolyte abnormality that did not respond to usual therapy.

Hypothermia was achieved by applying one or two soft, 12 x 12 cm, 250 g gel-packs (Penguin Manufacturers, International Health Care Distributors) around the head, and controlling the core body temperature with a servo-control radiant warmer (Servocrib, Servocare Medical Industries cc, Cape Town, South Africa) set at a target temperature of 34 ºC. This was the lowest target temperature that the warmer was capable of. The packs were stored in a refrigerator kept at 7–10 ºC and were replaced hourly when routine observations were done. The core temperature was measured and controlled using a temperature probe between the infant and the mattress. This method of core temperature measurement has been ratified in similar cooling studies [15].

A reflective perspex heat shield was placed over the head to prevent facial heating by the radiant warmer. If the infant core temperature was below 33 ºC, then the packs were removed for an hour. If the infant core temperature was above 34.5 ºC, then additional packs were added around the upper body. Infants were sedated with intravenous morphine at 8 μg/kg/hour unless they were sufficiently sedated with anticonvulsants.

A rectal temperature probe was inserted 4–5 cm into the rectum and two additional skin probes were attached to the surface abdomen over the left upper quadrant and to the middle of the back. These latter two probes were insulated with adhesive reflective coverings. Ambient temperature was monitored with a fourth probe attached to the side of the radiant warmer. The probes were connected to a dedicated data logger (Squirell 2010, Grant Instruments, Cambridge, UK) which logged temperature at these sites every 15 minutes for the duration of cooling.

After 72 hours of cooling, infants were re-warmed by removing the packs and increasing the target temperature on the radiant warmer by 0.2 ºC per hour until a temperature of 36.5 ºC was attainted. Then the heat shield was removed and core temperature was subsequently maintained at 36.5–37 ºC until the infant was well enough to be transferred to a bassinette.

Infants received routine monitoring of vital signs, acid base parameters, electrolytes, haematological parameters and sepsis indicators. Clinical seizures and subclinical status were treated with up to two loading doses of intravenous phenobarbital 20 mg/kg and thereafter by midazolam infusion, followed by lignocaine infusion for refractory seizures. A Thompson neurological assessment [16] was performed daily for the first 10 days and aEEG monitoring was continued for 96 hours or until normal background activity was present for 24 hours.

Results

Four infants had a history of bradycardia during labour and one infant had a history of meconium-stained liquor. Arterial blood gas analysis in the first hour of life was available in three infants and the base deficit ranged from 15.9 to 19.5 mmol/l. The other two infants were outborn but were still requiring assisted ventilation at age 10 minutes. Table 5.1 shows the clinical characteristics of all the infants. The median birth weight was 3 060 g, the median 5-minute Apgar score was 5 and the median age of onset of cooling was 5.5 hours. Two infants were assessed as Sarnat stage 3, two others were assessed as stage 2, and one infant was assessed as stage 1.

Cooling was commenced within a median time of 5.5 hours. Onset of cooling was delayed by the process of stabilising the infant after birth and placing the aEEG sensors. The two infants were then admitted to the unit after age 3 hours.

Table 5.1 Clinical characteristics

Infant	Wt (g)	5-min AS	aEEG suppression at recruitment	Sz	Age cooling started (hours)	Anticon-vulsant and opiates	Inotrope	Vent-ilation
1	2 700	6	Severe (FT)	Subtle +ESz	6	Phenobarbital	Yes	None
2	3 600	3	Severe (CLV)	Subtle	3	Phenobarbital Morphine	No	MV/ NCPAP
3	3 640	4	Moderate (DNV)	Tonic + ESz	5.5	Phenobarbital Morphine	Yes	MV/ NCPAP
4	2 800	6	Moderate (DNV)	None	5.5	Morphine	No	None
5	3 060	5	Severe (FT)	Subtle + ESz	4.25	Phenobarbital Midazolam Morphine	No	Nasal canulae

AS, Apgar score; BS, burst suppression; CNV, continuous normal voltage; ESz, electrical seizures; FT, lat trace; MV, mechanical ventilation; NCPAP, nasal continuous positive airway pressure; Sz, seizures; Wt, weight

Table 5.2 shows the temperature variation. A rectal temperature of 34 ºC was attained within a median time of 45 minutes from commencement of cooling. The back temperature tended to read 0.1–0.5 ºC higher than the rectal temperature at the time target temperature was attained, but subsequently the mean difference between rectal and back temperatures was 0.1 ºC. Mean rectal temperature during cooling was 33.9 ± 0.3 ºC in a mean ambient temperature of 24.6 ± 0.5 ºC.

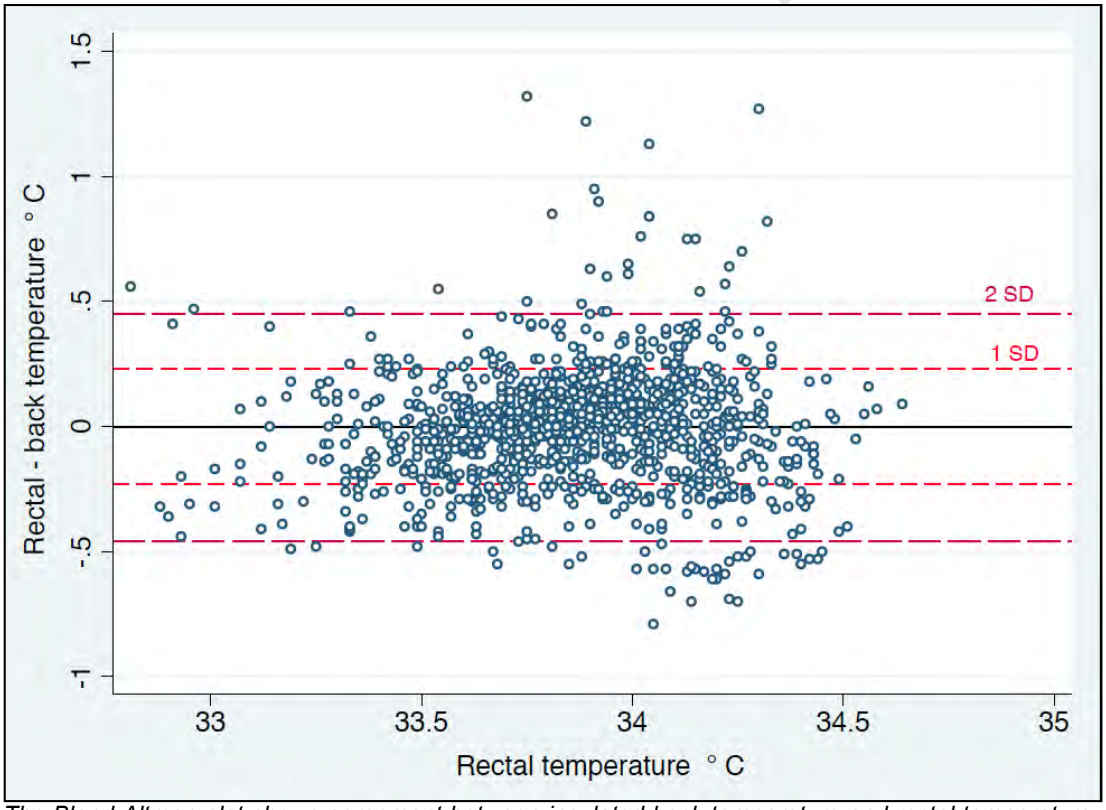
Table 5.2 Temperature variation

Infant	Ambient temperature during cooling (ºC) ±SD	Rectal temperature before cooling (ºC)	Intracool[a] rectal temperature (ºC) ±SD	Intracool[a] back temperature (ºC) ±SD	Time to reach rectal 34 ºC (minutes)	Back temperature when rectal reached target (ºC)
1	24 ± 0.4	35.7	33.7 ± 0.3	33.9 ± 0.3	30	34.5
2	23.7 ± 0.5	35.1	34.0 ± 0.2	34.1 ± 0.4	90	34.5
3	24 ± 0.5	36.1	33.9 ± 0.2	33.8 ± 0.3	45	34.1
4	26.5 ± 1.5	36.2	33.9 ± 0.2	33.8 ± 0.4	60	33.9
5	24.3 ± 0.4	34.7	33.7 ± 0.2	33.7 ± 0.2	30	34.4
ALL	24.6 ± 0.5	35.7 (median)	33.9 ± 0.3	33.9 ± 0.4	45 (median)	34.4 (median)

a, average temperature during cooling, from 1 to 71 hours

Figure 5.1 is a Bland-Altman plot showing the close agreement between rectal and insulated back temperatures during cooling in the entire group; with rectal temperature on the x-axis as the gold standard. The mean difference was – 0.01 ± 0.23 ºC and the limits of agreement were from – 0.46 to 0.45 ºC. Figure 5.2 shows a single case, demonstrating the correlation between rectal and back temperatures and the wide variation in the surface abdominal temperatures from 32.2 to 36.3 ºC. This case also demonstrates the ease and accuracy of re-warming the infant by increasing the radiant warmer target temperature by 0.2 ºC per hour, while keeping the heat shield in place. Figure 5.3 shows the rectal temperatures of all infants and demonstrates that the rectal temperatures remained at or below 34 ºC for the majority of the time.

Figure 5.1 Bland-Altman plot showing the agreement between rectal and insulated back temperatures during cooling



The Bland-Altman plot shows agreement between insulated back temperature and rectal temperature; the difference between the temperatures measured at the two sites (y-axis) is plotted against rectal temperature at the time (gold standard). The mean difference (black line) was 0.01 ± 0.23 ºC and the limits of agreement ranged from – 0.46 to 0.45 ºC. The limits of agreement correspond to the variation in temperature difference within2 standard deviations (SD) (long red dashed lines).

Figure 5.2 Regional temperature variation during cooling and re-warming of a single illustrative case (infant 5)

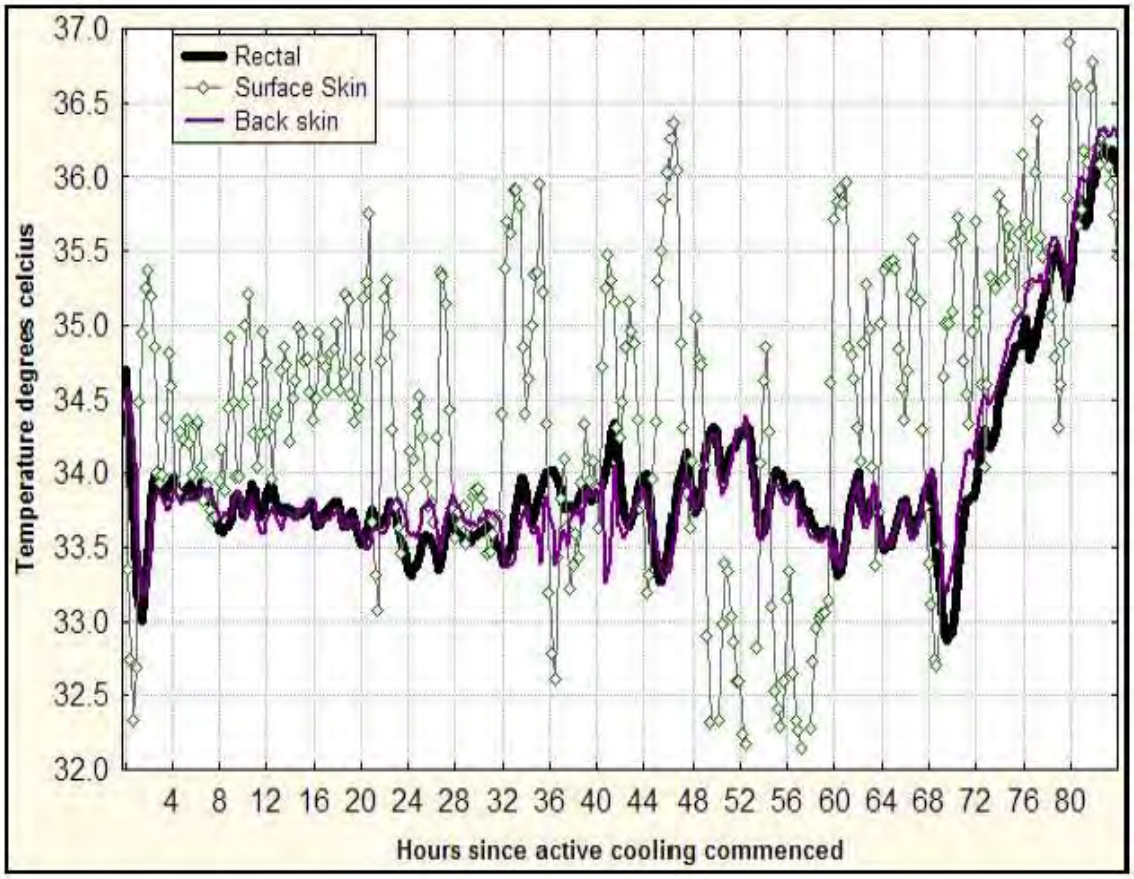
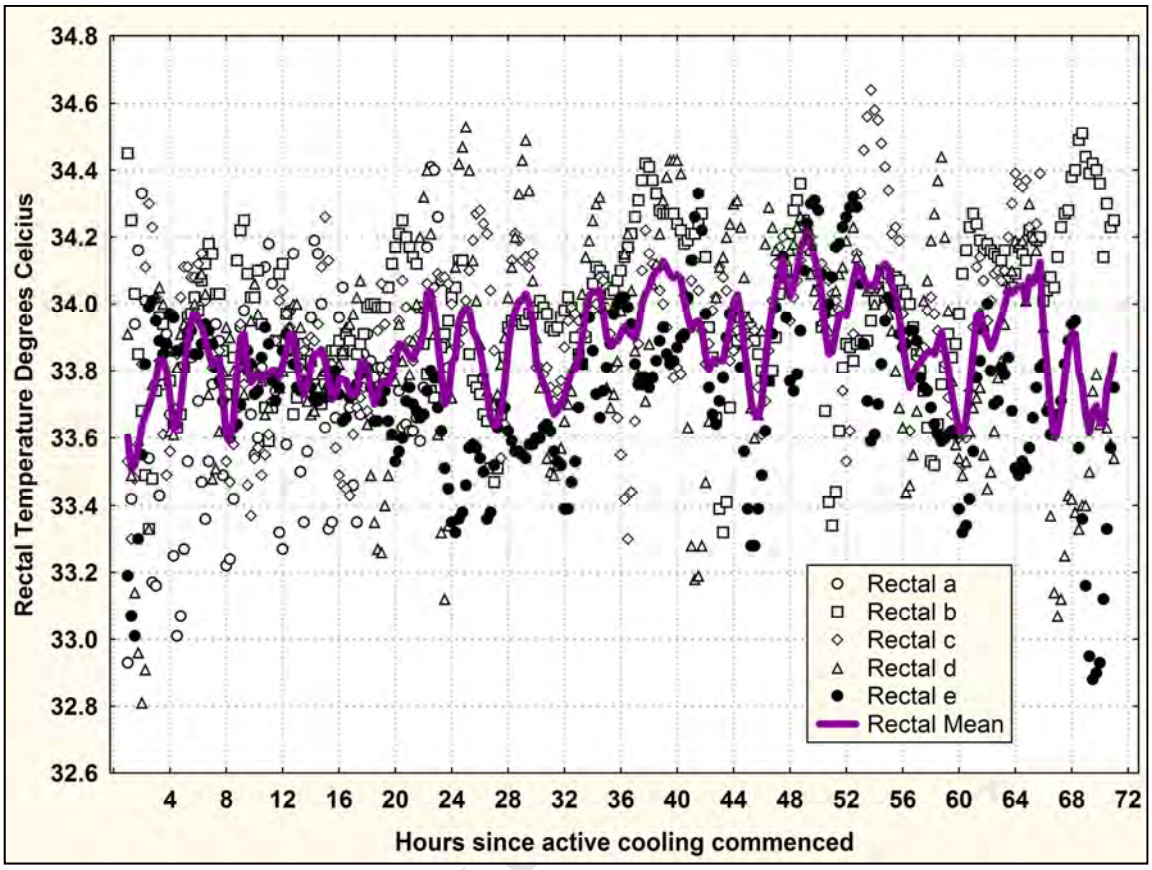


Figure 5.3 Scatter plot graph showing rectal temperature variation of all infants



There were very few adverse events during cooling. All infants had a physiological sinus bradycardia of < 100 beats per minute, but only two infants required inotropes for hypotension. Although the maximum creatinine ranged from 92 to 144 µmol/l (median 103), there were no electrolyte abnormalities. One infant had transient hypoglycaemia and one infant had transient pulmonary hypertension that resolved without the need for nitric oxide.

All infants survived to discharge. Three infants were sucking well enough to be discharged by day ten, despite the fact that two of these infants had severe suppression on aEEG at recruitment. Enteral feeding at a volume of 80 ml/kg/day was achieved by a median of four days (range 4–7 days).

Discussion

We have shown that hypothermia may be successfully achieved in term infants with HIE simply by reducing the target radiant warmer temperature to 34 ºC, covering the infant's head with a heat shield and by applying soft gel-packs around the head at a starting temperature of 7–10 ºC. We have also confirmed that a temperature measured at a point that is insulated between the mattress and the body reflects a rectal temperature in this setting.

Similar methods of inducing hypothermia have been used in other studies [17, 18], but in those studies, the radiant warmer was adjusted manually and the rectal temperature was measured directly. Manual temperature adjustments are more labour-intensive and require greater skill than our method of hourly bag changes.

The methodology used in our study is simpler and less costly than the CoolCap or mattress-based cooling methods described in the two largest randomised controlled studies published to date [4, 5]. Four of the five infants in our study achieved a rectal temperature of 34 ºC by 60 minutes or less, compared to average cooling times of 120 minutes and 90 minutes described using the more expensive methods above. Although only five infants were cooled, a total of 1 248 temperatures were measured during cooling and the standard deviation (SD) of ± 0.3 ºC shows good temperature stability.

The infants' heads were shielded during cooling and re-warming to prevent excessive facial and scalp heating which has been described in infants nursed under radiant warmers [19]. The facial vein drains via the ophthalmic vein to the cavernous sinus and hence heating of the face may cause relative heating of the base of the brain. It is similarly important to use lower humidifier temperature settings than usual when ventilating cooled infants. The humidifier used in the two infants who required ventilation (MR 850, Fisher and Paykel, New Zealand) was set at the lower of the two automated options, which resulted in the distal sensor reading 34–35 ºC instead of the usual 38 ºC.

Insulated skin temperature monitoring as the primary temperature control point is standard at MMH due to ease of application. Studies in piglets [20], pre-term infants [21] and term infants nursed in closed incubators [15], demonstrated the zero gradient principle whereby a point insulated between the body and mattress will equilibrate to the warmest part of the body. The good agreement between the insulated back temperature and the rectal temperature in our study, including 1 248 temperatures, confirms that this type of monitoring is reasonable.

Although the rectal temperatures achieved in this study were in the higher end of the range of cerebral neuroprotective temperatures, the regular application of gel-packs when rectal temperatures were above 33.5 °C may have decreased the scalp temperature sufficiently to achieve further mild cortical cooling. However, our method must be distinguished from uniform selective cerebral cooling [4] where rectal temperature is maintained at 34–35 °C and the constant low scalp temperature results in consistently lower brain temperatures.

Sedation during cooling is recommended [22-24] and we used intravenous morphine at low doses, to provide sedation to infants who did not require anticonvulsants, or who were not sufficiently sedated despite anticonvulsants. The morphine in the doses we used, did not cause apnoea but it may have enhanced the hypothermia [25].

Since completion of our study, a very basic method of cooling using hot water bottles filled with tepid tap water achieved good temperature control [26], if blankets were added intermittently to avoid excessive hypothermia. The infants were nursed in simple cots in a setting with limited electricity and no air-conditioning, which is very different to our setting.

Conclusion

This simple method of inducing and maintaining hypothermia achieved stable rectal temperatures within an acceptable time and the insulated back temperatures showed close agreement with rectal temperatures. The intervention was well defined and simple. This approach makes therapeutic hypothermia immediately accessible at virtually no cost. The method may be particularly suited to level-2 units with infants awaiting transfer and it might also be considered as a method of cooling in further trials. At the time the study was conducted, most models of radiant warmers and incubators did not permit a target temperature below 34 °C. If radiant warmers that allow a target temperature of 33.5 °C are used, then deeper cooling would probably be easily maintained using the same simple method.

References

1. Gunn AJ, Gluckman PD. Head cooling for neonatal encephalopathy: the state of the art. *Clin Obstet Gynecol* 2007;50(3):636-51.

2. Hoehn T, Hansmann G, Buhrer C, Simbruner G, Gunn AJ, Yager J, et al. Therapeutic hypothermia in neonates. Review of current clinical data, ILCOR recommendations and suggestions for implementation in neonatal intensive care units. *Resuscitation* 2008;78(1):7-12.

3. Perlman M, Shah P. Time to adopt cooling for neonatal hypoxic-ischemic encephalopathy: response to a previous commentary. *Pediatrics* 2008;121(3):616-8.

4. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460): 663-70.

5. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353(15):1574-84.

6. Jacobs S, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2007(4):CD003311.

7. Schulzke SM, Rao S, Patole SK. A systematic review of cooling for neuroprotection in neonates with hypoxic ischemic encephalopathy - are we there yet? *BMC Pediatr* 2007;7:30.

8. Shah PS, Ohlsson A, Perlman M. Hypothermia to treat neonatal hypoxic ischemic encephalopathy: systematic review. *Arch Pediatr Adolesc Med* 2007;161(10):951-8.

9. Higgins RD, Raju TN, Perlman J, Azzopardi DV, Blackmon LR, Clark RH, et al. Hypothermia and perinatal asphyxia: executive summary of the National Institute of Child Health and Human Development workshop. *J Pediatr* 2006;148(2):170-5.

10. Blackmon LR, Stark AR. Hypothermia: a neuroprotective therapy for neonatal hypoxic-ischemic encephalopathy. *Pediatrics* 2006;117(3):942-8.

11. Thayyil S, Costello A, Shankaran S, Robertson NJ. Therapeutic hypothermia for neonatal encephalopathy implications for neonatal units in India. *Indian Pediatr* 2009;46(4):283-9.

12. Simbruner G, Haberl C, Harrison V, Linley L, Willeitner AE. Induced brain hypothermia in asphyxiated human newborn infants: a retrospective chart analysis of physiological and adverse effects. *Intensive Care Med* 1999;25(10):1111-7.

13. World Medical Association Declaration of Helsinki. *World Med J* 2008;54(4):122-4.

14. Al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D. Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. *Pediatrics* 1999;103(6 Pt 1):1263-71.

15. Horn AR, Woods DL, Thompson C, Els I, Kroon M. Selective cerebral hypothermia for post-hypoxic neuroprotection in neonates using a solid ice cap. *S Afr Med J* 2006;96(9 Pt 2):976-81.

16. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

17. Jacobs SE, Stewart M, Inder TE, Doyle L, Morley C, editors. Feasability of a pragmatic randomised controlled trial of whole body cooling for term newborns with hypoxic-ischaemic encephalopathy. Paper presented at: Hot topics in Neonatology; 2002 December 9 - 10; Washington D.C.

18. Thoresen M, Whitelaw A. Cardiovascular changes during mild therapeutic hypothermia and rewarming in infants with hypoxic-ischemic encephalopathy. *Pediatrics* 2000;106(1 Pt 1):92-9.

19. Gunn AJ, Gunn TR. Effect of radiant heat on head temperature gradient in term infants. *Arch Dis Child Fetal Neonatal Ed* 1996;74(3):F200-3.

20. Dollberg S, Xi Y, Donnelly MM. A noninvasive transcutaneous alternative to rectal thermometry for continuous measurement of core temperature in the piglet. *Pediatr Res* 1993;34(4):512-7.

21. Dollberg S, Rimon A, Atherton HD, Hoath SB. Continuous measurement of core body temperature in preterm infants. *Am J Perinatol* 2000;17(5):257-64.

22. Thoresen M, Apricena F, Loberg EM. Post-hypoxic-ischaemic hypothermia reduces necrosis and apoptosis correspondingly in the newborn rat however restraint stress ameliorates neuroprotection. *Pediatric Research* 1998;44 421A.

23. Thoresen M, Satas S, Loberg EM, Whitelaw A, Acolet D, Lindgren C, et al. Twenty-four hours of mild hypothermia in unsedated newborn pigs starting after a severe global hypoxic-ischemic insult is not neuroprotective. *Pediatr Res* 2001;50(3):405-11.

24. Johnson EO, Kamilaris TC, Chrousos GP, Gold PW. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neurosci Biobehav Rev* 1992;16(2):115-30.

25. Ushijima I, Tanaka M, Tsuda A, Koga S, Nagasaki N. Differential effects of morphine on core temperature in stressed and non-stressed rats. *Eur J Pharmacol* 1985;112(3):331-7.

26. Robertson NJ, Nakakeeto M, Hagmann C, Cowan FM, Acolet D, Iwata O, et al. Therapeutic hypothermia for birth asphyxia in low-resource settings: a pilot randomised controlled trial. *Lancet* 2008;372(9641):801-3.

Chapter 6

Early clinical signs in newborn infants with hypoxic ischaemic encephalopathy predict an abnormal amplitude-integrated EEG at age 6 hours

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Author contributions

I was the principal investigator. I conceptualised the study, wrote the research proposal, supervised and participated in the data collection and analysed the data. I wrote all versions of the manuscript and submitted it to the journal. GHS, and LM participated in the design of the study and critically reviewed the draft manuscript. LLL, MSR, YJ, MCH, MC and NRR assisted with acquisition of data and critically reviewed the draft manuscript. NJR participated in the design of the study, assisted with acquisition of data and critically reviewed the draft manuscript.

All authors read and approved the final manuscript.

Notes

Minor amendments recommended by the examiners of the thesis have been included.

The consent form and data collection sheets are included at the end of the thesis as Appendices D and E respectively.

Abstract

Background

An early clinical score predicting an abnormal amplitude-integrated electroencephalogram (aEEG) or moderate-severe hypoxic ischaemic encephalopathy (HIE) may allow rapid triage of infants for therapeutic hypothermia. We aimed to determine if early clinical examination could predict either an abnormal aEEG at age 6 hours or moderate-severe HIE presenting within 72 hours of birth.

Methods

Sixty infants ≥ 36 weeks gestational age were prospectively enrolled following suspected intrapartum hypoxia and signs of encephalopathy. Infants who were moribund, had congenital conditions that could contribute to the encephalopathy or had severe cardio-respiratory instability were excluded. Predictive values of the Thompson HIE score, the modified Sarnat encephalopathy grade (MSEG) and the specific individual signs at age 3–5 hours were calculated.

Results

All of the 60 infants recruited had at least one abnormal primitive reflex. Visible seizures and hypotonia at 3–5 hours were strongly associated with an abnormal 6-hour aEEG (specificity 88% and 92%, respectively), but both had a low sensitivity (47% and 33%, respectively). Overall, 52% of the infants without hypotonia at 3–5 hours had an abnormal 6-hour aEEG. Twelve of the 29 infants (41%) without decreased level of consciousness at 3–5 hours had an abnormal 6-hour aEEG (sensitivity 67%; specificity 71%). The threshold Thompson score at age 3–5 hours that best predicted an abnormal 6-hour aEEG was a Thompson score of ≥ 7. A Thompson score of ≥ 7 and moderate-severe MSEG at 3–5 hours, both predicted an abnormal 6-hour aEEG (sensitivity 100 vs. 97% and specificity 67 vs. 71% respectively). Both assessments predicted moderate-severe encephalopathy within 72 hours after birth (sensitivity 90%, vs. 88%, specificity 92% vs. 100%). The 6-hour aEEG predicted moderate-severe encephalopathy within 72 hours (sensitivity 75%, specificity 100%) but with lower sensitivity ($p = 0.0156$) than the Thompson score (sensitivity 90%, specificity 92%). However, all infants with a normal 3- and 6-hour aEEG with moderate-severe encephalopathy within 72 hours who were not cooled had a normal 24-hour aEEG.

Conclusions

The encephalopathy assessment described by the Thompson score at age 3–5 hours is a sensitive predictor of either an abnormal 6-hour aEEG or moderate-severe encephalopathy presenting within 72 hours after birth. An early Thompson score may be useful to assist with triage and selection of infants for therapeutic hypothermia.

Background

Intrapartum fetal hypoxia followed by hypoxic ischemic encephalopathy (HIE) is a common cause of potentially avoidable brain injury in term infants [1, 2]. The incidence of HIE in developed countries is estimated to be 1.5 per 1,000 live births [3]. Estimates in developing countries range from 2.3 to 26.5 per 1,000 live births [4, 5]. A recent meta-analysis found that therapeutic hypothermia commenced by age 6 hours for infants with moderate or severe (moderate-severe) HIE, significantly reduces death or disability; in three studies an abnormal amplitude integrated electro-encephalogram (aEEG) was required as an additional criterion for cooling [6].

Moderate-severe HIE typically presents with worsening clinical signs after the first 1.5–18 hours and then a slow improvement after 4–5 days [7]. Early identification of infants at risk of developing moderate-severe encephalopathy is crucial: experimental studies emphasise that the sooner hypothermia is started, the better the therapeutic effect [8, 9]. During the first 6 hours after birth, a bi-parietal aEEG is the most sensitive and specific single indicator of long-term outcome after HIE [10-12]. In normothermic infants, a normal aEEG during the first 6 hours after birth has a sensitivity of 89% and a positive predictive value (PPV) of 96.2% for a normal long-term outcome [10]. Shalak et al. defined a modification of Sarnat's [7] encephalopathy grading (the modified Sarnat encephalopathy grade) [13]. They showed that the presence of at least one clinical sign of moderate-severe encephalopathy occurring in at least three of six separate components during the first 12 hours after birth, had a similar sensitivity but lower specificity for prediction of an abnormal outcome at discharge than a severely abnormal fronto-parietal aEEG. Importantly, Shalak found that a combination of both mild and moderate encephalopathic clinical signs identified infants with an abnormal outcome at discharge with a sensitivity of 100%. This is in keeping with our knowledge of the evolving nature of HIE [7, 14] and it suggests that infants can subsequently develop moderate-severe encephalopathy following initial signs of mild encephalopathy.

The Thompson score is derived from nine aspects of the neurological examination of infants with HIE: the total score ranges from 0 to 22 and the kappa coefficient is 0.87 [14]. This score allows a more precise description of infants than "mild", "moderate" or "severe" and recognises the prognostic significance of mixed signs within these three categories. In normothermic infants, a maximum score > 10 during the first 7 days of life, predicts an abnormal outcome with 100% sensitivity and 61% specificity [14]. A robust early clinical score that accurately predicts an abnormal aEEG by 6 hours after birth would allow rapid triage of specific babies for

therapeutic hypothermia. There are, however, no published data on the prognostic value of a Thompson score obtained < 6 hours after birth.

We studied a group of infants who were evaluated for therapeutic hypothermia with the primary objective of determining the threshold Thompson score at age 3–5 hours that predicted an abnormal 6-hour aEEG.

The secondary objectives were to determine the following:

(i) the predictive value of modified Sarnat encephalopathy grading (MSEG) at age 3–5 hours for an abnormal 6-hour aEEG;

(ii) the predictive value of specific clinical signs at age 3–5 hours for an abnormal 6-hour aEEG (the specific clinical signs we analysed were those used as entry criteria in the cooling trials with abnormal aEEG as an additional criterion [15-17]; these clinical signs included decreased level of consciousness, visible seizures, hypotonia, and abnormal reflexes); and

(iii) the ability of the Thompson score threshold to predict moderate-severe encephalopathy presenting within 72 hours after birth.

Methods

This prospective cohort study was approved by the University of Cape Town Health Sciences Faculty Human Research Ethics Committee and conforms to the principles of the 2008 Declaration of Helsinki [18]. Informed parental consent was obtained. The study was performed between June 2008 and March 2009 at three hospitals in Cape Town: Groote Schuur Hospital, a tertiary hospital and New Somerset Hospital and Mowbray Maternity Hospital, both secondary hospitals. Recruitment at New Somerset Hospital only commenced in September 2008.

Study sample

Infants ≥ 36 weeks gestation and birth weight ≥ 2000 g, with signs of encephalopathy after age 10 minutes but before age 5 hours were consecutively recruited using similar criteria for intrapartum hypoxia to those described in the cooling trial by Shankaran et al. [19].

Infants were included if they had one of the following signs suggesting intrapartum hypoxia:

(i) a base deficit of ≥ 16 mmol/l in the first hour of life on cord or infant arterial blood; *or*

(ii) an abnormal intra-partum course (eg: abnormal fetal heart rate, cord prolapse, uterine rupture, maternal haemorrhage, maternal trauma, maternal seizures, shoulder dystocia, maternal cardiorespiratory arrest, meconium-stained liquor, or prolonged second stage) and either a 10-minute Apgar score of < 7 or continued respiratory support at 10 minutes. A 10-minute Apgar score of < 7 was accepted as it corresponded with the need for continued respiratory support.

Exclusion criteria were any of the following: infection at birth; chorioamnionitis with prolonged rupture of membranes; chromosomal syndromes; cerebral malformations; lethal congenital malformations; neonatal abstinence syndrome; metabolic encephalopathies; moribund infants where demise was imminent; or infants with severe cardio-respiratory instability requiring high dose inotropes, a fractional inspired oxygen of ≥ 0.8, or high frequency oscillatory ventilation.

Sample size calculations were based on data from a previous analysis of HIE in our region [14]. We estimated that 33% of eligible infants would have a normal 6-hour aEEG, that 50–70% of these infants would have a Thompson score below threshold and that 2–10 % of the infants with an abnormal aEEG would have a Thompson score below threshold. We expected to recruit 60 infants within 12 months. Fisher's exact analysis of 2x2 tables populated with our estimates and a sample size of 60, yielded p-values ranging from 0–0.0026. According to Buderer's formula for sample size calculation for diagnostic tests [20], if the prevalence of abnormal aEEG at 6 hours is expected to be 67% and the Thompson score threshold is expected to have a minimum sensitivity of 90%, a minimum sample size of 51 is required if precision is set at 10%. We therefore recruited 60 infants to allow for a margin of error.

Neurological assessment

A clinical assessment was performed after initial stabilisation. A Thompson score was determined [14], and the encephalopathy was graded as mild, moderate or severe according to MSEG [13]: encephalopathy was defined as the presence of one or more abnormal signs in at least three of the following six categories; level of

consciousness, spontaneous activity, posture, tone, primitive reflexes (suck or Moro), and autonomic nervous system (pupils, heart rate, or respiration).

The grades were defined as follows:

Mild: hyperalert, normal tone and activity, exaggerated moro, normal autonomic function

Moderate: lethargic, decreased activity, distil flexion, hypotonia, weak primitive reflexes, constricted pupils, bradycardia or periodic breathing

Severe: stupor/coma, decerebrate posture, absent spontaneous activity, flaccid, absent primitive reflexes, non-reactive pupils, or apnoea

The grade with the most corresponding signs was assigned but if signs were equally distributed, the grade was based on the level of consciousness. Infants with seizures were graded as “moderate” unless severe signs predominated. These assessments were performed at 1 hour (in those presenting early enough), 3–5, 6, and 24 hours after birth and were continued daily until the tenth day or discharge, whichever occurred first. The assessments were performed by the attending paediatric residents or specialists who had received training in performing the Thompson score. After the clinical assessment, an aEEG was obtained on all infants and continuously recorded on a BrainZ BRM2 monitor (BrainZ Instruments Ltd, Auckland, NZ). The recording was continued until the single channel bi-parietal trace remained normal for 24 hours, or until 96 hours, or until the death of the infant, whichever occurred first. In addition to the biparietal aEEG, the BrainZ monitor records the bilateral raw EEG and bilateral aEEG between parietal and central electrodes. Two neonatologists (MC and NJR) with experience in aEEG interpretation and who were blind to the neurological assessments, reviewed the recordings offline. The assessors provided consensus opinion on the category of the bi-parietal background aEEG trace and the presence of seizures at age 3, 6, 24, 48, 72, and 96 hours.

The aEEG recording was classified by background voltage pattern according to the most severely abnormal trace at each of the assessment times ± 30 minutes. The classification was based on the modified system proposed by Hellström-Westas [21].

Five different aEEG patterns were described:

(i) Continuous normal voltage (CNV): continuous and variable activity with minimum voltage of 5–10 μV and maximum voltage of 10–50 μV

(ii) Discontinuous normal voltage (DNV): discontinuous activity with variable minimum amplitude - predominantly below 5 μV, and maximum amplitude above 10 μV

(iii) Burst-suppression (BS): discontinuous activity with minimum amplitude without variability at < 5 μV and bursts predominantly with amplitude ≥ 25 μV

(iv) Continuous low voltage (CLV): continuous and variable activity with maximum amplitude below 10 μV and minimum amplitude around or below 5 μV

(v) Flat trace (FT): primarily inactive (isoelectric) trace with both maximum and minimum background activity below 5 μV

Seizures on aEEG were defined by an abrupt rise in the minimum and maximum amplitude, confirmed on raw EEG showing repetitive spikes or sharp-wave activity with duration of at least 10 seconds.

The aEEG recordings were further graded as follows:

a) Normal: Background activity was CNV and EEG seizures were absent.

b) Abnormal: EEG seizures were present or background activity was DNV, BS, CLV or FT. A subgroup with severely abnormal background activity was defined as recordings showing BS, CLV or FT.

Medical management

All infants received routine monitoring and clinical care. The infants with seizures or an abnormal aEEG were treated with hypothermia within 6 hours of birth. These infants were cooled to a core temperature of 34 °C for 72 hours using gel-packs according to the limits of previously described basic cooling methods [22] and they were re-warmed at 0.2 °C per hour. All cooled infants were sedated with intravenous (IV) phenobarbital 20 mg/kg. Morphine 8 μg/kg/hour IV was given if infants were restless or agitated during cooling. Seizures were treated with a second dose of IV phenobarbital 20 mg/kg. Persistent seizures were treated with midazolam up to 0.1mg/kg/hour IV, followed by IV lignocaine if required.

Data collection and analysis

In addition to the clinical neurological assessments and aEEG, further data collection included perinatal characteristics, morbidity and short-term outcomes. The data included; maternal age; maternal HIV and Syphilis status; pregnancy complications including hypertension, hemorrhage, thyroid disease and diabetes; intrapartum complications including fetal heart rate abnormalities, cord prolapse, uterine rupture, maternal seizures, shoulder dystocia, maternal hemorrhage, meconium-stained liquor and prolonged second stage; delivery mode; infant characteristics at birth; resuscitation at birth; blood gas within 1 hour of birth; nosocomial sepsis; continuous positive airway pressure (CPAP); mechanical ventilation; inotropic support; pulmonary air leak; length of stay and death.

Data were analysed with Stata 12 (Stata Corporation, Texas, USA). Receiver operating characteristic (ROC) curve analysis was used to determine the threshold Thompson score that predicted an abnormal aEEG. The diagt module was used to calculate the sensitivity, specificity and likelihood ratio (LR) for the threshold Thompson score and moderate-severe encephalopathy at age 3–5 hours to predict an abnormal 6-hour aEEG. Inclusion in one group did not prevent inclusion in the other. The specific clinical signs utilised in the cooling trials were analysed using the same method. The short-term outcomes and morbidity were compared between infants with or without the threshold Thompson score. The Chi-square or Fisher's exact tests were used for categorical comparisons. The t-test and the Wilcoxon rank-sum tests were used to compare parametric and non-parametric continuous variables respectively.

To determine the extent to which earlier examinations differed from later assessments in their ability to predict an abnormal 6-hour aEEG, we performed post-hoc analysis of the subgroup of infants with a 1-hour clinical assessment and an aEEG at both 3 and 6 hours. ROC curve analysis was used to determine the threshold Thompson score at 1 and 3–5 hours predictive of an abnormal aEEG at 3 *and/or* 6 hours. Sensitivity, specificity and LR were calculated for moderate-severe encephalopathy and also for the most sensitive Thompson score thresholds at 1 and 3–5 hours.

We also determined the ability of early clinical and aEEG assessment to predict moderate-severe encephalopathy presenting within 72 hours after birth. Predictive values for a Thompson score of ≥ 7 at 3–5 hours, moderate-severe encephalopathy at 3–5 hours, abnormal aEEG at 6 hours and abnormal aEEG at 3 *and/or* 6 hours

were calculated. The McNemar test was used to confirm significant differences in sensitivity and specificity. All statistical tests are two-sided at alpha = 0.05.

Results

There were 80 infants with entry criteria: 20 met exclusion criteria and the remaining 60 infants were recruited. Eight infants (13%) died before discharge. Forty-one infants (68%) were cooled. Cooling was continued for 72 hours except in the six infants who died during cooling. The perinatal characteristics are shown in Table 6.1. The mother with antenatal syphilis was included because she completed treatment more than a month before delivery and the infant had no signs of congenital infection. The majority of infants (93%) were inborn. Despite the low number of outborn infants, 12 of the 60 infants (20%) did not have a cord or arterial blood gas within the first hour of life. A clinical neurological assessment was performed on all 60 infants at 3–5 hours (3.1 ± 0.4 hours) and only two infants had this assessment performed after 4 hours. Recordings of the 6-hour aEEG were available for 60 infants but a 3-hour aEEG was available for only 50 infants; 43 of these infants also had a 1-hour clinical assessment.

Table 6.1 Perinatal characteristics and short-term neonatal outcomes (N = 60)

Characteristics and short-term outcomes	n (%) / mean (± SD)
Maternal baseline characteristics	
Median maternal age, years (IQR)	23 (20–26)
HIV negative (Unknown in 3)	42 (70)
Antenatal Syphillis (treated)	1 (2)
Pregnancy complications[a]	11 (18)
Intrapartum complications[b]	54 (90)
Emergency caesarean section	24 (40)
Normal vertex delivery	26 (43)
Forceps / ventouse	10 (17)
Infant baseline characteristics	
Birth weight, g	3167 (± 517)
Male gender	34 (57)
Outborn	4 (7)
Median Apgar score (IQR)	
1 minute	3 (1–4)
5 minute	5 (4–6)
10 minute	6 (5–7)
Resuscitation	
Chest Compressions	19 (32)
Adrenaline given	6 (10)
Continued respiratory support at 10 minutes	47 (78)
Cord blood gas done	19 (32)
Arterial Blood gas in 1st hour or Cord Gas done	48 (80)
Worst pH in 1st hour (n = 48)	7.0 (± 0.2)
Worst base deficit in 1st hour	17.8 (± 4.4)

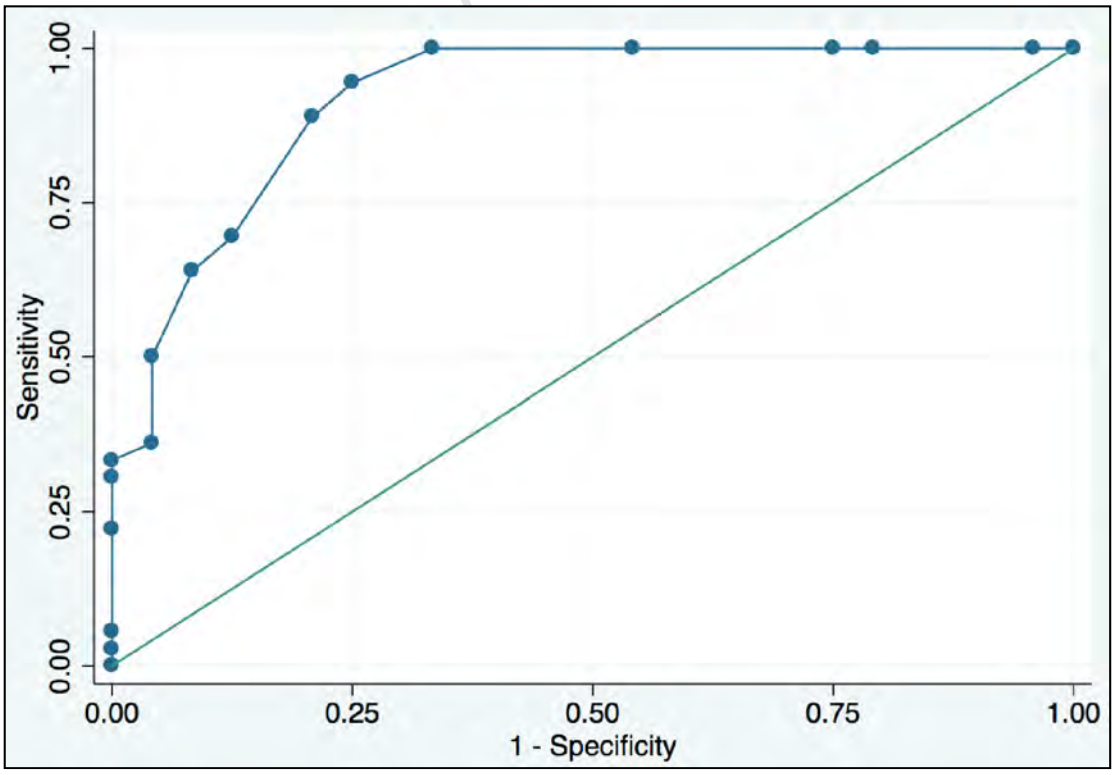
a, Includes hypertension, haemorrhage, thyroid disease and diabetes;
b, Includes abnormal fetal heart rate, cord prolapse, uterine rupture, maternal seizures, shoulder dystocia, maternal haemorrhage, meconium-stained liquor and prolonged second stage; SD, standard deviation; IQR, interquartile range

Clinical signs at 3–5 hours to predict an abnormal 6-hour aEEG

The ROC curve for the Thompson score at age 3–5 hours to predict an abnormal 6-hour aEEG (Figure 6.1) had an area under the curve (AUC) of 0.92 (95% confidence interval (CI) 0.84–0.99). The sensitivity, specificity and LR at different cut points are shown in Table 6.2. The Thompson score with a sensitivity of 100% and the highest specificity was a score of ≥ 7. The sensitivity, specificity and LR of the Thompson score at a cutoff of ≥ 7 vs. moderate-severe encephalopathy are shown in Table 6.3. There were no significant differences between the predictive values for these two assessment methods.

Univariate analysis of individual clinical signs at age 3–5 hours vs. abnormal 6-hour aEEG is shown in Table 6.4. At age 3–5 hours, all 60 infants had at least one abnormal primitive reflex. Twelve of the 29 infants (41%) *without* a decreased level of consciousness had an abnormal 6-hour aEEG. Hypotonia was a specific but not sensitive predictor for an abnormal aEEG: half of the infants *without* hypotonia (52%) had an abnormal 6-hour aEEG.

Figure 6.1 ROC curve: the Thompson score at 3–5 hours to predict an abnormal 6-hour aEEG



ROC, receiver operating characteristic

Table 6.2 ROC detail: Thompson score at 3–5 hours to predict an abnormal 6-hour aEEG

Thompson score cut point	Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+	LR-
≥ 2	100	0.00	60.00	1.0000	
≥ 3	100	4.17	61.67	1.0435	0.0000
≥ 4	100	20.83	68.33	1.2632	0.0000
≥ 5	100	25.00	70.00	1.3333	0.0000
≥ 6	100	45.83	78.33	1.8462	0.0000
≥ 7	100	66.67	86.67	3.0000	0.0000
≥ 8	94.44	75.00	86.67	3.7778	0.0741
≥ 9	88.89	79.17	85.00	4.2667	0.1404
≥ 10	69.44	87.50	76.67	5.5556	0.3492
≥ 11	63.89	91.67	75.00	7.6667	0.3939
≥ 12	50.00	95.83	68.33	12.0000	0.5217
≥ 13	36.11	95.83	60.00	8.6667	0.6667

LR, likelihood ratio; ROC, receiver operating characteristic

Table 6.3 Prediction of abnormal 6-hour aEEG with different encephalopathy assessment methods at 3–5 hours (N = 60)

Encephalopathy assessment		n	Abnormal aEEG n (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR + (95% CI)	LR - (95% CI)	LR Test (OR) (95% CI)
Moderate-severe encephalopathy	Yes	42	35 (83)	97	71	3.2 [a]	0.06 [a]	55.22 [a]
	No	18	1 (6)	(86–100)	(49–87)	(1.75–5.84)	(0.01–0.28)	(8.73–349.15)
Thompson score ≥ 7	Yes	44	36 (82)	100	67	2.9 [a]	0.02 [a]	141.71 [a]
	No	16	0 (0)	(90–100)	(45–84)	(1.7–5)	(0–0.33)	(7.71–2603.42)

aEEG, amplitude-integrated EEG; CI, confidence interval; LR, likelihood ratio; OR, odds ratio,
a, when zero count cells are present, LR is estimated using a substitution formula (0.5 is added to all cell frequencies before calculation)

Table 6.4 Univariate analysis: specific clinical signs at 3–5 hours as predictors of abnormal 6-hour aEEG (N = 60)

Predictor		n	Abnormal aEEG n (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR + (95% CI)	LR - (95% CI)	LR Test (OR) (95% CI)
Decreased LOC	Yes	31	24 (77)	67	71	2.29	0.47	4.86
	No	29	12 (41)	(49–81)	(49–87)	(1.18–4.44)	(0.28–0.8)	(1.61–14.61)
Seizures visible	Yes	20	17 (85)	47	88	3.78	0.6	6.26
	No	40	19 (48)	(30–65)	(68–97)	(1.24–11.5)	(0.43–0.85)	(1.67–23)
Hypotonia	Yes	14	12 (86)	33	92	3.38 [a]	0.74 [a]	4.59 [a]
	No	46	24 (52)	(19–51)	(73–99)	(0.96–11.9)	(0.56–0.96)	(1.05–20.04)
Stretch reflexes abnormal	Yes	40	31(78)	86	63	2.3	0.22	10.33
	No	20	5 (25)	(71–95)	(41–81)	(1.35–3.91)	(0.09–0.53)	(3.02–35.2)
Primitive reflexes abnormal	Yes	60	36 (100)	100	0	1.01 [a]	0.68 [a]	1.49 [a]
	No	0	0 (0)	(90–100)	(0–14)	(0.94–1.08)	(0.01–32.95)	(0.03–77.62)

aEEG, amplitude-integrated EEG; CI, confidence interval; LOC, Level of consciousness; LR, likelihood ratio; OR, odds ratio;
a , when zero count cells are present, LR is estimated using a substitution formula (0.5 is added to all cell frequencies before calculation)

Morbidity and short-term outcomes at the threshold Thompson score

Morbidity and short-term outcomes were compared between infants with a Thompson score of ≥ 7 and those with a Thompson score of < 7 at age 3–5 hours. Forty-four of the 60 infants (73%) had a Thompson score of ≥ 7. Nosocomial sepsis, pulmonary air leak and pulmonary hypertension were uncommon events and there was no significant difference in the incidence between the groups. More infants in the group with a Thompson score of ≥ 7 required CPAP (p = 0.036), mechanical ventilation (p = 0.002) and inotropic support (p = 0.048), than did those with a lower Thompson score. Forty of the 44 infants with a Thompson score of ≥ 7 (91%) met criteria for therapeutic hypothermia and were cooled. Only one infant with a Thompson score of < 7 was cooled. This infant had an abnormal 3-hour aEEG showing a DNV background, but the background normalised by 6 hours.

Predicting an abnormal aEEG at 3 and/or 6 hours

Subgroup analysis was performed on the 43 infants who had a 1-hour clinical assessment as well as an aEEG at both 3 and 6 hours. Twenty-eight infants in this subgroup had an abnormal aEEG at 3 *and/or* 6 hours and all of these 28 infants were cooled. The remaining 15 infants were not cooled. ROC curve analysis of the Thompson score at age 1 hour vs. an abnormal aEEG at 3 *and/or* 6 hours determined that the highest predictive Thompson score with a sensitivity of 100% was a score of ≥ 6 (AUC 0.93, 95% CI 0.86–1.00). This threshold therefore also identified all the infants in this subgroup who were cooled. However, specificity at this threshold was only 33% and most infants were correctly classified at a Thompson score of ≥ 7. ROC curve analysis of the Thompson score at age 3–5 hours vs. an abnormal aEEG at 3 *and/or* 6 hours determined that the highest predictive Thompson score with a sensitivity of 100% was a score of ≥ 5 (AUC 0.91, 95% CI 0.82–1.00) but specificity at this threshold was only 27%. The sensitivities, specificities and LRs for Thompson score and moderate-severe encephalopathy at 1 and 3–5 hours to predict an abnormal aEEG at 3 *and/or* 6 hours are shown in Table 6.5.

Table 6.5 Predicting abnormal aEEG at 3 or 6 hours: assessments at 1 and 3–5 hours (N = 43)

Encephalopathy assessment method		n	Abnormal aEEG n (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR+ (95% CI)	LR - (95% CI)	LR Test (OR) (95% CI)
Moderate-severe encephalopathy	Yes	31	25 (81)	89	60	2.23	0.18	12.5
age 1 hour	No	12	3 (25)	(72–98)	(32–84)	(1.19–4.2)	(0.06–0.56)	(2.71–56.89)
Moderate-severe encephalopathy	Yes	29	26 (90)	93	80	4.64	0.09	52
age 3 – 5 hours	No	14	2 (14)	(77–99)	(52–96)	(1.68–12.84)	(0.02–0.35)	(8.24–321.56)
Thompson score ≥ 8	Yes	28	25 (89)	89	80	4.46	0.13	33.33
age 3 – 5 hours	No	15	3 (20)	(72–98)	(52–96)	(1.61–12.38)	(0.04–0.4)	(6.16–180.87)
Thompson score ≥ 7	Yes	31	26 (84)	93	67	2.79	0.11	26
age 3 – 5 hours	No	12	2 (17)	(77–99)	(38–88)	(1.35–5.74)	(0.03–0.43)	(4.67–139.13)
Thompson score ≥ 6	Yes	34	26 (76)	93	47	1.74	0.15	11.38
age 3 – 5 hours	No	9	2 (22)	(77–99)	(21–73)	(1.07–2.83)	(0.04–0.65)	(2.15–57.78)
Thompson score ≥ 5	Yes	39	28 (72)	100	27	1.37 [a]	0.06 [a]	22.3 [a]
age 3 – 5 hours	No	4	0 (0)	(88–100)	(8–55)	(1–1.86)	(0–1.07)	(1.11–448.4)
Thompson score ≥ 8	Yes	30	26 (87)	93	73	3.48	0.1	35.75
age 3 – 5 hours	No	13	2 (15)	(77–99)	(45–92)	(1.5–8.11)	(0.02–0.38)	(6.13–201.31)
Thompson score ≥ 7	Yes	32	27 (84)	96	67	2.76 [a]	0.08 [a]	35 [a]
age 3 – 5 hours	No	11	1 (9)	(82–100)	(38–88)	(1.39–5.46)	(0.02–0.39)	(5.03–243.64))
Thompson score ≥ 6	Yes	38	28 (74)	100	33	1.5 [a]	0.05 [a]	29.86 [a]
age 1 hours	No	5	0 (0)	(88–100)	(12–62)	(1.05–2.14)	(0–0.85)	(1.52–587.98)
Thompson score ≥ 5	Yes	39	28 (72)	100	27	1.37 [a]	0.06 [a]	22.3 [a]
age 1 hours	No	4	0 (0)	(88–100)	(8–55)	(1–1.86)	(0–1.07)	(1.11–448.4)

aEEG, amplitude integrated electro-encephalogram; CI, confidence interval; LR, likelihood ratio, OR, odds ratio,
a, when zero count cells are present, LR is estimated using a substitution formula (0.5 is added to all cell frequencies before calculation)

Neurological short-term outcomes

Comparison of neurological short-term outcomes in infants at or below the threshold Thompson score at 3–5 hours are shown in Table 6.6. Most infants (86%) with a Thompson score of ≥ 7 had an abnormal aEEG at 3 and/or 6 hours. All of the infants with a Thompson score of < 7 had a normal 6-hour aEEG, but two of them had an abnormal 3-hour aEEG. The 3-hour aEEG in both infants was DNV and it corrected to a normal background by 6 hours. One of these infants was cooled on the basis of the abnormal aEEG, but in the other infant the aEEG had normalised by the time cooling was considered and cooling was not commenced. None of the infants with a Thompson score of < 7 had seizures. Ninety-eight per cent of infants (43 of 44) with a Thompson score of ≥ 7 developed moderate-severe encephalopathy within 72 hours of birth, but the majority of these infants (41 of 43) were cooled.

The predictive values of MSEG, Thompson score and aEEG for moderate-severe encephalopathy within 72 hours are shown in Table 6.7. The differences between the predictive values for the Thompson score and MSEG were not significant, but the sensitivity of the Thompson score (90%) was significantly higher (Exact McNemar p = 0.0156) than that of the 6-hour aEEG (75%).

Table 6.6 Short-term neurological outcomes

Outcome	Whole cohort N = 60 (100%)	Thompson < 7 at 3–5 hours n = 16 (100%)	Thompson ≥ 7 at 3–5 hours n = 44 (100%)
Mild Encephalopathy [a]	12 (20)	11 (69)	1 (2) *
Moderate Encephalopathy [a]	27 (45)	5 (31)	22 (50) *
Severe Encephalopathy [a]	21 (35)	0	21 (48) *
aEEG or Clinical Seizure	35 (58)	0	35 (80) *
Mean core temperature age 3 hours (± SD)	35.3 (± 1.1)	36.3 (± 0.4)	34.9 (± 1.0) *
aEEG normal at 6 and 24 hours	23 (38)	16 (100)	7 (16) *
aEEG abnormal age 3 hours (n = 50)	33 (66)	2(13)	31(71) *
aEEG abnormal age 6 hours	36 (60)	0	36 (82) *
aEEG abnormal age 3 or 6 hours	40 (67)	2(13)	38 (86) *
aEEG severely abnormal age 6 hours	25 (42)	0	25 (57) *
aEEG severely abnormal age 24 hours	18 (30)	0	18 (41) **
Dead or aEEG severely abnormal age 48 hours	12 (20)	0	12 (27) †
Maximum Thompson score ≥ 15	21 (35)	0	21 (48) **
Dead or maximum Thompson Score ≥ 15	22 (37)	0	22 (50) *
Dead	8 (13)	0	8 (19) ‡
Median Age at death, (Range) (n = 8)	2 (1.5–5)	-	2 (1.5–5)
Thompson score normal (0) by day 7	22 (42)	13 (81)	9 (25) *

aEEG, amplitude-integrated EEG; LOC, level of consciousness; SD, standard deviation;
a, the highest modified Sarnat encephalopathy grade recorded in the first 72 hours;
** p < 0.0001; ** p = 0.001; † p = 0.025; ‡ p = 0.095*

Table 6.7 Prediction of moderate-severe HIE presenting in the first 72 hours after birth

Encephalopathy assessment		n	moderate-severe HIE n (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR + (95% CI)	LR - (95% CI)	LR Test (OR) (95% CI)
aEEG abnormal age 6 hours (N = 60)	Yes	36	36 (100)	75 (60–86)	100 (74–100)	19.4 [a] (1.3–294.9)	0.27 [a] (0.16–0.43)	73 [a] (4–1325)
	No	24	12 (50)					
Moderate-severe encephalopathy age 3–5 hours (N = 60)	Yes	42	42 (100)	88 (75–95)	100 (74–100)	23 [a] (1.5–342.4)	0.1 [a] (0.1–0.3)	163.5 [a] (8.6–3106.8)
	No	18	6 (33)					
Thompson score ≥ 7 age 3–5 hours (N = 60)	Yes	44	43 (98)	90 (77–97)	92 (62–100)	7.7 [a] (1.7–34.8)	0.1 [a] (0.1–0.3)	60.6 [a] (8.9–413.1)
	No	16	5 (31)					
aEEG abnormal age 3 hours and/or 6 hours (N = 50)	Yes	40	39 (98)	81 (67–91)	50 (1–99)	1.6 [a] (0.5–5)	0.4 [a] (0.1–1.4)	4.2 [a] (0.4–44.5)
	No	10	9 (90)					

aEEG, amplitude integrated electro-encephalogram; CI, confidence interval; HIE, hypoxic ischaemic encephalopathy; LOC, level of consciousness; LR, likelihood ratio; OR, odds ratio;
a, when zero count cells are present, LR is estimated using a substitution formula (0.5 is added to all cell frequencies before calculation)

Moderate-severe encephalopathy during the first 72 hours occurred in five of 16 infants (31%) with a Thompson score of < 7 at 3–5 hours. All five infants had a normal aEEG at both 6 and 24 hours and none of these infants were cooled. Thirty-three per cent of infants (6 of 18) *without* moderate-severe encephalopathy at 3–5 hours, developed moderate-severe encephalopathy within 72 hours. However, five of the six infants had a normal aEEG at 24 hours: the sixth infant had an abnormal aEEG at 6 hours and was cooled. In comparison, 50% of the infants with a normal aEEG at 6 hours (12 of 24) developed moderate-severe encephalopathy within 72 hours, but three of these infants had an abnormal aEEG at 3 hours. A 3-hour aEEG was not available in one infant and one infant was cooled on the basis of suspected clinical seizures. The remaining seven infants with moderate-severe encephalopathy by 72 hours, but a normal aEEG at both 3 and 6 hours, were not cooled and all had a normal aEEG at 24 hours.

Overall, within the entire cohort of 60 infants there were eight infants with moderate-severe encephalopathy by 72 hours who were not cooled: all of these infants had a normal aEEG at both 6 and 24 hours and the median day of discharge (with normal nutritive suck) was day 5 (inter quartile range, day 4–7). Neurological examination on day 7 or at discharge, which ever occurred first, was normal in five infants. The Thompson score in the other three infants ranged from 1 to 2.

Discussion

Our data suggest that a Thompson score of ≥ 7 at age 3–5 hours identifies all infants with an abnormal 6-hour aEEG with a specificity of 67%. The presence of moderate-severe encephalopathy at the same age had similar specificity and sensitivity but failed to identify one infant with an abnormal 6-hour aEEG. Individual clinical signs previously used as entry criteria in clinical cooling trials had low sensitivity and/or low specificity for an abnormal 6-hour aEEG. A Thompson score of ≥ 5 at 1 *or* 3–5 hours identified all infants with an abnormal aEEG at 3 *and/or* 6 hours but with low specificity. A Thompson score of ≥ 7 at 3–5 hours predicted moderate-severe encephalopathy presenting within 72 hours after birth. However, the hypothermia and the sedating medication received by 83% (40 of 48) of the infants with moderate-severe encephalopathy may have exaggerated the abnormal signs; it was for this reason that the aEEG at 6 hours was chosen as a primary outcome rather than moderate-severe encephalopathy within 72 hours.

We defined an abnormal aEEG as one that qualifies an infant for cooling according to published protocols [15-17]. This definition allows inclusion of infants with DNV. In a retrospective study of aEEG in cooled vs. normothermic infants, Thoresen et al. defined a normal aEEG as one with CNV *or* DNV [23]. However, following their observation that three of the nine normothermic infants with DNV had a severely abnormal outcome vs. none of the eight cooled infants with DNV, they conceded it was reasonable to cool infants with DNV at 3–6 hours.

The predictive values for an abnormal outcome are lower for an aEEG at 3 vs. 6 hours [12] and an early abnormal assessment might inappropriately select infants for cooling therapy who were destined to be normal without cooling. Post-hoc analysis of the subgroup of infants with 1-hour assessments (Table 6.6) showed that an earlier Thompson score was more sensitive but less specific in predicting an abnormal aEEG at 3 *and/or* 6 hours (and therefore the need for cooling). If a single Thompson score threshold to be used at 1 *or* 3–5 hours is to be defined, a score of ≥ 7 had the best overall combination of high sensitivity and high diagnostic odds ratios of 35 and 26 respectively. The Thompson score of ≥ 5 at 1 *or* 3–5 hours identified all infants with an abnormal aEEG at 3 *and/or* 6 hours (and all the infants who were cooled). Although this threshold may be suitable to identify infants for referral or further assessment, the low specificity and LR makes it unsuitable as a sole indication for cooling.

Several cooling trials required the presence of a decreased level of consciousness and/or hypotonia as minimum evidence of moderate or severe encephalopathy before further assessment [15-17, 24, 25]. In our study, a significant proportion of the infants without either a decreased level of consciousness or hypotonia at 3–5 hours had an abnormal 6-hour aEEG. Our data suggest that the absence of specific individual clinical signs should not be grounds for excluding infants from aEEG assessment or cooling therapy.

Shalak et al. studied 50 infants with suspected intra-partum hypoxia [13]. They determined the ability of the moderate-severe encephalopathy at age 5 ± 3 hours and an abnormal fronto-parietal aEEG acquired within an hour of the examination, to predict moderate-severe encephalopathy persisting until the fifth day. The clinical assessments were performed slightly later than in our study and the aEEG position was fronto-parietal, but similar to our study, they found that 23% of infants *without* all the criteria for moderate-severe encephalopathy had an abnormal aEEG and progressed to moderate encephalopathy persisting on the fifth day. The sensitivity and specificity of early moderate-severe encephalopathy to predict moderate-severe

encephalopathy persisting to the 5th day were both 78%, but the combination of an abnormal aEEG and encephalopathy including clinical signs of both mild and moderate encephalopathy increased the specificity to 94%. In our study, only one of the infants (6%) without all the criteria for moderate-severe encephalopathy at 3–5 hours had an abnormal aEEG at 6 hours and developed persistent moderate-severe encephalopathy by the fifth day. By comparison, none of the infants with a Thompson score of < 7 had an abnormal aEEG at 6 hours, and moderate-severe encephalopathy did not persist to the fifth day in any of those infants. One infant with a Thompson score of < 7 was cooled, but data from a secondary analysis of the CoolCap cooling trial data suggest that the hypothermia is unlikely to have influenced the improvement in grade of encephalopathy in this infant [26].

Sarkar et al. have questioned whether a 6-hour aEEG should be used to identify infants for cooling [27]. In a retrospective analysis, they reported that 13 of 24 infants with a normal aEEG had abnormal magnetic resonance imaging (MRI), but the rates of abnormal outcome were similar between the cooled and normothermic groups. In a prospective study, Shankaran et al. found that a fronto-parietal aEEG at < 9 hours did not enhance the predictive value of HIE grade at < 6 hours [28]. Twelve of 71 infants with moderate HIE had a CNV background at < 9 hours and three of these infants had an abnormal outcome. The data of both Sarkar and Shankaran suggests that a normal aEEG in the first 6–9 hours does not guarantee a normal outcome. However it is still unclear whether cooling infants with a normal aEEG is beneficial.

The Thompson score of ≥ 7 at 3–5 hours identified more infants with moderate-severe encephalopathy than did the 6-hour aEEG, but the additional infants identified by the Thompson score were predominantly those who were not cooled. The presence of CNV or DNV background voltage on aEEG at 24 hours is significantly associated with normal outcomes in both cooled and normothermic infants [23, 29]. The finding of a normal 24-hour aEEG, very low or normal Thompson scores by day 7, and a median discharge age of 5 days, in the infants with moderate-severe encephalopathy who were not cooled suggests that these infants did not require cooling. Thus, although clinical assessment with either MSEG or the Thompson score identified significantly more infants with moderate-severe encephalopathy, this may not be clinically significant.

This study has several limitations. The exclusion of sick infants may alter the sensitivity and specificity of the Thompson score – the findings of this study cannot be applied to the infants whom were excluded. It is a significant weakness of the study that we did not determine the kappa coefficient for the multiple assessors

(raters) in this particular study. However, all clinicians were trained, the kappa coefficient for two raters of the Thompson score in a previous study at Groote Schuur Hospital is known to be high [14], and the use of multiple raters replicates the setting in which this particular diagnostic approach would ultimately be implemented. The lack of data from age 1 hour in all infants compromised the strength of our conclusions regarding very early assessments. Although the primary objective was to study the prediction of an abnormal 6-hour aEEG, the availability of MRI or long-term follow up data would have allowed further validation and interpretation of the threshold Thompson scores, particularly in the infants with moderate-severe encephalopathy who were not cooled. A further important limitation is that the use of phenobarbital and morphine may have resulted in over-diagnosis of moderate-severe encephalopathy in the infants who were cooled.

The strengths of our study are that we prospectively recruited infants with all grades of HIE and obtained at least one clinical and one aEEG assessment by age 6 hours. We compared previously published neurological assessments to a validated aEEG assessment method as a gold standard. We blinded the clinicians' assessments by comparing their clinical assessment with a later aEEG recording and neonatologists who were blind to the clinical assessments subsequently read the aEEG recordings.

Conclusions

We have shown that, in a cohort of term infants with all grades of HIE, a Thompson score of ≥ 7 or the presence of a moderate-severe modified Sarnat encephalopathy grade at age 3–5 hours both independently predict an abnormal aEEG at 6 hours, with similar predictive values. A Thompson score of ≥ 7 at age 3–5 hours was a more sensitive predictor of moderate-severe HIE presenting within 72 hours than a 6-hour aEEG. This data may assist in providing a threshold for intervention and/or benchmarking early neurological assessments of infants being considered for neuroprotective hypothermia. A Thompson score of ≥ 5 at 1 or 3 hours identified all infants with an abnormal aEEG at 3 *and/or* 6 hours – this may be an appropriate threshold to guide early referral to cooling centers for further assessment. An abnormal 6-hour aEEG can occur in infants without a decreased level of consciousness or hypotonia and these single signs may not be appropriate as minimum criteria for cooling. The Thompson score thresholds we have identified should be validated in larger studies with long-term outcomes and MRI. Further research should investigate long-term outcomes of infants with signs of moderate HIE but normal aEEG who are not cooled.

References

1. Amiel-Tison C. Cerebral damage in full-term new-born. Aetiological factors, neonatal status and long-term follow-up. *Biol Neonat* 1969;14(3):234-50.

2. Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 2003;361(9359):736-42.

3. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic–ischaemic encephalopathy. *Early Hum Dev* 2010;86(6):329-38.

4. Lawn JE, Lee AC, Kinney M, Sibley L, Carlo WA, Paul VK, et al. Two million intrapartum-related stillbirths and neonatal deaths: Where, why, and what can be done? *Int J Gynaecol Obstet* 2009;107(S):S5-S19.

5. Horn AR, Swingler GH, Myer L, Harrison MC, Linley LL, Nelson C, et al. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. *J Perinat Med* 2013; 41(2):211-7.

6. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for Neonatal Hypoxic Ischemic Encephalopathy: An Updated Systematic Review and Meta-analysis. *Arch Pediatr Adolesc Med* 2012;166(6):558-66.

7. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;33(10): 696-705.

8. Gunn AJ, Bennet L, Gunning MI, Gluckman PD, Gunn TR. Cerebral hypothermia is not neuroprotective when started after postischemic seizures in fetal sheep. *Pediatr Res* 1999;46(3):274-80.

9. Iwata O, Iwata S, Thornton JS, De Vita E, Bainbridge A, Herbert L, et al. "Therapeutic time window" duration decreases with increasing severity of cerebral hypoxia-ischaemia under normothermia and delayed hypothermia in newborn piglets. *Brain Res* 2007;1154:173-80.

10. Hellstrom-Westas L, Rosen I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. *Arch Dis Child Fetal Neonatal Ed* 1995;72(1):F34-8.

11. Spitzmiller RE, Phillips T, Meinzen-Derr J, Hoath SB. Amplitude-integrated EEG is useful in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischemic encephalopathy: a meta-analysis. *J Child Neurol* 2007;22(9):1069-78.

12. Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 1999;81(1):F19-23.

13. Shalak LF, Laptook AR, Velaphi SC, Perlman JM. Amplitude-integrated electroencephalography coupled with an early neurologic examination enhances prediction of term infants at risk for persistent encephalopathy. *Pediatrics* 2003;111(2):351-7.

14. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

15. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361(14):1349-58.

16. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460): 663-70.

17. Simbruner G, Mittal RA, Rohlmann F, Muche R, neon.EURO.network Trial Participants. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics* 2010;126(4):e771-8.

18. World Medical Association Declaration of Helsinki. *World Med J* 2008;54(4):122-4.

19. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353(15):1574-84.

20. Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. *Acad Emerg Medicine* 1996;3(9):895-900.

21. Hellstrom-Westas L, Rosen I, de Vries LS, Greisen G. Amplitude-integrated EEG Classification and Interpretation in Preterm and Term Infants. *Neoreviews* 2006;7(2):e76-87.

22. Horn AR, Harrison MC, Linley LL. Evaluating a Simple Method of Neuroprotective Hypothermia for Newborn Infants. *J Trop Pediatr* 2010;56(3):172-7.

23. Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics* 2010;126(1):e131-9.

24. Zhou W-h, Cheng G-q, Shao X-m, Liu X-z, Shan R-b, Zhuang D-y, et al. Selective head cooling with mild systemic hypothermia after neonatal hypoxic-ischemic encephalopathy: a multicenter randomized controlled trial in China. *J Pediatr* 2010;157(3):367-72, 72.e1-3.

25. Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch Pediatr Adolesc Med* 2011;165(8):692-700.

26. Gunn AJ, Wyatt JS, Whitelaw A, Barks J, Azzopardi D, Ballard R, et al. Therapeutic hypothermia changes the prognostic value of clinical evaluation of neonatal encephalopathy. *J Pediatr* 2008;152(1):55-8.

27. Sarkar S, Barks JD, Donn SM. Should amplitude-integrated electroencephalography be used to identify infants suitable for hypothermic neuroprotection? *J Perinatol* 2008;28(2):117-22.

28. Shankaran S, Pappas A, McDonald SA, Laptook AR, Bara R, Ehrenkranz RA, et al. Predictive value of an early amplitude integrated electroencephalogram and neurologic examination. *Pediatrics* 2011;128(1):e112-20.

29. Van Rooij LG, Toet MC, Osredkar D, van Huffelen AC, Groenendaal F, de Vries LS. Recovery of amplitude integrated electroencephalographic background patterns within 24 hours of perinatal asphyxia. *Arch Dis Child Fetal Neonatal Ed* 2005;90(3):F245-51.

Chapter 7

Early clinical predictors of a persistently abnormal amplitude-integrated electroencephalogram at 48 hours in cooled neonates with hypoxic ischaemic encephalopathy

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Author contributions

I was the principal investigator. I conceptualized the study, wrote the research proposal, supervised and participated in the data collection and analysed the data. I wrote all versions of the manuscript and submitted it to the journal. GHS, and LM participated in the design of the study and critically reviewed the draft manuscript. LLL and MC assisted with acquisition of data and critically reviewed the draft manuscript. NJR participated in the design of the study, assisted with acquisition of data and critically reviewed the draft manuscript. All authors read and approved the final manuscript.

Notes

Minor amendments recommended by the examiners of the thesis have been included.

The data consent form and collection sheets were the same as those used in the research described in chapter 6 and they are included at the end of the thesis as Appendices D and E respectively.

Abstract

Aim

There is a need to identify infants with hypoxic ischaemic encephalopathy who have a poor outcome despite therapeutic hypothermia. A severely abnormal amplitude-integrated electroencephalogram at 48 hours predicts death or disability. Our aim was to determine if clinical assessment at age 3–5 hours predicts a severely abnormal amplitude-integrated electroencephalogram at 48 hours or death in cooled infants.

Methods

This study is a pre-planned secondary analysis. Forty-one cooled infants, ≥ 36 weeks gestation, with moderate-severe hypoxic ischaemic encephalopathy, were prospectively enrolled. Infants who were moribund, had congenital conditions associated with encephalopathy or had severe cardio-respiratory instability were excluded. The Predictive abilities of the Thompson encephalopathy score and individual signs at age 3–5 hours were assessed,

Results

All infants with a Thompson score of ≥ 16 at 3–5 hours had a severely abnormal amplitude-integrated electroencephalogram at 6 hours and an abnormal short-term outcome. At 48 hours, 75% had a severely abnormal aEEG or died vs. 18% with a score of < 16 ($p = 0.004$). Multivariate analysis did not find a significant independent association with any of the individual signs.

Conclusion

The Thompson score could be useful to identify infants who will have a poor outcome despite cooling. A score of ≥ 16 should be validated as a pre-specified variable in prospective studies.

Background

There is strong evidence showing the benefit of therapeutic hypothermia for infants born with moderate or severe hypoxic ischaemic encephalopathy (HIE) [1] and the benefit is still evident at age 7 years [2]. Hypothermia is now recommended as a standard of care by international consensus of developed countries [3]. However there remains a need to develop bedside methods to identify in the first hours of life those infants whose brain injury is so severe that they will have a poor outcome despite cooling [4]. This is particularly relevant for resource-limited areas where the incidence of HIE is highest [5, 6]. Although simple, low-cost methods of cooling have been published [7-9] there are few data to guide the use of hypothermia in these settings [10].

In cooled infants with HIE, the amplitude-integrated electroencephalogram (aEEG) has low predictive values at < 6 hours but at 48 hours a severely abnormal background is strongly predictive of death or disability [11]. A severely abnormal aEEG could therefore be used as a proxy for later abnormal outcome where there are insufficient resources for magnetic resonance imaging and long-term follow-up is difficult. This option is particularly relevant in the Western Cape, South Africa, where long-term follow-up is difficult. This is partly because a high proportion of mothers relocate from other provinces to peri-urban regions of Cape Town for the birth of their infants and return to their home region after giving birth [12].

The Thompson HIE score is derived from nine aspects of the neurological examination and it ranges from 0 to 22 [13]. We hypothesised that the Thomson score assessed < 6 hours after birth could predict a poor outcome in cooled infants. This study is a preplanned secondary analysis of cooled infants within a previously described cohort of infants who were evaluated for therapeutic hypothermia [14]. Our objectives were:

(i) to determine the Thompson score at age 3–5 hours that best predicted a severely abnormal aEEG at 48 hours or death in cooled infants; and

(ii) to determine if specific individual clinical signs at age 3–5 hours predict a severely abnormal aEEG at 48 hours or death.

Patients and methods

The University of Cape Town Health Sciences Faculty Human Research Ethics Committee approved the study. The study conforms to the principles of the 2008 Declaration of Helsinki [15] and informed parental consent was obtained. The study was performed between 1 June 2008 and 31 March 2009 at three hospitals in Cape Town: Groote Schuur Hospital, a tertiary hospital and New Somerset Hospital and Mowbray Maternity Hospital, both secondary hospitals.

Study sample

Infants at ≥ 36 weeks gestation and birth weight ≥ 2000 g, with signs of encephalopathy after age 10 minutes but before age 5 hours were consecutively recruited if they had a base deficit of ≥ 16 in the first hour of life on cord or infant arterial blood or if they had an abnormal intra-partum course *with* either a 10-minute Apgar score of < 7 *or* continued respiratory support at age 10 minutes. If the aEEG background was abnormal or if seizures were present, cooling to a core (rectal) temperature of 34 °C was initiated within the first 6 hours after birth. The lower limit of the basic servo-controlled gel-pack method of cooling in use in the department at the time was 34 °C. This method has previously been described in more detail where the mean core temperature achieved was 33.9 ± 0.3 °C which is comparable to other commercial methods in use at the time [8]. The cooled infants were the research subjects in this analysis.

Exclusion criteria were any of the following: infection at birth, chorioamnionitis with prolonged rupture of membranes, chromosomal syndromes, cerebral malformations, lethal congenital malformations, neonatal abstinence syndrome due to maternal substance abuse, metabolic encephalopathies, moribund infants where demise was imminent or infants with severe cardio-respiratory instability requiring high dose inotropes, a fractional inspired oxygen of ≥ 0.8, or high frequency oscillatory ventilation.

Neurological assessment

The neurological assessment has been reported previously [14]. In brief, a neurological assessment evaluating clinical signs described by Sarnat [16] and Thompson [13] was performed at age 3–5 hours, 6, and 24 hours after birth and then daily until the tenth day or discharge, whichever occurred first. A Thompson HIE score [13] was assigned (Box S7.1) and the encephalopathy was also graded as

mild, moderate or severe according to a modified Sarnat encephalopathy grading (MSEG) [17]. The attending paediatric residents or researchers with experience in neonatal neurological examination performed the assessments.

Box S7.1: The Thompson HIE score [13]

Sign \ Score	1	2	3	Score
Limb tone	Generally hypertonic	Generally hypotonic	Flaccid	
Level of consciousness	Hyper-alert, staring, or excessive irritability	Lethargic	Comatose or stuporose	
Visible fits	Infrequent; < 3/day	Frequent; > 2/day		
Posture	Fisting and / or cycling	Strong distil flexion	Decerebrate	
Moro	Partial	Absent		
Grasp	Poor	Absent		
Suck	Poor	Absent and/or bites		
Respiratory effort	Hyperventilation	Transient apnoea	Apnoea + IPPV	
Fontanel	Full	Tense		
TOTAL				

IPPV: Intermittent Positive Pressure Ventilation

The maximum score, based on the infant's clinical signs since the previous assessment, is recorded in each category and then totaled to obtain the final score.

An aEEG was recorded on a BrainZ BRM2 monitor [BrainZ Instruments Ltd, Auckland, NZ] until the background remained normal for 24 hours or until 96 hours, or until the infant demised, whichever occurred first. The aEEG and associated raw EEG recordings were assessed by two neonatologists (MC and NJR) who were experienced in interpreting aEEGs and who were blind to the neurological assessments. The assessors provided consensus opinion on the category of the bi-parietal background aEEG trace and the presence of seizures at 6, 24 and 48 hours. The classification of the aEEG background voltage pattern was based on the modified system proposed by Hellström-Westas [18] and the aEEG background activity was further graded as follows:

(i) *Normal/moderately abnormal:* Background activity showed continuous normal voltage (CNV) or discontinuous normal voltage (DNV); and

(ii) *Severely abnormal:* Background activity showed burst-suppression (BS), continuous low voltage (CLV) or a flat trace (FT).

Medical management

All infants received routine clinical care and monitoring. Fluid administration was restricted to 40 ml/kg/day on the first day; inotropes were commenced if the mean blood pressure was below 35–40 mmHg. All infants were sedated with intravenous (IV) phenobarbital 20 mg/kg when cooling started. Morphine 8 µg/kg/hour IV was commenced if infants were restless or agitated during cooling. Seizures were treated with a second dose of IV phenobarbital 20 mg/kg. Persistent seizures were treated with midazolam at 0.05–0.1 mg/kg/hour (0.8–1.6 mcg/kg/minute) IV, which was replaced by IV lignocaine at half standard doses, if seizure control was not maintained with midazolam.

Data collection and analysis

Data collection included perinatal characteristics and events, morbidity and short-term outcomes. Data were analysed with Stata 12 (Stata Corporation, Texas, USA). Receiver operating characteristic (ROC) curve analysis was used to determine the threshold Thompson score at 3–5 hours predicting a severely abnormal aEEG at 48 hours or death. The threshold score that correctly classified the most infants with the most appropriate balance between sensitivity and specificity was chosen. The diagt module was used to calculate the sensitivity, specificity and likelihood ratio (LR) for the threshold Thompson score. Short-term outcomes and morbidity during the hospital stay were compared between infants with or without the threshold score. The Chi-square or Fisher's exact tests were used for categorical comparisons. The Student's t-test and the Wilcoxon rank-sum tests were used to compare the parametric and non-parametric continuous variables respectively. Univariate analysis determined the sensitivity, specificity and LR of individual clinical signs at age 3–5 hours to predict a severely abnormal aEEG at 48 hours or death. The specific signs were those typically associated with severe encephalopathy (coma, stupor or coma, flaccidity, decerebrate posturing, seizures, ventilatory support due to apnoea or gasping, and absent primitive reflexes). The signs that were significant predictors on univariate analysis were further examined with multivariate analysis using logistic regression. All statistical tests are two-sided at alpha = 0.05.

Results

The study cohort comprised 41 cooled infants. Thirty-nine infants were cooled because of an abnormal aEEG within the first 6 hours and two infants were cooled on the basis of clinical seizures alone. Eight infants (20%) died before discharge. All the infants who died had an aEEG that remained severely abnormal from 6 hours until the time of death. The cause of death was attributed to HIE in all cases and the age at death ranged from 1.5 to 5 days: three infants died before 48 hours. A severely abnormal aEEG was recorded in 25 of 41 (61%) infants at 6 hours and in nine of 38 surviving infants (24%) at 48 hours. The highest MSEG recorded in the first 72 hours was moderate in 19 of 41 (46%) and severe in 21 of 41 (51%) infants. A significantly higher proportion of infants who did not die or have a severely abnormal aEEG at 48 hours, received sedation with morphine than those who did (21 of 29 vs. 4 of 12 respectively; Fisher's exact p = 0.034).

The perinatal characteristics are shown in Table 7.1. Most infants (93%) had a history of intrapartum complications. Severe metabolic acidosis was present in all 30 infants who had a blood gas measured in the first hour of life. The ROC curve for the Thompson score at age 3–5 hours for death or a severely abnormal aEEG at 48 hours (Figure 7.1) had an area under the curve (AUC) of 0.73 (95% confidence interval (CI) 0.53–0.93). The sensitivity, specificity and LR at different cut points are shown in Table 7.2. The Thompson score threshold of ≥ 16, correctly classified the most infants with the highest specificity.

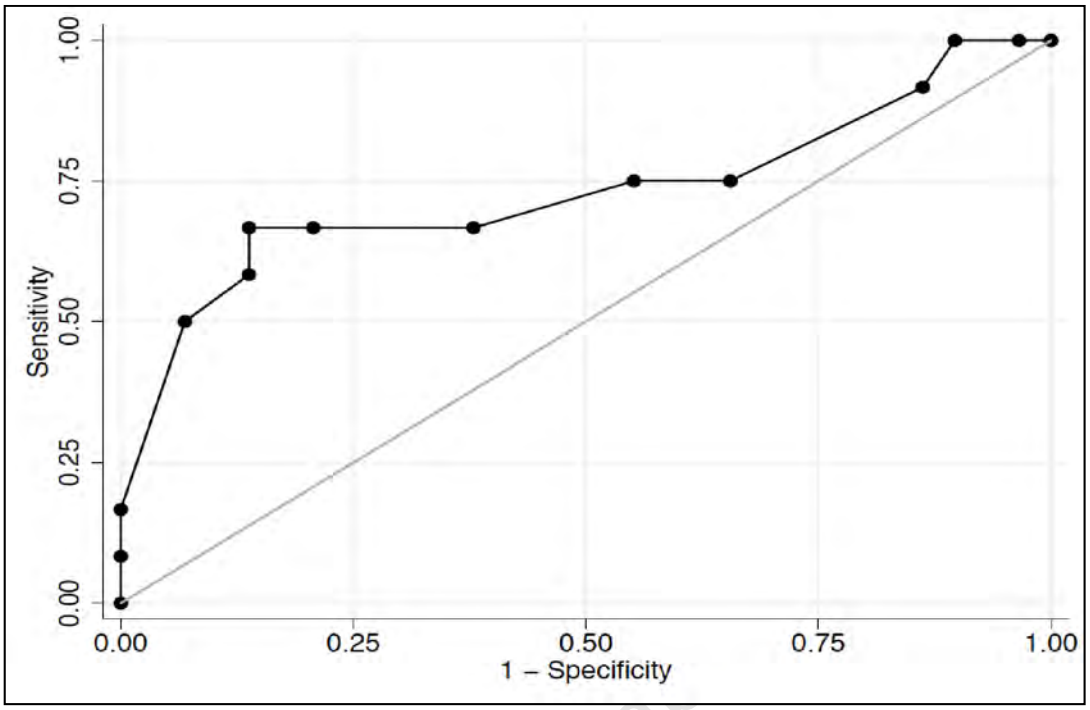
The univariate analysis of individual clinical signs at age 3–5 hours is shown in Table 7.3. The presence of stupor/coma, flaccidity and apnoea/gasping were significantly predictive of death/severely abnormal aEEG at 48 hours, but multivariate logistic regression did not find a significant independent association with any of these signs.

Table 7.1 Perinatal characteristics of all cooled infants (N = 41)

Characteristics	n (%) / mean (± SD) / IQR)
Maternal baseline characteristics	
Maternal age in years	23 (± 5)
HIV negative (Unknown in 2)	27 (66)
Antenatal Syphillis (treated)	1 (2)
Pregnancy complications[a]	4 (10)
Intrapartum complications[b]	38 (93)
Emergency caesarean section	14 (34)
Normal vertex delivery	19 (46)
Forceps / ventouse – assisted delivery	8 (20)
Infant baseline characteristics	
Birth weight, g	3220 (± 407)
Male gender	23 (56)
Outborn	4 (10)
Median Apgar score (IQR)	
1 minute	3 (1–4)
5 minute	4 (3–5)
10 minute	6 (5–6)
Resuscitation	
Chest compressions	15 (36)
Adrenaline given	6 (15)
Continued respiratory support at 10 minutes	34 (83)
Cord blood gas done	11 (27)
Arterial blood gas in 1st hour or cord gas done	30 (73)
Worst pH 1st hour (n = 30)	7.02 (± 0.16)
Worst base deficit 1st hour	17.6 (15.9–22.6)

IQR, interquartile range; SD, standard deviation
a, includes hypertension, haemorrhage, thyroid disease and diabetes.
b, includes abnormal fetal heart rate, cord prolapse, uterine rupture, maternal seizures, shoulder dystocia, maternal haemorrhage, meconium-stained liquor and prolonged second stage.

Figure 7.1 ROC curve for the Thompson score at 3–5 hours to predict death or a severely abnormal 48-hour aEEG



ROC, Receiver operating characteristic

Table 7.2 Cut point detail of ROC curve for Thompson score at 3–5 hours to predict death or a severely abnormal aEEG

Thompson score cut point	Sensitivity (%)	Specificity (%)	Correctly Classified (%)	LR+	LR-
≥ 5	100.00	0.00	29.27	1.0000	
≥ 7	100.00	3.45	31.71	1.0357	0.0000
≥ 8	100.00	10.34	36.59	1.1154	0.0000
≥ 9	91.67	13.79	36.59	1.0633	0.6042
≥ 10	75.00	34.48	46.34	1.1447	0.7250
≥ 11	75.00	44.83	53.66	1.3594	0.5577
≥ 12	66.67	62.07	63.41	1.7576	0.5370
≥ 13	66.67	79.31	75.61	3.2222	0.4203
≥ 14	66.67	86.21	80.49	4.8333	0.3867
≥ 15	58.33	86.21	78.05	4.2292	0.4833
≥ 16	50.00	93.10	80.49	7.2500	0.5370
≥ 17	16.67	100.00	75.61		0.8333
≥ 18	8.33	100.00	73.17		0.9167
> 18	0.00	100.00	70.73		1.0000

aEEG, amplitude-integrated electroencephalogram; LR, likelihood ratio; ROC, receiver operating characteristic

Table 7.3 Univariate analysis: clinical signs at 3–5 hours as predictors of death or severely abnormal 48-hour aEEG (N = 41)

Predictor	n		Death / Severely Abnormal 48-hour aEEG n (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR + (95% CI)	LR – (95% CI)	LR Test / Odds ratio (95% CI)
Coma	Yes	3	1 (33)	8.3	93.1	1.21	0.98	1.23
	No	38	11 (29)	(0.2–38.5)	(77.2–99.2)	(0.12–12.1)	(0.81–1.2)	(0–10.65)
Stupor/Coma	Yes	10	6 (60)	50	86.2	3.62 *	0.58	6.25 *
	No	31	6 (19)	(21.1–78.9)	(68.3–96.1)	(1.24–10.58)	(0.32–1.04)	(1.4–27.98)
Flaccid	Yes	5	4 (80)	33.3	96.6	9.67 *	0.69	14 [a, *]
	No	36	8 (22)	(9.9–65.1)	(82.2–99.9)	(1.2–77.8)	(0.46–1.04)	(1.09–713.03)
Decerebrate	Yes	3	2 (67)	16.7	96.6	4.83	0.86	5.6 [a]
	No	38	10 (26)	(2.1–48.4))	(82.2–99.9)	(0.48–48.41)	(0.66–1.12)	(0.26–341.81)
Seizures	Yes	20	5 (25)	41.7	48.3	0.81	1.21	0.67
	No	21	7 (33)	(15.2–72.3)	(29.4–67.5)	(0.38–1.72)	(0.66–2.22)	(0.18–2.5)
Ventilation for Apnoea or Gasping	Yes	13	7 (54)	58.3	79.3	2.82 *	0.53	5.37 *
	No	28	5 (18)	(27.7–84.8)	(60.3–92)	(1.2–6.65)	(0.26–1.05)	(1.3–22.27)
Primitive reflexes Absent	Yes	13	5 (38)	41.7	72.4	1.51	0.81	1.88
	No	28	7 (25)	(15.2–72.3)	(52.8–87.3)	(0.62–3.69)	(0.47–1.37)	(0.48–7.39)

aEEG, amplitude-integrated electroencephalogram; CI, confidence interval; LR, likelihood ratio;
a, when zero count cells are present, LR is estimated using a substitution formula: 0.5 is added to all cell frequencies before calculation.
** $p < 0.05$*

The comparison of morbidity and short-term outcomes between infants with a Thompson score of ≥ 16 and those with a Thompson score of < 16 at age 3–5 hours is shown in Table 7.4. Eight infants (19.5%) had a Thompson score of ≥ 16. There were no significant differences in the incidence of death, ventilatory support, inotrope requirement or acquired hospital sepsis. The incidence of both moderate and severe MSEG was significantly higher in the infants with a Thompson score ≥ 16 (p = 0.05). All of the infants with a Thompson score of ≥ 16 at 3–5 hours had a severely abnormal aEEG at 6 hours vs. only ten of the 13 infants with severe MSEG at 3–5 hours. The five infants with a Thompson score of < 16 who died, all showed rapid deterioration and recurrent seizures after the first 6 hours: two of these infants had a Thompson score of ≥ 14 at 3–5 hours.

By 48 hours 75% of the infants with a Thompson score of ≥ 16 at 3–5 had a severely abnormal aEEG or had died vs. 18% of the infants with a Thompson score of < 16 (p = 0.004). The two infants with a Thompson score of ≥ 16 who did not die nor had a severely abnormal aEEG at 48 hours, both had an abnormal Thompson score at 7 days. In the whole cohort of 41 infants, the sensitivity and specificity of a Thompson score of ≥ 16 *and* a severely abnormal aEEG at 6 hours to predict a severely abnormal outcome at 48 hours was identical to the Thompson score alone. However, ROC analysis of the Thompson score at 3–5 hours only in the 25 infants with a severely abnormal aEEG at 6 hours, found that a Thompson score of ≥ 14 correctly classified the most infants (76%) as having a poor outcome at 48 hours, with a specificity of 78.6% (95% CI 49–95%), a sensitivity of 72.7% (95% CI 39–94%), a LR+ of 3.39 (95% CI 1.17–9.86) and LR- of 0.35 (95% CI 0.13–0.95). For comparison, the sensitivity, specificity and LR of severe MSEG and a Thompson score of ≥ 16 at 3–5 hours for a severely abnormal aEEG at 48 hours or death, are shown in Table 5. The Thompson score had higher specificity than that of severe MSEG, but the difference was not significant (Exact McNemar, p = 1.000).

Table 7.4 Morbidity, short-term outcome and associated variables

	Whole cohort	Thompson score at 3–5 hours		p-value
		< 16	≥ 16	
	N = 41 (100%)	n = 33 (100%)	n = 8 (100%)	
Morbidity				
Mechanical ventilation	25 (61)	18 (55)	7 (88)	0.12
Continuous positive airway pressure	20 (49)	16 (49)	4 (50)	1
Inotropes used	14 (34)	9 (27)	5 (63)	0.097
Pulmonary airleak	2 (5)	2 (6)	0 (0)	1
Acquired sepsis	1 (2)	1 (3)	0 (0)	1
Pulmonary hypertension	3 (7)	3 (9)	0 (0)	1
Short-term outcomes and associated variables				
Birth weight in grams (± SD)	3220 (± 407)	3212 (± 407)	3251 (± 434)	0.8
10-minute Apgar score (range)	6 (3–8)	6 (3–8)	5 (3–6)	0.07
Mild MSEG [a]	1 (2)	1 (3)	0 (0)	1
Moderate MSEG [a]	19 (46)	18 (55)	1 (13)	0.05
Severe MSEG [a]	21 (51)	14 (42)	7 (88)	0.045
Severe MSEG at 3–5 hours	13 (32)	6 (18)	7 (88)	0.001
Severe MSEG at 72 hours or dead	16 (39)	10 (30)	6 (75)	0.04
Seizures (aEEG and/or clinical)	35 (85)	28 (85)	7 (88)	1
Mean core temperature at 3 hours (± SD)	34.8 (± 0.93)	34.6 (± 0.95)	34.8 (± 0.86)	0.8
aEEG severely abnormal at 6 hours	25 (61)	17 (52)	8 (100)	0.014
aEEG severely abnormal at 24 hours	18 (44)	12 (36)	6 (75)	0.109
Dead or severely abnormal 48-hour aEEG	12 (29)	6 (18)	6 (75)	0.004
Dead before discharge	8 (20)	5 (15)	3 (38)	0.172
Thompson score zero by day 7 (n = 33)	7 (21)	7/28 (25)	0/5 (0)	0.559

aEEG, amplitude-integrated electroencephalogram; MSEG, modified Sarnat encephalopathy grading; SD, standard deviation;
a, highest modified Sarnat grade of encephalopathy recorded in the first 72 hours

Table 7.5 Prediction of severely abnormal 48-hour aEEG or death, with different assessment methods at age 3–5 hours (N = 41)

Encephalopathy assessment	n		Severely abnormal 48-hour aEEG or death n (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	LR + (95% CI)	LR - (95% CI)	LR Test / Odds ratio (95% CI)
Severe MSEG	Yes	13	7 (54)	58.3	79.3	2.8	0.53	5.37
	No	28	5 (18)	(28–85)	(60–92)	(1.2–6.65)	(0.26–1.05)	(1.3–22.27)
Thompson score ≥ 16	Yes	8	6 (75)	50	93.1	7.25	0.54	13.5
	No	33	6 (18)	(21–79)	(77–99)	(1.7–30.97)	(0.3–0.95)	(2.39–73.64)

aEEG, amplitude-integrated electroencephalogram; CI, confidence interval; LR, likelihood ratio; MSEG, modified Sarnat encephalopathy grade

Discussion

Our data suggest that infants with HIE and a Thompson score of ≥ 16 assessed as early as 3–5 hours, have a very poor prognosis despite being cooled, but sensitivity of the assessment is low. The sensitivity and specificity of the Thompson score to predict a poor outcome was not altered by adding the presence of a severely abnormal aEEG at 6 hours. In addition, the proportion of infants with a Thompson score ≥ 16 at 3–5 hours with severe encephalopathy, both at age 3–5 hours and as a maximum grade during the first 72 hours, was double that of infants with a score of < 16. The sample was not adequately powered to detect a significant difference between the ability of severe MSEG vs. a Thompson score of ≥ 16 at 3–5 hours to predict death or a severely abnormal aEEG at 48 hours.

Shankaran et al. found that 83% of infants with severe encephalopathy persisting until 72 hours after birth died or were disabled and the proportions were similar in both cooled and normothermic infants [19]. A meta-analysis of the CoolCap, TOBY and Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) hypothermia trials, found that hypothermia was less effective in infants with severe encephalopathy [20]. The lack of response to hypothermia is postulated to be due to a more rapid progression of delayed neuronal loss [21] and/or the presence of established injury with lack of recovery of the cerebral oxidative metabolism [22].

A secondary analysis of the CoolCap trial, suggested that in the control group, a birthweight < 25th percentile was associated with better outcomes; conversely, in the cooled group, there was a highly significant increased cooling effect in the ≥ 25th percentile group, with a lower number needed to treat than the smaller infants [23]. Similarly, in a secondary analysis of the NICHD trial, Laptook et al. found that a birth weight of ≥ 3.2 kg predicted a better outcome in hypothermic infants with 10-minute Apgar scores of 3 or 4 [24]. There was no significant difference in birth weight between infants above or below the threshold Thompson score in our study.

Laptook et al. also explored the association of the 10-minute Apgar score with death or disability in cooled infants: they found that an Apgar score of 3 or 4 was associated with death or disability in 44% vs. 71% for infants with a 10-minute Apgar of ≤ 2. An Apgar score of 0 did not discriminate further. Sarkar et al. retrospectively reviewed the records of infants who were cooled in their institution [25]: they determined that a 10-minute Apgar score of 0 was independently associated with death from HIE, odds ratio (OR) 51.7 (95% CI 9.9–269.5). In our cohort, the infants with a Thompson score of ≥ 16 at 3–5 hours had lower 10-minute Apgar score, but

the difference was not significant (p = 0.07). None of the infants enrolled in our study had 10-minute Apgar scores below 3: infants with 10-minute Apgar scores of 1 or 2 may have comprised the group of infants considered to be “moribund” and would therefore have been excluded.

In a retrospective single centre study including cooled infants, two factors were found to be independently predictive of death or disability at age 1.5 hours: base deficit (OR 1.13, 95% CI 0.99–1.28) for each mmol increase and the need for respiratory support (OR 11.62, 95% CI 1.17–115.51) [26]. Seizures before 6 hours were also independently predictive of poor outcome (OR 6.07, 95% CI 1.25–29.46). We did not make comparisons at these specific times and blood gas analysis was not available for all infants in our cohort. However, there were no significant differences in the incidence of respiratory support or seizures, between the infants who were above or below the threshold Thompson score at 3–5 hours. Moreover, univariate analysis of clinical signs in our cohort showed that the presence of clinically visible seizures at 3–5 hours did not predict death or a severely abnormal aEEG at 48 hours. Similarly, Wyatt et al. [23] found that the presence of aEEG seizures at randomisation did not predict death or disability in cooled infants (OR 0.63, 95% CI 0.36–1.09). Ambalavanan et al. analysed the predictive value of early neurological signs for death or disability in a combined cohort of both cooled and normothermic infants in the NICHD trial. The only components that were identified as independently contributing to prognosis were distal flexion, decerebrate posture, absent spontaneous activity and absent suck [27]. In our cohort, multivariate logistic regression did not find a significant independent association with similar signs but the very wide confidence intervals did not exclude clinically meaningful associations.

Cooling had already commenced in all the infants in our study at the time of the 3–5 hour assessment. The influence of cooling on clinical assessment is not entirely clear. In a secondary analysis of the CoolCap trial, Gunn et al. found that hypothermia did not affect the severity of encephalopathy on day 4, but the cooled infants with moderate encephalopathy at this time had a better prognosis than those who were not cooled [28]. In 2012, Shankaran et al. reported on the evolution of encephalopathy during cooling in the NICHD trial [19]. Similar to the findings of Gunn et al., they found no difference in the stage of encephalopathy at 72 hours between cooled and non-cooled infants. However, infants in the hypothermia group had less severe encephalopathy at 24 and 48 hours. Our assessments are unlikely to have been confounded by cooling as there were no significant differences between the temperatures at assessment of infants above or below the Thompson score threshold.

Our study has some important limitations. It was a secondary analysis and was also limited by the small sample size. The cooling method used and the population studied may limit the applicability of the results in other populations and further research is needed in these settings. The morphine and anticonvulsants may have influenced the clinical findings and the aEEG background, but the aEEG background improved more frequently in those infants who received morphine and several studies have shown that the doses we used are unlikely to have significantly influenced the aEEG background [29].

The lack of long-term follow-up is a further limitation that is difficult to address as a result of the transient interprovincial migration of many mothers [12]. However, a recent systematic review of prognostic tests in term neonates with HIE showed similar sensitivity and specificity of aEEG to that of magnetic resonance imaging [30]; moreover, in cooled infants a severely abnormal aEEG (BS/CLV/FT) at 48 hours predicts a very poor outcome [11, 31]. Thoresen et al. followed a cohort of 43 cooled infants with moderate-severe HIE [11]. All of the 14 infants with a severely abnormal aEEG at 48 hours either died or were severely disabled, while only three of the 29 infants whose aEEG corrected to CNV or DNV before 48 hours had an abnormal outcome. Hallberg et al. followed a much smaller cohort and all four infants whose aEEG did not normalise by 48 hours had a poor outcome [31]. These data are limited by the small number of infants and the lack of confidence intervals around predictive values for abnormal aEEG at 48 hours for an abnormal outcome, but the strong association of death or disability with an abnormal aEEG at ≥ 48 hours was consistent across both studies. In our setting, a persistently severely abnormal aEEG at age 48 hours in cooled infants profoundly influences subsequent medical management decisions. The strengths of our study are that we prospectively and consecutively recruited infants and the Thompson score was used as an assessment method: this method has an inter-observer reliability coefficient of 0.87 and was already in established use in our department [13].

In conclusion, a Thompson score of ≥ 16, assessed at 3–5 hours, identified a subgroup of infants with severe hypoxic ischaemic encephalopathy, who had a very poor short-term outcome, despite cooling. The Thompson threshold we have identified could be useful in practice and it should be validated as a pre-specified variable in larger prospective studies providing more detailed follow-up data.

References

1. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for Neonatal Hypoxic Ischemic Encephalopathy: An Updated Systematic Review and Meta-analysis. *Arch Pediatr Adolesc Med* 2012;166(6):558-66.
2. Shankaran S, Pappas A, McDonald SA, Vohr BR, Hintz SR, Yolton K, et al. Childhood Outcomes after Hypothermia for Neonatal Encephalopathy. *The New England journal of medicine* 2012;366(22):2085-92.
3. Perlman JM, Wyllie J, Kattwinkel J, Atkins DL, Chameides L, Goldsmith JP, et al. Part 11: Neonatal Resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. *Circulation* 2010;122(16_suppl_2):S516-S38.
4. Perlman M, Shah PS. Hypoxic-Ischemic Encephalopathy: Challenges in Outcome and Prediction. *J Pediatr* 2011;158(2):e51-e4.
5. Lawn JE, Lee AC, Kinney M, Sibley L, Carlo WA, Paul VK, et al. Two million intrapartum-related stillbirths and neonatal deaths: Where, why, and what can be done? *Int J Gynaecol Obstet* 2009;107(S):S5-S19.
6. Horn AR, Swingler GH, Myer L, Harrison MC, Linley LL, Nelson C, et al. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. *J Perinat Med* 2013;41(2):211-7.
7. Robertson NJ, Nakakeeto M, Hagmann C, Cowan FM, Acolet D, Iwata O, et al. Therapeutic hypothermia for birth asphyxia in low-resource settings: a pilot randomised controlled trial. *Lancet* 2008;372(9641):801-3.
8. Horn AR, Harrison MC, Linley LL. Evaluating a Simple Method of Neuroprotective Hypothermia for Newborn Infants. *J Trop Pediatr* 2010;56(3):172-7.
9. Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch Pediatr Adolesc Med* 2011;165(8):692-700.
10. Thayyil S, Costello A, Shankaran S, Robertson NJ. Therapeutic hypothermia for neonatal encephalopathy implications for neonatal units in India. *Indian Pediatr* 2009;46(4):283-9.

11. Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics* 2010;126(1):e131-9.

12. Human I, Kroon M, Bergman N, Fawcus S, Ntwana B. Patient population movement in a Cape Town obstetric service. *S Afr Med J* 2003;93(9):634.

13. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

14. Horn AR, Swingler GH, Myer L, Linley LL, Raban MS, Joolay Y, et al. Early clinical signs in neonates with hypoxic ischemic encephalopathy predict an abnormal amplitude-integrated electroencephalogram at age 6 hours. *BMC Pediatr* 2013;13:52.

15. World Medical Association Declaration of Helsinki. *World Med J* 2008;54(4):122-4.

16. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;33(10): 696-705.

17. Shalak LF, Laptook AR, Velaphi SC, Perlman JM. Amplitude-integrated electroencephalography coupled with an early neurologic examination enhances prediction of term infants at risk for persistent encephalopathy. *Pediatrics* 2003;111(2):351-7.

18. Hellstrom-Westas L, Rosen I, de Vries LS, Greisen G. Amplitude-integrated EEG Classification and Interpretation in Preterm and Term Infants. *Neoreviews* 2006;7(2):e76-87.

19. Shankaran S, Laptook AR, Tyson JE, Ehrenkranz RA, Bann CM, Das A, et al. Evolution of Encephalopathy during Whole Body Hypothermia for Neonatal Hypoxic-Ischemic Encephalopathy. *The Journal of pediatrics* 2012;160(4):567-72.

20. Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, et al. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* 2010;340:c363.

21. Gunn AJ, Thoresen M. Hypothermic neuroprotection. *NeuroRx* 2006;3(2):154-69.

22. Azzopardi D, Wyatt JS, Cady EB, Delpy DT, Baudin J, Stewart AL, et al. Prognosis of newborn infants with hypoxic-ischemic brain injury assessed by phosphorus magnetic resonance spectroscopy. *Pediatr Res* 1989;25(5): 445-51.

23. Wyatt JS, Gluckman PD, Liu PY, Azzopardi D, Ballard R, Edwards AD, et al. Determinants of Outcomes After Head Cooling for Neonatal Encephalopathy. *Pediatrics* 2007;119(5):912-21.

24. Laptook AR, Shankaran S, Ambalavanan N, Carlo WA, McDonald SA, Higgins RD, et al. Outcome of term infants using apgar scores at 10 minutes following hypoxic-ischemic encephalopathy. *Pediatrics* 2009;124(6):1619-26.

25. Sarkar S, Bhagat I, Dechert RE, Barks JD. Predicting death despite therapeutic hypothermia in infants with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 2010;95(6):F423-8.

26. Thoresen M. Patient selection and prognostication with hypothermia treatment. *Semin Fetal Neonatal Med* 2010;15(5):247-52.

27. Ambalavanan N, Carlo WA, Shankaran S, Bann CM, Emrich SL, Higgins RD, et al. Predicting outcomes of neonates diagnosed with hypoxemic-ischemic encephalopathy. *Pediatrics* 2006;118(5):2084-93.

28. Gunn AJ, Wyatt JS, Whitelaw A, Barks J, Azzopardi D, Ballard R, et al. Therapeutic hypothermia changes the prognostic value of clinical evaluation of neonatal encephalopathy. *J Pediatr* 2008;152(1):55-8.

29. Csekő AJ, Bangó M, Lakatos P, Kárdási J, Pusztai L, Szabó M. Accuracy of amplitude-integrated electroencephalography in the prediction of neurodevelopmental outcome in asphyxiated infants receiving hypothermia treatment. *Acta Paediatr* 2013;102(7):707-11.

30. van Laerhoven H, de Haan TR, Offringa M, Post B, van der Lee JH. Prognostic tests in term neonates with hypoxic-ischemic encephalopathy: a systematic review. *Pediatrics* 2013;131(1):88-98.

31. Hallberg B, Grossmann K, Bartocci M, Blennow M. The prognostic value of early aEEG in asphyxiated infants undergoing systemic hypothermia treatment. *Acta Paediatr* 2010;99(4):531-6.

Chapter 8 Discussion and conclusions

8.1 Study endpoints and applicability

The overall aims of this thesis were: (i) to develop strategies for the early diagnosis and management of moderate-severe hypoxic ischaemic encephalopathy (HIE) in a setting where, although resources are limited, neonatal intensive care provision is available; (ii) to review the bedside tools for early identification or prediction of moderate-severe HIE in this setting; and (iii) to describe the epidemiology and management challenges of HIE specific to the Southern Cape Peninsula, South Africa. The validation of the low-cost cooling method in use in the region at the time, to maintain appropriate core temperature, was an integral component of the research. The determination of the incidence of HIE in the region was important to establish the burden of disease for health management and to demonstrate the importance of benchmarking.

8.1.1 A systematic review of early clinical or serum predictors of moderate-severe encephalopathy

Many biomarkers of HIE have been identified and reviewed in the literature [1]. However, for the optimal use of biomarkers in clinical practice, biomarker measurements must be rapidly accessible with specific cut-off levels which can robustly predict moderate-severe HIE. No previous systematic review specifically addressing these aspects of prediction with biomarkers was identified in a systematic literature search of papers published in English.

Using a systematic search strategy, seven papers describing 29 index test studies that had sufficient data to populate 2x2 tables were identified. Only bedside clinical examination and serum tests were included; tests using a bedside amplitude-integrated electroencephalogram (aEEG) were not included.

A high potential for bias was present in all 29 tests and all tests derived a predictive threshold rather than investigating a pre-specified value. The reasons for risk of bias were variable. Frequently, the methods of patient selection were not clear, sample handling was inadequately described and blinding was poorly reported. Quantitative analysis revealed very wide confidence intervals of the measures of diagnostic accuracy for the majority of the tests. A combined early clinical and blood gas assessment (the Logistic Score) was the strongest predictor (sensitivity 93%,

specificity 88%, and diagnostic odds ratio (DOR) 106). However, the precise timing of the assessments (age 30 minutes), the very small number of infants with moderate-severe HIE, and the potential for bias around the patient selection and assessment methods, limit the immediate application of the Logistic Score in clinical practice. Importantly, the finding of this systematic review, that moderate-severe HIE can occur at base deficit thresholds lower than 12 mmol/l, highlights the implications of excluding infants with lower base deficits if the history and clinical assessment suggest HIE. The improvement in diagnostic accuracy following the addition of a clinical assessment support the conclusion that a combination of clinical examination and other variables indicating intrapartum abnormality, will be required to achieve appropriate power and accuracy of tests predicting moderate-severe HIE.

8.1.2 Defining HIE and benchmarking in a South African population

The several different published criteria defining "significant intra-partum hypoxia" and "HIE" [2-7], present challenges when describing the epidemiology. The only population-based study of HIE in South Africa published prior to this thesis was published in abstract format [8], and it did not benchmark against international consensus definitions or against criteria that were used in the major cooling studies.

This thesis presents the first study of HIE where different definitions of intra-partum hypoxia and encephalopathy were applied to the same population to determine the variation in incidence of HIE with different definitions. The data provides a benchmark for our region, showing that the incidence of HIE varied from 2.3–4.3 per 1 000 live births depending on which defining criteria were used. While the incidence is specific to the Southern Cape Peninsula, the extent to which it varies can plausibly be extrapolated to similar settings and the variation is likely to be more pronounced in less-resourced settings.

The importance of measuring the incidence of HIE, particularly moderate-severe HIE, is two-fold: it is an indicator of antenatal and obstetric care [9] and it indicates the anticipated requirement for therapeutic hypothermia and/or specialised neonatal care. There is often a paucity of diagnostic data in settings where there are inadequate staffing ratios and/or where late clinical presentation is frequent [10, 11]. However, the pitfall in assigning a diagnosis of HIE where the evidence of intra-partum hypoxia is not clear, is that the encephalopathy may in fact be due to a different aetiology [12]. Diagnostic confidence is important for medicolegal purposes. The American Academy of Pediatrics (AAP) [13] and the International Cerebral Palsy Task Force (ICPTF) [2] defined rigid criteria to define intra-partum hypoxia of

sufficient severity to cause brain damage. An essential component of these criteria was an early blood gas. In the cohort that I studied, only 56% of the infants had a blood gas done in the first hour of life. In addition, meconium-stained liqor was the only intrapartum abnormality in 24% of infants and a prolonged second stage of labour was the only indication of intrapartum hypoxia in 11%. While it is accepted that these variables are not, on their own, strong indications of intrapartum hypoxia, if they are not accepted as sufficient evidence for hypoxia when the infant presents with typical signs of HIE, in our setting, this would result in over estimating the quality of obstetric care and also potentially denying over a third of the infants access to therapeutic hypothermia. Moreover, intrapartum hypoxia is the commonest cause of neonatal encephalopathy (NE) or seizures in term and near-term infants [14] and in settings with a high incidence of intrapartum hypoxia, this may be the cause of NE proportionately more often than in settings where the incidence of intrapartum hypoxia is lower.

Most cooling trials used very similar criteria to those described by the AAP and the ICPTF – these criteria helped to ensure homogenous groups of infants with an expected high incidence of adverse outcome [15]. Although the researchers do not claim that these criteria are all required to define HIE, the International Liason Committee on Resuscitation suggest that similar criteria to those used in the randomised controlled cooling trials should be used when treating infants with hypothermia [16].

The findings from our South African cohort suggest that either a less restrictive definition of HIE should be used or an alternative term should be adopted. The importance of the definition in correctly identifying cases of HIE may have more impact in low-income countries with a high neonatal mortality rate. The term, “intrapartum-related neonatal encephalopathy” could be used to define neonates with encephalopathy and an abnormal intrapartum course, without other obvious cause for the encephalopathy, but not necessarily meeting the published criteria of HIE required for medico-legal purposes [2, 13]. The use and definition of this term would need to be approved at national and international forums before it could be formally adopted. There is a pressing need for consensus definitions of HIE and NE to be established, accepted and used in studies across the world for us to be able to make valid comparisons and assessments of interventions.

8.1.3 Evaluating a simple method of neuroprotective hypothermia

Before a prospective study evaluating the predictive value of clinical signs could be carried out in our setting, the existing method of therapeutic hypothermia had to be evaluated to ensure that appropriate core (rectal) temperatures were maintained. The method in use in Cape Town utilised a very similar approach to that used in the Infant Cooling Evaluation (ICE) study [17], by applying soft refrigerated gel-packs to the heads of the infants and further controlling their core temperature using a radiant warmer. However, in the ice study, the power of the radiant warmer was manually controlled, whereas in Cape Town the radiant warmer was servo controlled to a proxy core temperature.

The report on the Cape Town method is the first to describe the thermal efficacy of this method of cooling: in subsequent studies it is referred to as "servo-assisted gel-pack cooling" [18]. The cooling method maintained a mean rectal temperature of 33.9 ± 0.3 °C, which was comparable to other published data at the time [19, 20]. These findings suggest that the outcome data derived from the infants who were prospectively studied during and after cooling is comparable to that obtained from infants cooled to a similar core temperature in other settings using other methods.

The environmental temperature in the neonatal unit during this study was 24.6 ± 0.5 °C. Two subsequent studies in India, using *frozen* gel-bags, found that a radiant warmer was not required during cooling with their method where the environmental temperature is 28 °C or more [21, 22]. This is particularly relevant for warm equatorial countries in sub-Saharan Africa and it emphasizes the need to do pilot studies to ensure the validity of the method in the specific environment.

8.1.4 Early clinical signs predicting an abnormal aEEG at age 6 hours

This study prospectively recruited 60 infants with signs of encephalopathy after suspected intrapartum hypoxia. The sample included infants with mild HIE who did not require cooling and infants with moderate-severe HIE, most of whom were cooled. Infants were only cooled if they had seizures or an abnormal aEEG background diagnosed before age 6 hours. This aim of this study was to determine if individual clinical signs or a threshold Thompson HIE score could predict an abnormal aEEG by age 6 hours, with the intention that infants with an abnormal aEEG by age 6 hours should be treated with hypothermia. It was not a study of cooling efficacy. No previous studies investigating this correlation before age 6 hours have been published.

Although some hypothermia trials have treated infants on the basis of clinical signs of moderate-severe HIE alone [3, 17], others also required an abnormal aEEG by 6 hours [4, 5], because a normal aEEG at 6 hours is strongly associated with a normal outcome [23].

The results of this study showed that none of the individual clinical signs had a high predictive accuracy, but a Thompson score of ≥ 7 at age 3–5 hours identified all the infants with an abnormal 6-hour aEEG, with a specificity of 67%. The score at this time also predicted the presence of moderate-severe encephalopathy presenting in the first 72 hours with 90% sensitivity and 92% specificity. A modified Sarnat encephalopathy grading (MSEG) system [6] was also compared to aEEG and encephalopathy outcomes and showed similar predictive values to those of the Thompson score, but the sample was not large enough to determine if one assessment method was significantly superior to the other.

An abnormal 6-hour aEEG and a Thompson score of ≥ 7, both individually predicted moderate-severe HIE to occur within 72 hours after birth. The aEEG was more specific (100% vs. 92%) but less sensitive (75% vs. 90%) than the Thompson score, but all the infants with a normal aEEG at 3 or 6 hours who also had moderate-severe HIE, had a normal 24-hour aEEG and normal or near-normal neurological examination at discharge. This finding is in keeping with other published data suggesting that the aEEG does not identify all cases of moderate-severe HIE [24]. However, it also supports an approach of conservative treatment of such infants as the majority of the infants in question had normal short-term outcomes.

The accuracy of the Thompson scores at age 1 or 3 hours to predict an abnormal aEEG at 3 or 6 hours was also assessed in those infants who presented early enough: a score of ≥ 5, identified all affected infants but with very low specificity. The Thompson HIE score is a widely recognised assessment method and has been used in several studies of HIE outside of the centers in this study, in both developed [25, 26] and resource-limited settings [27, 28].

Applying these findings from this study in clinical practice would involve a screening Thompson assessment of infants with suspected HIE as soon as possible after birth and no later than age 6 hours. Infants with a score of ≥ 5 should be assessed with aEEG if available, or re-assessed frequently until age 6 hours. If the score is ≥ 7 at any stage during the first 6 hours, the infant can be expected to have or develop moderate-severe HIE, and should be treated with hypothermia if it is feasible or appropriate to that setting.

However, larger prospective studies are required to validate the Thompson score at a pre-specified threshold. It could be argued that since it has similar predictive values to Shalak's MSEG system, that the latter is sufficient. But staff in some centers may already be trained in the use of the Thompson score and the small differences between the methods that this study identified, may reach significance in larger studies. Moreover, the similarity in likelihood ratios provides interim benchmarking data that could assist when comparing studies.

8.1.5 Early clinical signs predicting death or a severely abnormal aEEG at 48 hours in cooled infants.

The 41 cooled infants in the previous paper were studied to determine the predictive accuracy of clinical signs for a severely abnormal short-term outcome. This novel approach aimed to provide a method of assessment, without the need to refer to long-term follow-up data, that could identify infants who had very severe HIE and a poor outcome despite cooling.

A Thompson score of ≥ 16 at 3–5 hours predicted either death or an abnormal aEEG at 48 hours with 93% specificity, but only 50% sensitivity. The low sensitivity suggests that there are infants with a lower score who will still have an abnormal outcome, but in this setting, specificity is most important because the response to the assessment is that of redirected care. Seventy-five percent of infants with a Thompson score of ≥ 16 died or had a severely abnormal aEEG at 48 hours and the remaining 25% infants had an abnormal neurological examination at 7 days. Individual clinical signs were not significantly predictive.

It is important to ensure that treatment is offered to those with the highest chance of benefit and not to continue with the treatment of futile cases; this issue is especially relevant in situations where there are limited resources. To this end, a Thompson score of ≥ 15 may be a more pragmatic and cost effective threshold, because it predicts either death or an abnormal aEEG at 48 hours with almost the same specificity (86%) but higher sensitivity (67%), than a score of ≥ 16. Similar to the previous study, the simple assessment methods in this study were specifically used to ensure applicability to similar resource-limited settings. Larger prospective studies comparing these thresholds to outcome are required before firm recommendations can be made, but the thresholds could be used as an interim measure in resource-limited centers without access to aEEG while awaiting further data.

8.2 Limitations

8.2.1 A systematic review of early clinical or serum predictors of moderate-severe encephalopathy

The systematic review of early predictors was limited to English language publications listed in Medline. A wider search with broader inclusion criteria may have revealed more predictors, but no such predictor was referred to in the identified literature.

8.2.2 Defining HIE and benchmarking in a South African population

The retrospective method of deriving the incidence of HIE was limited by the quality of the data in the hospital record and all cases in the cohort had already been diagnosed with HIE by attending clinicians. It is possible that some cases of HIE may have been missed, however HIE was diagnosed using less restrictive criteria than are generally recommended, so the impact of this limitation is likely to be small. While infants with known metabolic encephalopathies were not included, it is possible that infants with apparently occult metabolic encephalopathies and a similar clinical course to HIE were included. Cowan et al. showed a high incidence of peripartum hypoxic brain injury (80%) on MRI, in association with neonatal encephalopathy [14] despite including neonates with less rigid evidence of intrapartum hypoxia than is required by the AAP [13] and ICPTF [2]. The study by Cowan and colleagues was done in a well-resourced setting, but in the relatively resource-limited setting of our study, the contribution of "non-hypoxic" encephalopathies may be expected to be less. The availability of long-term outcomes or magnetic resonance imaging (MRI) would have allowed further interpretation of the appropriateness of the less restrictive definitions referred to in our setting.

8.2.3 Evaluating a simple method of neuroprotective hypothermia

The study evaluating the simple cooling method had few patients and was not powered nor intended to study neurological outcomes. A greater level of certainty regarding the efficacy of the method would have been achieved with a randomised controlled trial, however the method was already well established and was very similar to that used in the ICE trial [17].

The small size of the study also limited the identification of unanticipated problems with the approach, however a subsequent larger case series from our unit using the same method at a lower target core temperature of 33.5 °C showed excellent temperature control, comparable to the currently available state-of-the-art cooling devices [18].

8.2.4 Early clinical signs predicting an abnormal aEEG at age 6 hours

The high confidence intervals around the DOR for the Thompson score threshold indicate that a larger sample would have been more appropriate, however in a screening scenario, sensitivity is most important and the confidence intervals around sensitivity were appropriately narrow (90–100%). The kappa score for the Thompson score for multiple assessors was not calculated in this study, but a score for two raters had previously been derived at Groote Schuur Hospital and was found to be very high (0.87).

A further limitation is the potential effect of phenobarbital and morphine on the aEEG voltage as well as the clinical diagnosis of HIE. It is possible that moderate-severe HIE may have been over-diagnosed by clinical assessment during the first 72 hours. However, this limitation will apply to all similar studies. The morphine is unlikely to have affected the aEEG background significantly as the dose used was very low (8 μg/kg/hour). Doses up to 20 μg/kg/hour had no effect on the background voltage or incidence of sleep-wake cycling in a cohort of 26 neonates after cardiac surgery [29], nor in a cohort of 47 infants after non-cardiac surgery [30]. The same authors reported only transient effects of midazolam at doses up to 0.25 mg/kg/hour, but profound suppression following a bolus of fentanyl, at a dose of 10 μg/kg, was observed [29].

Eken et al. reported a minimal and transient effect of phenobarbital, at a dose of 20 mg/kg, on the aEEG background of infants with mild HIE [31] and more recently, in 2008, Shany et al. reported that although the majority of infants treated with anticonvulsants will not show any significant effect on the aEEG voltage pattern, phenobarbital can affect the trace in a minority of cases and the effect is transient and variable. Csekö et al. studied the effects of anticonvulsant and sedative drugs on aEEG recovery in a large cohort of 70 asphyxiated infants treated with hypothermia [32]. They showed that morphine at a dose of 10 μg/kg/hour, midazolam at a dose of 0.1 mg/kg/hour and phenobarbital at a dose of 20 mg/kg did not delay recovery of the aEEG. Importantly, they showed that there was no

difference in the cumulative drug doses of these three medications between the normal and poor outcome groups or between the first and second day aEEG recovery groups.

In our study, the clinicians who performed the neurological assessments were effectively blinded to the aEEGs, because the comparison was made between clinical assessments performed at 3–5 hours and the aEEG at 6 hours. However, in some cases the clinicians would have had knowledge of the condition of the infant at birth and may have had sight of earlier aEEG recordings – this aspect could introduce bias, but it was difficult to avoid. The assessors of the aEEG were more effectively blinded as they only read the aEEGs and did not have access to other clinical data.

The availability of MRI and/or long-term follow-up would have allowed further validation and interpretation of the threshold Thompson scores in the infants with moderate-severe encephalopathy and normal 6-hour aEEGs.

8.2.5 Early clinical signs predicting death or a severely abnormal aEEG at 48 hours in cooled infants

This study was a sub-study that was limited by a small sample size. However, there were narrow confidence intervals around specificity and the positive likelihood ratio (LR) was clinically and statistically significant. The response to a severely abnormal aEEG at 48 hours, particularly in a resource-limited setting, is likely to include limiting intensive care: in this setting, the specificity and LR+ are most important.

Both this study and the one preceeding it derived a threshold Thompson score. The process of deriving a predictive threshold is itself subject to bias [33]. Similar concerns regarding the effect on the aEEG of anticonvulsants and sedative agents apply to this study as to the previous study, but as described above, it is unlikely that there was a significant effect from these medications.

Similar to previous studies, an important limitation of this study is that neither long-term outcomes nor MRI were available: a severely abnormal aEEG was used as a proxy for later abnormal outcome. Although the predictive value of aEEG is altered when infants are treated with therapeutic hypothermia [34, 35], both Hallberg et al.[35] and Thoresen et al.[34] reported that all cooled infants with moderate-severe HIE, whose aEEG background had normalised to continuous/discontinuous normal voltage by 24 hours had a normal outcome, most infants whose aEEG had normalised between 36 and 48 hours had a normal outcome, and all infants whose

aEEG normalised after 48 hours had a poor outcome. Interpretation of these data is limited by the small number of cooled infants with an abnormal outcome at follow-up (a total of 22 of 66 cooled infants) and the lack of provision of confidence intervals around predictive values for abnormal aEEG at specified time intervals for abnormal outcome, however the strong association of death or disability in infants with a severely abnormal aEEG persisting ≥ 48 hours was consistent across both studies. A further limitation is that the absence of the abnormality at 48 hours does not confirm a normal outcome. However, most infants who do not have a severely abnormal background at 48 hours, will have a normal long-term outcome [34, 35].

8.3 Further research

8.3.1 Predictors of moderate-severe encephalopathy

In low- and middle-income countries, predictors will be most useful if they are simple and do not require expensive, complicated equipment. The history of the peripartum period combined with an early clinical examination is likely to be the most appropriate in this setting. There are very few data of this type in resource-limited settings and future studies should consider combining accessible markers with a clinical examination. Publications should include sufficient raw data to populate 2x2 tables and should describe inclusion and exclusion criteria precisely. Methods of patient enrolment along with other methods to reduce bias should be clearly stated.

8.3.2 Epidemiology and definitions of HIE

In settings where intensive care is not available; the burden of HIE, standardised neonatal care, and neurodevelopmental follow-up should be established before research with neuroprotective therapies such as hypothermia commences. Further epidemiological studies of HIE and NE in resource-limited areas should be prospective. To avoid exclusion on the basis of lack of data, these studies should consider broad inclusion criteria, such as those defined by intrapartum-related encephalopathy. Subsequent analysis could then be done according to grade of encephalopathy and related parameters; the grade of encephalopathy should ideally be correlated with MRI imaging and preferably long-term neurodevelopmental outcome. If the study is done prospectively, regular Thompson HIE scores, starting at < 6 hours could then be correlated with outcome. Kaufman et al have shown that early signs of severe HIE predict an abnormal MRI, but predictions within the moderate group remain elusive [36].

8.3.3 Cooling methods

Cooling is a standard of care in high-income settings; a high level of care and monitoring for maintenance of physiological homeostasis is recommended for optimal safety and cooling efficacy [27, 37]. Further research needs to focus on finding a robust, simple, energy-efficient cost-effective method, and then studying these devices in randomised controlled trials. Cooling is not generally recommended in low-resource settings where neonatal practices are not consistent and where there is no provision of neonatal intensive care such as ventilation, blood pressure support and prompt treatment of seizures. In the mid-resourced setting of South Africa, intensive care is available in some areas, however provision of therapeutic hypothermia is especially challenging: the incidence of neonatal encephalopathy is high, leading to a high demand on intensive care cot spaces and limited resources [38]. In addition, the population has high rates of infection and poverty [39]. Simple, reliable cooling methods are needed in such settings and are being developed: a modified version of the gel-pack method we described has been found to be effective in one trial [22]; other simple methods including phase-changing material [40] and fan-cooling [41] have also been shown to be feasible in pilot studies.

Some animal data suggests that deeper cooling will provide greater benefit than mild cooling. In adult gerbils, cooling to a core temperature of 32 °C was more beneficial than cooling to 34 °C [42]; however, data from piglet studies suggest that deep cerebral structures do not benefit from deep cooling as much as superficial cortical regions [43] and deep hypothermia to 15 °C post cardiac arrest in adult dogs was not beneficial [44]. Furthermore, systemic side effects were increased with deeper cooling to 30 °C compared to 33.5 °C in the piglet model [45].

Further research using different temperatures and cooling techniques is needed to find the optimal balance between efficacy, cost and feasibility. The high burden of HIE in low-resource settings prompts the question of how research into the role of therapeutic hypothermia should be carried out in these settings. Initially, pilot feasibility studies should be done using basic cooling techniques at intended research sites to establish consistent care practices, assess the impact on resources and assess the feasibility of follow-up.

8.3.4 Predicting outcomes using the Thompson score

The threshold Thompson score of ≥ 7 to predict abnormal aEEG or moderate-severe encephalopathy and the threshold Thompson scores of ≥ 16 and / or ≥ 15 to predict poor long-term outcome with or without cooling should be validated in large prospective studies with long-term neurodevelopmental follow up and MRI. A close correlation would support the use of the Thompson score as a surrogate for poor long-term outcome: this information could guide appropriate allocation of resources. These studies can be performed in both high- and low-resource settings, but studies in low-resource settings will be essential before therapeutic hypothermia is adopted in that setting as a routine.

The use of a parental questionnaire-based assessment as a tool to facilitate improved follow-up rates should be researched in settings where long-term follow-up is likely to be suboptimal. There is already extensive research into the use of the ages and stages questionnaire as a developmental screening tool [46]. This questionnaire has also been translated and validated as a developmental screening tool in several low- or middle-income countries [47, 48]. A study in Australia found that the questionnaire was extremely effective in detecting severe developmental delay after HIE [49]. It's application following HIE in the South African setting as well as other less-resourced settings should be researched further.

8.4 Final conclusions

The most frequently cited definitions of HIE do not successfully identify all infants who are affected; consensus standardised definitions and the benchmarking of HIE against a comparable definition is a global priority. Research in low-resource settings is difficult because of lack of infrastructure, but this baseline data is needed before the benefit of neuroprotective strategies can be established.

This thesis describes the feasibility, benefits and limitations of a simple and inexpensive method of cooling that can be used in resource-limited settings with intensive care provision. In an upper-middle income country such as South Africa where intensive care is increasingly available, it is likely that other servo-controlled commercial methods of cooling will be adopted. However, the low-tech nature of the servo-controlled gel-pack method of cooling makes it very attractive for similar settings.

The process of deriving novel data correlating aEEG with the Thompson score in this thesis, also facilitated the introduction of aEEG to South Africa. Since commencing the research in this thesis, the aEEG has become increasingly available in many other hospitals in the country. The aEEG facilitates accurate identification of seizures and provides prognostic data that facilitates appropriate treatment. Although aEEG should be used when cooling infants in an intensive care setting, international consensus guidelines acknowledge that it may not be immediately available at the time that hypothermia may be indicated [50, 51]. The guidelines recommend that cooling can be initiated on the basis of clinical signs. In settings where aEEG is not easily or immediately accessible, but cooling is feasible, the Thompson score at age 3–5 hours could be used to select infants with intrapartum-related encephalopathy for cooling, after prospective research in larger studies further validates the accuracy of this assessment.

This is a unique study of infants with HIE in a mid-resourced setting. These data have application to both high- and low-resource settings; they demonstrate the critical importance of clear and consistent criteria for the diagnosis of HIE, while also outlining the challenges and limitations of research in this context. The application of benchmarking strategies, the study of simple cooling methods and basic clinical assessment are all crucial components of global solutions towards the treatment of hypoxic ischaemic encephalopathy.

8.5 References

1. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. *Pediatr Neurol* 2009;40(3):215-26.

2. MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. *BMJ* 1999;319(7216):1054-9.

3. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353(15):1574-84.

4. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365(9460): 663-70.

5. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009;361(14):1349-58.

6. Shalak LF, Laptook AR, Velaphi SC, Perlman JM. Amplitude-integrated electroencephalography coupled with an early neurologic examination enhances prediction of term infants at risk for persistent encephalopathy. *Pediatrics* 2003;111(2):351-7.

7. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86(7):757-61.

8. Linley LL, Thompson CM, Fawcus S. Incidence, morbidity and mortality of hypoxic ischaemic encephalopathy in the PMNS. Proceedings of the 23rd Conference on Priorities in Perinatal Care in Southern Africa; 2004 March 9-12; Limpopo, South Africa. P.144-6.

9. Buchmann EJ, Velaphi SC. Confidential enquiries into hypoxic ischaemic encephalopathy. *Best Pract Res Clin Obst Gynaecol* 2009;23(3):357-68.

10. Lawn JE, Lee AC, Kinney M, Sibley L, Carlo WA, Paul VK, et al. Two million intrapartum-related stillbirths and neonatal deaths: Where, why, and what can be done? *Int J Gynaecol Obstet* 2009;107(S):S5-S19.

11. Fawcus S, Linley LL, Thompson CM, Greenfield D. Obstetric risk factors for neonatal encephalopathy. Proceedings of the 23rd Conference on Priorities in Perinatal Care in Southern Africa; 2004 March 9-12; Limpopo, South Africa. P.141-3.

12. Hankins GDV, Speer M. Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. *Obstet Gynecol* 2003;102(3):628-36.

13. Committee on Obstetric Practice. ACOG Committee Opinion. Number 326, December 2005. Inappropriate use of the terms fetal distress and birth asphyxia. *Obstet Gynecol* 2005;106(6):1469-70.

14. Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 2003;361(9359):736-42.

15. Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA. Hypothermia for Neonatal Hypoxic Ischemic Encephalopathy: An Updated Systematic Review and Meta-analysis. *Arch Pediatr Adolesc Med* 2012;166(6):558-66.

16. Perlman JM, Wyllie J, Kattwinkel J, Atkins DL, Chameides L, Goldsmith JP, et al. Part 11: Neonatal Resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. *Circulation* 2010;122(16_suppl_2):
S516-S38.

17. Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch Pediatr Adolesc Med* 2011;165(8):692-700.

18. Horn AR, Joolay Y, Tooke L, Harrison MC. A servo-assisted gel-pack cooling method for newborn infants with hypoxic-ischemic encephalopathy. *J Trop Pediatr* 2012;58(3):236-8.

19. Shankaran S, Laptook A, Wright LL, Ehrenkranz RA, Donovan EF, Fanaroff AA, et al. Whole-Body Hypothermia for Neonatal Encephalopathy: Animal Observations as a Basis for a Randomized, Controlled Pilot Study in Term Infants. *Pediatrics* 2002;110(2):377-85.

20. Gunn AJ, Gluckman PD, Gunn TR. Selective head cooling in newborn infants after perinatal asphyxia: a safety study. *Pediatrics* 1998;102(4 Pt 1):885-92.

21. Thomas N, George KC, Sridhar S, Kumar M, Kuruvilla KA, Jana AK. Whole body cooling in newborn infants with perinatal asphyxial encephalopathy in a low resource setting: a feasibility trial. *Indian Pediatr* 2011;48(6):445-51.

22. Bharadwaj SK, Bhat BV. Therapeutic hypothermia using gel packs for term neonates with hypoxic ischaemic encephalopathy in resource-limited settings: a randomized controlled trial. *J Trop Pediatr* 2012;58(5):382-8.

23. De Vries LS, Toet MC. Amplitude integrated electroencephalography in the full-term newborn. *Clin Perinatol* 2006;33(3):619-32

24. Sarkar S, Barks JD, Donn SM. Should amplitude-integrated electroencephalography be used to identify infants suitable for hypothermic neuroprotection? *J Perinatol* 2008;28(2):117-22.

25. Simbruner G, Mittal RA, Rohlmann F, Muche R, neon.EURO.network Trial Participants. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics* 2010;126(4):e771-8.

26. Azzopardi D, Strohm B, Edwards AD, Halliday H, Juszczak E, Levene M, et al. Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. *Arch Dis Child Fetal Neonatal Ed* 2009;94(4):F260-4.

27. Robertson NJ, Nakakeeto M, Hagmann C, Cowan FM, Acolet D, Iwata O, et al. Therapeutic hypothermia for birth asphyxia in low-resource settings: a pilot randomised controlled trial. *Lancet* 2008;372(9641):801-3.

28. Ellis M, Shrestha L, Shrestha PS, Manandhar DS, Bolam AJ, Costello AMD. Clinical predictors of outcome following mild and moderate neonatal encephalopathy in term newborns in Kathmandu, Nepal. *Acta Paediatr* 2001;90(3):316-22.

29. Bernet V, Latal B, Natalucci G, Doell C, Ziegler A, Wohlrab G. Effect of sedation and analgesia on postoperative amplitude-integrated EEG in newborn cardiac patients. *Pediatr Res* 2010;67(6):650-5.

30. Olischar M, Davidson AJ, Lee KJ, Hunt RW. Effects of morphine and midazolam on sleep-wake cycling in amplitude-integrated electroencephalography in post-surgical neonates ≥ 32 weeks of gestational age. *Neonatology* 2012;101(4):293-300.

31. Eken P, Toet MC, Groenendaal F, de Vries LS. Predictive value of early neuroimaging, pulsed Doppler and neurophysiology in full term infants with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 1995;73(2):F75-80.

32. Csekő AJ, Bangó M, Lakatos P, Kárdási J, Pusztai L, Szabó M. Accuracy of amplitude-integrated electroencephalography in the prediction of neurodevelopmental outcome in asphyxiated infants receiving hypothermia treatment. *Acta Paediatr* 2013;102(7):707-11.

33. Leeflang MMG, Moons KGM, Reitsma JB, Zwinderman AH. Bias in sensitivity and specificity caused by data-driven selection of optimal cutoff values: mechanisms, magnitude, and solutions. *Clin Chem* 2008;54(4):729-37.

34. Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics* 2010;126(1):e131-9.

35. Hallberg B, Grossmann K, Bartocci M, Blennow M. The prognostic value of early aEEG in asphyxiated infants undergoing systemic hypothermia treatment. *Acta Paediatr* 2010;99(4):531-6.

36. Kaufman SA, Miller SP, Ferriero DM, Glidden DH, Barkovich AJ, Partridge JC. Encephalopathy as a predictor of magnetic resonance imaging abnormalities in asphyxiated newborns. *Pediatr Neurol* 2003;28(5):342-6.

37. Thayyil S, Costello A, Shankaran S, Robertson NJ. Therapeutic hypothermia for neonatal encephalopathy implications for neonatal units in India. *Indian Pediatr* 2009;46(4):283-9.

38. Joolay Y, Harrison MC, Horn AR. Therapeutic hypothermia and hypoxic ischemic encephalopathy: opinion and practice of pediatricians in South Africa. *J Perinat Med* 2012;40(4):447-53.

39. StatsSA. Census 2011 Statistical release P0301.4. statssagovza [Internet]. 2013 [updated 2013 February 8; cited 2013 April 3]; Available from: http://www.statssa.gov.za/Publications/P03014/P030142011.pdf.

40. Iwata S, Iwata O, Olson L, Kapetanakis A, Kato T, Evans S, et al. Therapeutic hypothermia can be induced and maintained using either commercial water bottles or a “phase changing material” mattress in a newborn piglet model. *Arch Dis Child* 2009;94(5):387-91.

41. Horn A, Thompson C, Woods D, Nel A, Bekker A, Rhoda N, et al. Induced hypothermia for infants with hypoxic- ischemic encephalopathy using a servo-controlled fan: an exploratory pilot study. *Pediatrics* 2009;123(6):e1090-8.

42. Colbourne F, Corbett D. Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. *J Neurosci* 1995;15(11):7250-60.

43. Iwata O, Thornton JS, Sellwood MW, Iwata S, Sakata Y, Noone MA, et al. Depth of delayed cooling alters neuroprotection pattern after hypoxia-ischemia. *Ann Neurol* 2005;58(1):75-87.

44. Schubert A. Side effects of mild hypothermia. *J Neurosurg Anesthesiol* 1995;7(2):139-47.

45. Kerenyi A, Kelen D, Faulkner SD, Bainbridge A, Chandrasekaran M, Cady EB, et al. Systemic effects of whole-body cooling to 35 °C, 33.5 °C, and 30 °C in a piglet model of perinatal asphyxia: implications for therapeutic hypothermia. *Pediatr Res* 2012;71(5):573-82.

46. Schonhaut L, Armijo I, Schonstedt M, Alvarez J, Cordero M. Validity of the ages and stages questionnaires in term and preterm infants. *Pediatrics* 2013;131(5):e1468-74.

47. Juneja M, Mohanty M, Jain R, Ramji S. Ages and Stages Questionnaire as a screening tool for developmental delay in Indian children. *Indian Pediatr* 2012;49(6):457-61.

48. Amir Ali Akbari S, Montazeri S, Torabi F, Amiri S, Soleimani F, Alavi Majd H. Correlation between anthropometric indices at birth and developmental delay in children aged 4-60 months in Isfahan, Iran. *Int J Gen Med* 2012;5:683-7.

49. Lindsay NM, Healy GN, Colditz PB, Lingwood BE. Use of the Ages and Stages Questionnaire to predict outcome after hypoxic-ischaemic encephalopathy in the neonate. *J Paediatr Child Health* 2008;44(10):590-5.

50. British Association of Perinatal Medicine. BAPM Position Statement on Therapeutic Cooling for Neonatal Encephalopathy [Internet]. 2010 [cited 2013 June 6]. Available from: http://www.bapm.org/publications/documents/guidelines/Position_Statement_Therapeutic_Cooling_Neonatal_Encephalopathy_July%202010.pdf.

51. Olsen SL, DeJonge M, Kline A, Liptsen E, Song D, Anderson B, et al. Optimizing Therapeutic Hypothermia for Neonatal Encephalopathy. *Pediatrics* 2013;131(2):e591-e603.

Appendix A Data capture form for retrospective folder review

Surname**……**Infant Folder no**……**DOB**……** TOB**……**

MATERNAL DETAILS

Age**…..** ***yr*** Grav**……** HIV ***pos / neg / UK*** VDRL ***pos / neg / UK***

Medical Problems during pregnancy: YES / NO *if Yes: -*

Smoker ***Y / N*** Alcohol Abuse ***Y / N*** Drug Abuse ***Y / N*** Hypertension ***Y /N***

Thyroid disorder ***Y / N*** Bleeding ***Y / N*** Infection (other than HIV / Syphillis) ***Y / N***

Diabetes ***Y / N*** TB ***Y / N*** Anaemia ***Y / N*** Pyrexial illness ***Y / N***

Other ***Y / N***……..

LABOUR FH recorded continuously until delivery ***Y / N / UK***

Evidence of potential intrapartum hypoxia YES / NO *if Yes: -*

Abruptio ***Y / N*** APH ***Y / N*** Ut Rup ***Y / N*** Prolapse Cord ***Y / N*** MSL ***Y / N*** Mat Sz ***Y / N***

FH Abnormal **Y / N**…….. ……..fetal bradycardia ***Y / N*** Prol 2nd Stg ***Y / N / UK***

Should Dystocia ***Y / N*** Head entrapment ***Y/N*** Breech ***Y / N*** Maternal arrest ***Y / N***

Other Sentinal Event ***Y / N***……

Evidence of potential congenital sepsis YES / NO *if Yes: -*

PROM > 18 hours ***Y / N*** Chorioamnionitis ***Y / N*** Maternal sepsis ***Y / N***

INFANT

Hospital giving Care: ***GSH / MMH / NSH / MOU/Ambulance / Home / Other*** ……..

Name mou……

Birth site: ***GSH / MMH / NSH / MOU/Ambulance / Home / Other***……..

Name mou……..

Birth weight**……..*****gm*** Birth COH:**……..*****cm*** Gender: ***Male / Female***

Gestation**…… *****wks***

Apgar Score 1min: **…….** 5min **……..** 10min **…….**

Chest Comp ***Y / N / UK*** Adren ***Y / N / UK*** Resp support at 10 min ***Y / N***

Delivery: ***CS / NVD / ventouse / forceps / breech / Other***……

Presented < 6h Y / N **NE < 6h Y / N**

Blood Gases First Day Cord Gas: ***Y / N*** Infant gas within 60 mins : ***Y / N***

Worst Gas – inc cord (Lowest BE) in 1st hr: pH**……** CO_2**……** Base excess **…….** Lactate**…..**

Neuroassess

Clin Sz ***Y / N***

ThompHIE: **D1…….D2…….D3……D4…… D5……D6……D7…… D10……Max……**

Sarnat moderate-severe HIE grade **Y / N**

Shankaran moderate-severe HIE grade **Y / N**

Thompson moderate-severe grade (Sc > 10) **Y / N**

Exclusion criteria

Suspected Congenital Sepsis **Y / N ……..**

Chromosomal Syndrome **Y / N ……**

Congenital Malformation **Y / N ……**

Neonatal Abstinence **Y / N ……**

Metabolic encephalopathy **Y / N ……..**

Final outcome

Alive at Discharge Y / N **Discharge Age…… d**

Appendix B

Consent and information form: evaluating a simple method of hypothermia

To the parent(s) of……

Your baby has had some problems with breathing at birth and is at risk of having problems with brain function. International research shows that cooling to body temperatures of 33–34 °C is able to protect the brain in 15 to 25% of infants with these problems and these temperatures are safe.

Although cooling can help some infants, it is not effective in all infants and is most likely to be of benefit if started early. It is therefore the standard of care at Mowbray Maternity Hospital, to commence a simple method of cooling in all infants that might benefit. The method involves placing cold soft bags from a refrigerator around your baby's head and monitoring your baby's temperature with a temperature probe between the baby and the mattress. A radiant warmer prevents your baby from cooling down too much and a heat shield prevents the warmer from heating your baby's head.

We are seeking your permission to continue cooling in your infant and to enhance the monitoring of your infant by monitoring the temperature in your baby's rectum continuously, using a thin flexible temperature probe. Rectal temperature monitoring is the preferred method of monitoring cooled babies, but has not previously been available in this hospital and is not yet available to all infants. The other studies that used rectal monitoring did not show any problems with this method of monitoring, and in most other centers in the world, this type of monitoring and procedure is standard. We are also seeking your permission to attach an additional temperature probe between baby and mattress and an additional probe on the skin. These additional probes will help us to be sure that the existing monitoring that we use is safe and accurate.

The information we obtain from the extra monitoring will help us make recommendations to other neonatal units about feasible methods of cooling and we may also take photographs and videos to help us describe the method.

After the cooling period (normally 72hours), your baby will be warmed up slowly at 0.2 °C per hour. All other care and monitoring of your baby will be done according to the routine on the neonatal unit.

CONSENT STATEMENT

Your baby's participation in this study is entirely voluntary on your part and if you refuse to participate or if you withdraw your baby from participation at any time, there will be no prejudice to the quality of your baby's subsequent clinical management and care.

I/We……

do consent to my/our baby…….....,whose date of birth is.....,…….and whose folder number is ….....being entered into the cooling study as explained above. And I/We consent to the publication, in scientific literature, of all data, videos and photographs of my/our baby acquired during the study. *(Presentations will not name the patients)*

Signed (Mother)…….Signed (Father)……...

Witness 1…….Print name……

Witness 2……..Print name……

Date……/……./2008 Place…Mowbray Maternity Hospital , Cape Town

This study has been approved by the UCT health sciences ethics committee, who may be contacted at the following address: E52 Room 23, Old Main Building, Groote Schuur Hospital. Tel: 021 406 6338 Fax: 021 406 6411

Appendix C Data sheet: cooling method evaluation

Surname……Study Case Number……

Infant Folder no…… DOB……..

MATERNAL DEMOGRAPHIC DATA

Age……yr Gravidity…… HIV pos/neg/UK VDRL pos/neg/UK

Pregnancy morbidity: None / Hypertension / Thyroid disorder / Bleeding / Infection

Other……

Pre-existing Maternal Medical Conditions or treatment.

…….....

INFANT DEMOGRAPHICS

Birth weight……gm COH:…….cm Gender: Male / Female

Gestation……wks Apgar Score 1min: …… 5min …… 10min ……

Chest Compressions Yes / No Adrenaline Yes / No

Respiratory support at 10mins? Yes / No

Birth site; GSH / MMH / NSH / MOU/Ambulance / Home /

Other……

Intrapartum fetal heart abnormalities: None / fetal brady/ other abnormal

(detail……)

Other intrapartum complications:

None/ maternal seizures / abruptio / prolonged 2nd stage / shoulder distocia / MSL/

Other……

Mode of delivery: CS / NVD / ventouse / forceps / breech /

Other…….

Cord gas avaiable: Yes / No Result: pH……CO_2……Base excess……Lactate……

First arterial blood gas: Age in minutes; …….....

pH……CO_2…….. Base excess ……..Lactate…….

Worst arterial blood gas within 60 minutes of birth (Including cord blood) available:

Yes / No pH……CO_2…….. Base excess ……Lactate…….

COOLING DATA

Pre-Cool starting Temp……..°C

Age cooling started ……hr

Duration of cooling…….hr

Mean rectal temperature during cooling…….°C (from processed logger data)

Time to reach rectal 34°C……°C (from processed logger data)

Back temperature when rectal reached 34°C ……..°C (from processed logger data)

Duration of rewarm (till rectal 36.5°C)……hr (from processed logger data)

ADVERSE EVENTS DURING COOLING AND REWARMING

Seizures	Yes / No
Seizures during re-warming	Yes / No
Hospital aquired sepsis	Yes / No
Meconium aspiration	Yes / No
Bradycardia < 100bpm	Yes / No
Pulmonary Hypertension	Yes / No
Hypoglycaemia	Yes / No
Progressive metabolic acidosis	Yes / No
Hyponatremia	Yes / No
Hypocalcaemia	Yes /No
Thrombocytopaenia	Yes / No
Active Bleeding	Yes / No

DIAGNOSES/PROBLEMS DURING ENTIRE ADMISSION

Sepsis at birth	Yes / No
Acquired hospital sepsis	Yes / No
Pulmonary Hypertension	Yes / No
Meconium aspiration	Yes / No
Pulmonary Airleak	Yes / No
Pulmonary Haemorrhage	Yes / No
Necrotising enterolcolitis	Yes / No

Hypoglycaemia	Yes / No
Hypokalaemia	Yes/ No
Hyponatremia	Yes / No
Hypocalcaemia	Yes /No
Hypomagnesaemia	Yes / No
CPAP	Yes / No
Mechanical ventilation	Yes / No
Hypotension	Yes / No
Inotropic support	Yes / No
Thrombocytopaenia	Yes / No
Active Bleeding	Yes / No
Bradycardia	Yes / No
Other arrhythmia	Yes / No
Max Creatinine during 1st 96 hours:	……

aEEG Data during 1st 96 hours

aEEG Background at recruitment	CNV/ DNV / BS / FT
aEEG voltage at recruitment	normal/moderate abn/ suppressed
aEEG Sz at recruitment	present / absent
aEEG Background at 6hr	CNV/ DNV / BS / FT
aEEG voltage at 6hr	normal/moderate abn/ suppressed
aEEG Sz at 6hr	present / absent
aEEG Background at 24hr	CNV/ DNV / BS / FT
aEEG voltage at 24hr	normal/moderate abn/ suppressed
aEEG Sz at 24hr	present / absent
aEEG Background at 48hr	CNV/ DNV / BS / FT
aEEG voltage at 48hr	normal/moderate abn/ suppressed
aEEG Sz at 48hr	present / absent
aEEG Background at 72hr	CNV/ DNV / BS / FT
aEEG voltage at 72hr	normal/moderate abn/ suppressed
aEEG Sz at 72hr	present / absent
aEEG Background at 96hr	CNV/ DNV / BS / FT
aEEG voltage at 96hr	normal/moderate abn/ suppressed
aEEG Sz at 96hr	present / absent

Cerebral ultrasound assessments

……
……
……
……
……

Fluids and Feeds 1st 10 days

Age(hours)	24	48	72	96	120	144	168	192	216	240
Total ml/kg/day										
Feeds ml/kg/day										

Outcome at Discharge

Alive/demised Age in days at Discharge/Demise…… (decimal if < 48hours)

Peak Thompson HIE score: ……..on Day……. (first appearance)

Thomson HIE score D7:……

Thompson HIE score D10 or D/C – whichever occurred first……

Worst Sarnat Grade …….on Day……(first appearance)

Thompson HIE Score and anticonvulsants

	Pre-Cool 1 – 6h	**24h**	**48h**	**72h**	**96h**	**D5**	**D6**	**D7**	**D8**	**D9**	**D 10**
Date											
Limb Tone											
LOC											
Visible Fits											
Posture											
Moro											
Grasp											
Suck											
Respiration											
Fontanel											
TOTAL											
Anticonvulsants or sedation P=Phenobarb M = Midazolam L = Lignocaine Mo = Morphine											

Sarnat Grade

Time	**Precool**	**24h**	**48h**	**72h**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
Grade											

THOMPSON SCORE REFERENCE CHART

Score / Sign	1	2	3
Limb Tone	Generally Hypertonic	Generally Hypotonic	Flaccid
Level of Consciousness	Hyper-alert, staring, or excessive irritability	Lethargic	Comatose or Stuporose
Visible Fits	Infrequent; < 3/day	Frequent; > 2/day	
Posture	Fisting and / or Cycling	Strong distil flexion	Decerebrate
Moro	Partial	Absent	
Grasp	Poor	Absent	
Suck	Poor	Absent and/or bites	
Respiratory effort	Hyperventilation	Transient apnoea	Apnoea + IPPV
Fontanel	Full	Tense	
TOTAL			

IPPV: Intermittent Positive Pressure Ventilation

The maximum score, based on the infant's clinical signs since the previous assessment, is recorded in each category and then totaled to obtain the final score.

DEFINITIONS

Hypocalcaemia: < 2mmol/L corrected or < 1mmol/L ionised
Hypotension: Mean blood pressure < 40mmHg
Hyponatraemia: < 130mmol/L
Hypoglycaeamia: < 2.5mmol/L
Hypokalaemia: < 3.5 mmol/l
Pulmonary hypertension: Severe hypoxaemia disproportionate to the severity of lung disease and evidence of right to left shunt
Meconium aspiration syndrome: The presence of meconium stained liquor at birth and severe respiratory distress within 1 hour of birth and compatible X-ray changes.
Necrotising enterocolitis: Abdominal distension, gastric aspirate and/or blood in stools together with abdominal X-ray showing bowel oedema, pneumatosis or pneumoperitoneum .
Pulmonary airleak: Radiologically confirmed airleak serious enough to affect management (including pneumothorax, pulmonary interstitial emphysema, pneumopericardium, pneumoperitoneum and pneumomediastinum).
Pulmonary haemorrhage: Copious bloody secretions with clinical deterioration requiring change(s) in ventilatory management.
Sepsis: Culture positive
Thrombocytopaenia: < 100 000/ μL

Appendix D Parental information and consent form: aEEG study

To the parent(s) of…….

Your baby must be monitored closely for the next few days, because of problems with breathing at the time of birth.

In many hospitals, brain monitoring is performed routinely on babies with breathing problems to help learn how best to look after them. We are therefore performing careful clinical examinations of all babies who have required significant resuscitation at regular intervals, and babies who have abnormalities (even if these are only mild abnormalities) in these examinations will have brain monitoring and some may have video recording performed. The benefit of the brain monitoring is that we are able to detect changes in the brain earlier and we are able to predict the likely outcome much more accurately than would otherwise be possible.

We are asking your permission to collect data from our observations of your baby that may help us to learn how to predict the outcome of babies with similar problems. This is called research.

All the examinations, monitoring and management in this study are routine, so there is no added risk being in this study. Although the brain monitor is not always available, if your baby takes part in the study, brain monitoring will be performed at least 2 times. When we attach a brain monitor to your baby's scalp we will need to shave a small amount of hair on each side of the head. This can be upsetting for parents, but your baby's hair will grow back quickly.

All the information we collect will be kept confidential and only the research doctors will have access to the records. Your name will not appear in any reports which we might publish about this research.

If you have any questions or do not understand anything in this consent form, please tell one of the research doctors who will answer your questions and give you any other information you might want. If you have any questions about your rights as a research subject, you can contact Professor Marc Blockman (021 406 6496) who is the Chairperson of the Research Ethics Committee of the University of Cape Town, which approved this study.

Taking part in this study is entirely voluntary and if you decide not to take part or if you withdraw your baby from the research at any time, it will not affect the quality of your baby's future clinical management and care.

CONSENT STATEMENT

I/We…….,

do consent to my/our baby……...,whose date of birth is.....,

and whose folder number is ….....,being monitored as explained above.

I/We consent to the presentation in academic literature or lectures, of all data acquired during the study.

Signed (Mother)……...Signed (Father)……

Signature of person obtaining consent……..Print name……

Witness ……..…… Print name……

Date……/……/200…….Place:…… , Cape Town

Appendix E Data sheet: aEEG and early clinical signs study

Surname…… Study Case Number……

Infant Folder no…… DOB……

MATERNAL DEMOGRAPHIC DATA

Age……yr Gravidity……. HIV pos/neg/UK VDRL pos/neg/UK

Pregnancy complications:

None / Hypertension / Thyroid disorder / Bleeding / Infection

Other……

INFANT DEMOGRAPHICS

Birth weight……gm COH:……cm Gender: Male / Female

Gestation……wks Apgar Score 1min: …… 5min …… 10min ……

Chest Compressions Yes / No Adrenaline Yes / No

Respiratory support at 10mins Yes / No

Birth site: GSH / MMH / NSH / MOU/Ambulance / Home /

Other……..

Intrapartum fetal heart abnormalities:

None / bradycardia / other ……

Other intrapartum complications suggesting fetal hypoxia :

None / maternal seizures / abruptio / prolonged 2nd stage / shoulder distocia / MSL /

Other……

Mode of delivery: CS / NVD / ventouse / forceps / breech / Other……

Cord gas available: Yes / No pH……CO_2…… Base deficit……Lactate……

First arterial blood gas: Age in minutes; …….....

pH……CO_2…….. Base Excess ……..Lactate…….

Worst arterial blood gas within 60 minutes of birth (inc cord) available: Yes / No

pH……CO_2…….. Base Excess ……..Lactate…….

DAILY GENERAL MONITORING DATA

Cooled Yes / No **:** If Cooled, **Pre-Cool starting Temp……at age……hr**

DAY 1 *(Cooling temps only completed if infant meets cooling criteria and is cooled)*

Hours from start of cooling	**"Back" temp °C**	**Hours from start of cooling**	**"Back" temp °C**	**Clinical info1st day**	
1		13		Sepsis	Yes / No
2		14		Hypoglycaemia	Yes / No
3		15		CPAP	Yes / No
4		16		Mechanical ventilation	Yes / No
5		17		Hypotension	Yes / No
6		18		Inotropic support	Yes / No
7		19		Clinical Bleeding	Yes / No
8		20		Bradycardia	Yes / No
9		21		Other arrhythmia	Yes / No
10		22			
11		23			
12		24			

DAY 2

Hours from start of cooling	**"Back" temp °C**	**Hours from start of cooling**	**"Back" temp °C**	**Clinical info 2nd day**	
25		37		Sepsis	Yes / No
26		38		Hypoglycaemia	Yes / No
27		39		CPAP	Yes / No
28		40		Mechanical ventilation	Yes / No
29		41		Hypotension	Yes / No
30		42		Inotropic support	Yes / No
31		43		Clinical Bleeding	Yes / No
32		44		Bradycardia	Yes / No
33		45		Other arrhythmia	Yes / No
34		46			
35		47			
36		48			

DAY 3

Hours from start of cooling	"Back" temp °C	Hours from start of cooling	"Back" temp °C	**Clinical info 3rd day**	
49		61		Sepsis	Yes / No
50		62		Hypoglycaemia	Yes / No
51		63		CPAP	Yes / No
52		64		Mechanical ventilation	Yes / No
53		65		Hypotension	Yes / No
54		66		Inotropic support	Yes / No
55		67		Clinical Bleeding	Yes / No
56		68		Bradycardia	Yes / No
57		69		Other arrhythmia	Yes / No
58		70			
59		71			
60		72			

DAY 4

Hours from start of cooling	"Back" temp °C	Hours from start of cooling	"Back" temp °C	**Clinical info 4th Day:**	
73		85		Sepsis	Yes / No
74		86		Hypoglycaemia	Yes / No
75		87		CPAP	Yes / No
76		88		Mechanical ventilation	Yes / No
77		89		Hypotension	Yes / No
78		90		Inotropic support	Yes / No
79		91		Clinical Bleeding	Yes / No
80		92		Bradycardia	Yes / No
81		93		Other arrhythmia	Yes / No
82		94			
83		95			
84		96			

DIAGNOSES/BIOCHEMISTRY DURING ADMISSION

Sepsis at birth	Yes / No
Acquired hospital sepsis	Yes / No
Pulmonary Hypertension	Yes / No
Meconium aspiration	Yes / No
Pulmonary Airleak	Yes / No
Pulmonary Haemorrhage	Yes / No
Necrotising enterolcolitis	Yes / No
CPAP	Yes / No
Mechanical ventilation	Yes / No
Hypotension	Yes / No
Inotropic support	Yes / No
Bradycardia	Yes / No
Other arrhythmia	Yes / No
Clinical Bleeding	Yes / No
Thrombocytopaenia	Yes / No
Hypoglycaemia	Yes / No
Severe Hypoglycaemia	Yes / No
Hypokalaemia (< 3.5mmol/L)	Yes/ No
Hyponatremia	Yes / No
Severe Hyponatremia	Yes / No
Hypocalcaemia	Yes /No
Max Creatinine:	……
Max Base Excess after age 4 hours:	……
Hyperoxia	Yes/No
Hypocarbia	Yes/No

DEFINITIONS

Bradycardia: < 100bpm
Hyperoxia: > 200mmHg/26.6KPa
Hypocarbia: < 25mmHg/3.3Kpa
Hypocalcaemia: < 2mmol/L corrected or < 1mmol/L ionised
Hypotension: Mean blood pressure < 40mmHg
Hyponatraemia: < 130mmol/L **Severe Hyponatraemia:** < 120mmol/L
Hypoglycaeamia: < 2.6mmol/L **Severe Hypoglycaemia:** < 1.6mmol/L
Meconium aspiration syndrome: The presence of meconium stained liquor at birth and severe respiratory distress within 1 hour of birth and compatible X-ray changes.
Necrotising enterocolitis: Abdominal distension, gastric aspirate and/or blood in stools together with abdominal X-ray showing bowel oedema, pneumatosis or pneumoperitoneum
Pulmonary hypertension: Severe hypoxaemia disproportionate to the severity of lung disease and evidence of a right to left shunt
Pulmonary airleak: Any radiologically confirmed airleak serious enough to affect management (including pneumothorax, pulmonary interstitial emphysema, pneumopericardium, pneumoperitoneum and pneumomediastinum).
Pulmonary haemorrhage: Copious bloody secretions with clinical deterioration requiring change(s) in ventilatory management.
Sepsis: Blood culture positive
Thrombocytopaenia: < 100 000/ μL

Fluids and Feeds 1st 10 days

Age(hours)	24	48	72	96	120	144	168	192	216	240
Total ml/kg/day										
Feeds ml/kg/day										

Cerebral ultrasound

1st……..Day:……

……
…… ……..
……
……
……
……
……

2nd …….Day……

……
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…….
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……
……

Clinical neurological outcome by discharge

Alive/demised Age in days at Discharge/Demise…… (use decimal if < 48hours)

Thomson HIE score D7:……

Thompson HIE score D10……

D/C befor D 10 Y / N

Thompson HIE score @ D/C if D/C before day 10……

Peak Thompson HIE score: …….

Blinded aEEG Data during 1st 96 hours

Case no…….

Code Background as: CNV/ DNV/ BS/ CLV /FT

Code Voltage as:

Normal / Moderately Abnormal / Suppressed / Severely Suppressed

(all activity < 5 μV)

Code Seizures as: Present / Absent / Status

Code Sleep Wake Cycling (SWC) as: Present/Absent

Date	Age (Hours)	Actual Time	Background	Voltage	Seizures	SWC
	3					
	6					
	24					
	48					
	72					
	96					

Duration of any abnormal pattern other than DNV……

Duration Severe Suppression……..

Age when SWC (with either DNV or CNV) first seen……

NEURO-ASSESSMENT CHART

Core Temp°C……... Date:……..TIME:……Actual Age in hours……

Age in hours: 1 , 3 , 6 , 12 , 24 , 48 , 72 , 96 **then Day……Current ventilation:** none / nasal canulae / ncpap / ippv / hfov **Inotropes:** None / Dop / Dob / Adren

Anticonvulsants/sedatives used in the last 48 hrs: phenobarbital / phenytoin / midazolam / lignocaine / opiate / other…… ……/ None

SARNAT CLINICAL ASSESSMENT : tick each sign

GRADE		0	1	2		3	
Level of Consciousness		**Normal**	**Hyperalert OR staring OR low threshold to rouse and irritable**	**Lethargic** *(Increased threshold to rouse, decreased spontaneous movts, some response to handling, may be irritable)*	**Obtunded** *(Difficult to rouse, absent spontaneous mvts, minimal response to handling)*	**Stuporous** *(Cannot rouse, only responds to strong noxious stimuli eg pain)*	**Coma** (*No response to painful stimuli*)
Muscle tone ***upper limbs***		**Normal**	**Increased**	**Decreased**		**Flaccid** ***(no tone)***	**-**
Muscle tone ***lower limbs***		**Normal**	**Increased**	**Decreased**		**Flaccid** ***(no tone)***	**-**
Stretch reflexes ***(Knee J.)***		**Normal**	**Overactive/Ankle clonus**	**Decreased** ***(felt not seen)***			**Absent**
Posture *(ring the relevant posture)*		**Normal**	**Mild distal flexion** *(intermittent thumb adduction)*	**Strong distal flexion** *(continuous, thumb adduction or continuous big toe extension or continuous flexion of all toes)*		**Other abnormal posture Describe:**	**Decerebrate or opisthotonic or decorticate**
Stim sens Myoclonus		**Absent**		**Present**			
Suck		**Normal**	**-**	**Weak** ***(or normal + bites)***		**Absent (±bites)**	**-**
Moro		**Normal**	**Strong; low threshold**	**Weak, high threshold or partial**		**Absent**	**-**
Grasp		**Normal**	**Weak**	**Absent**			
Pupils		**Normal**	**-**	**Fixed Miosis** *(Constricted, pin point)*		**Unequal or sluggish**	**Fixed mid/dilated**
Heart Rate		**Normal**	**Tachycardia** ***(> 160)***	**Bradycardia** ***(< 120) : State rate:***		**-**	**-**
Salivary Secretions		**Normal**	**-**	**Increased**		**-**	**-**
Visible Seizures *(since last assess)*		**Absent**	**-**	**Present (Describe):**		**-**	**-**
Number AC last 48h		**0**	**1**	**2**		**3**	**4 or more**
Fontanel		**Normal**		**Full**		**Tense**	
Cycling movements		**Absent**	**Intermittent/brief /few** *(≤ 2 episodes)*	**Recurrent/sustained**			**-**
Respiratory effort		**Normal**	**Hyperventilation** (> 65/min)	**Transient apnoea / shallow respiration** *(since last assesment)*		**Brainstem Gasps since assess ± normal breaths between.**	**Apnoea req ippv since assess.**

Thompson Score

Score	0	1	2	3	Final TOTAL
Limb Tone	Normal	Generally HypErtonic	Generally HypOtonic	Flaccid	
Level of Consciousness	Normal	Hyper-alert or staring	Lethargic/Obtunded	Comatose/Stuporose	
Visible Fits	None	Infrequent < 3/day	Frequent > 2/day		
Posture	Normal/Other	Fisting and / or Cycling	Strong distil flexion	Decerebrate	
Moro	Normal	Partial	Absent		
Grasp	Normal	Poor	Absent		
Suck	Normal	Poor	Absent and/or bites		
Respiration	Normal	Hyperventilation	Transient apnoea	Apnoea *requiring* IPPV	
Fontanel	Normal	Full	Tense		

Shankaran / Shalak Modified Sarnat Encephalopathy Grade (MSEG)

Shankaran –Sarnat Chart	Mild	Moderate Encephalopathy	Severe Encephalopathy	Final Shankaran/Shalak MSEG Category - Ring
Level of Consciousness	Hyper	Lethargic	Obtunded/Stuporous/Coma	Score **Normal** if no positive Signs.
Spontaneous activity		Decreased spontaneous activity (ex. clonus/cycling)	No spontaneous activity (ex. clonus/cycling)	**Mild** if mod/severe signs in 1-2 categories only And/or signs in mild category.
Muscle tone	Hyper	Hypotonia	Flaccid (profound hypotonia)	
Posture		Distil flexion or extensor posture	Decerebrate	
Suck or Moro *(score worst)*		Weak suck or partial moro	Absent	**Moderate or Severe** according to most signs per cat (if equally distributed then assign per LOC)
Autonomic *Pupils*		Miosis (fixed pinpoint)	Fixed dilated, slow, unequal or deviated.	**Normal Mild Moderate Severe**
Heart Rate		Bradycardia (< 100 beats per min)	-	
Respiration	Hyper	Shallow/Periodic breathing/gasping	Apnoea req ippv	