# A COMPARATIVE STUDY OF NEUROPROTECTIVE STRATEGIES AND OUTCOMES IN NEONATAL HYPOXIC ISCHAEMIC ENCEPHALOPATHY

by

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Thesis presented for the degree of

**DOCTOR OF PHILOSOPHY (PhD)**

in the Department of Paediatrics and Child Health

Faculty of Medicine and Health Sciences at Stellenbosch University

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March 2021

## DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own original work, that I am the sole author thereof save to the extent explicitly reported otherwise in the statement below. I have not previously in its entirety or in part submitted it for obtaining any other qualification.

## PLAGIARISM DECLARATION

This thesis/dissertation has been submitted to the Turnitin module and I confirm that my supervisors have seen my report and any concerns revealed by such have been resolved with my supervisors.

Date: March 2021

## THE NATURE AND SCOPE OF CONTRIBUTIONS

Gugu Kali conceptualized all the studies and developed the protocols, recruited most of the patients for the prospective studies, was involved in management of all the infants, and performed data analysis. I drafted and finalized all manuscripts for publication in chapters 4-7; reviewed and edited the manuscript in chapter 8 (addendum).

All co-authors agreed that the papers would form part of my PhD.

Johan Smith assisted with conceptualizing all the studies and reviewing the study protocol, reviewed all manuscripts for publication, and co-supervised the MMED degree.

Jeanetta Van Zyl conducted follow up and performed developmental assessments on all the study participants. She reviewed all the manuscripts.

Johann M Van Zyl assisted with conceptualizing the morphine studies (chapter 6-7). He was responsible for storage, processing and analysis of the samples together with the Central Analytical Facilities at Stellenbosch University. He assisted with analysis of the morphine data, and with reviewing and editing of the morphine manuscripts.

Mary Rutherford assisted with conceptualizing all the studies and reviewing the study protocol. She reported all the MRIs of the imaged infants. She reviewed and edited all the manuscripts.

Miriam Martinez Biarge assisted with data collection and analysis, and with reviewing and editing the manuscripts of the 2 published articles.

Moleen Zunza assisted with statistical analysis in the study in chapter 6. She reviewed the manuscript for publication.

Lunga Mfingwana was an MMED student that I co-supervised with Johan Smith. He wrote the protocol, collected and analysed data for the study in the addendum (chapter 8). He drafted and revised the manuscript, which is in preparation for publication.

## SUMMARY

### *Background*

Hypoxic ischaemic encephalopathy affects 1.15 million neonates annually worldwide, the majority of whom are in low to middle income countries. It is the leading cause of death of term neonates in South Africa. 40% of infants survive with disability, which places a significant burden on family and state resources.

The only effective therapy that reduces mortality and disability is therapeutic hypothermia, but its effect is limited with many still surviving with disability.

Therapeutic hypothermia is now standard of care in high income countries, but is recommended with caution in resource-constricted countries. This is due to concerns about safety and whether a therapy tested in a different setting is directly applicable in these environments.

### *Methods*

To address the safety and applicability concerns, we conducted a retrospective study to assess the feasibility and safety of therapeutic hypothermia after introducing it into routine care in our hospital.

The second study documented the outcomes of infants treated after the introduction of therapeutic hypothermia.

To assess whether the benefits of therapeutic hypothermia could be improved upon, the third study compared the outcomes of infants treated with therapeutic hypothermia only to those treated with therapeutic hypothermia plus morphine.

The fourth study described the pharmacokinetic profile of morphine in serum and cerebrospinal fluid in the infants treated with therapeutic hypothermia plus morphine at a dose of 25 µg/kg/h for 72 hours.

### *Results*

Study 1: we reviewed the management of 100 neonates treated with therapeutic hypothermia over 3 years. The majority could commence cooling within the therapeutic window of 6 hours, with a mean admission time of 4.9 hours. Rectal temperature was maintained within target range 83% of the time. Complications were transient and did not occur more frequently than in published trials.

Study 2: we documented the outcomes of 99 cooled infants. 17 infants died, 33 were lost to follow up. Of the 50 survivors that could be assessed at 1 year of age, 82% were normal and 18% had significant impairment. A severely abnormal aEEG background, severe HIE and an abnormal MRI were associated poor outcome. A good suck, mild HIE, primiparity and normal MRI were associated with good outcome.

Study 3: 45 neonates were included in the randomised trial comparing therapeutic hypothermia with therapeutic hypothermia plus morphine. No significant differences were found in later outcome between the groups, but infants in the therapeutic hypothermia plus morphine group had less liver dysfunction and a lower seizure burden in the early clinical course.

Study 4: morphine concentrations were measured at 24, 72 and 96 hours in serum; and at 72 hours in cerebrospinal fluid. Toxic concentrations were not found at the administered dose of morphine. There was no increased length of stay, need for ventilation or inotropic support as found in other studies.

***Conclusion***

Therapeutic hypothermia is feasible and safe in this setting. Survivors have good outcomes. Combining morphine with therapeutic hypothermia at 25 µg/kg/h is tolerated well, and may confer some added neuroprotection that needs further exploration.

# OPSOMMING


### *Agtergrond*

Hipoksiese isgemiese enkefalopatie(HIE) affekteer 1.15 miljoen neonate jaarliks wêreldwyd, die meerderheid van hulle is afkomstig van laer en middelinkomstelande. Dit is die hoofoorsaak van sterftes in voltermynbabas in Suid Afrika. 40% van hierdie babas oorleef met gestremdheid, wat ‘n betekenisvolle las op families en staatshulpbronne plaas.

Die enigste effektiewe terapie wat mortaliteit en gestremdheid verminder is terapeutiese hipotermie. Die effek is egter beperk met baie wat steeds met gestremdheid oorleef. Terapeutiese hipotermie is nou standaardsorg in hoë-inkomstelande, maar dit word met versigtigheid aanbeveel in lande met beperkte hulpbronne. Dit is weens besorgdheid oor veiligheid en die vraag of terapie wat in spesifieke omgewings getoets is direk toepasbaar is op omgewings met ander omstandighede.

### *Metodes*

‘n Retrospektiewe studie is gedoen om die veiligheidsaspekte en lewensvatbaarheid van terapeutiese hipotermie te evalueer nadat dit ingestel is as deel van roetinesorg.

Die tweede studie het die uitkomste van babas wat behandel is met terapeutiese hipotermie beskryf.

‘n Derde studie is gedoen om te evalueer of die uitkomste in terapeutiese hipotermie verder verbeter kan word deur morfien by die behandeling te voeg.

Die vierde studie beskryf die farmakologiese profiel van morfien in serum en serebrospinale vog in die babas wat behandel is met terapeutiese hipotermie plus morfiendosis van 25µg/kg/uur vir 72 uur.

### *Resultate*

Studie 1: ons het die hantering van 100 neonate behandel met terapeutiese hipotermie oor ‘n 3 jaar periode hersien. Die meerderheid het afkoeling begin binne die terapeutiese vensterperiode van 6 uur, met ‘n mediaan toelatingstyd van 4,9 ure. Rektale temperature is gehandhaaf binne die teikenreikwydte 83% van die tyd.

Komplikasies was tydelik en het nie meer gereeld gebeur as in gepubliseerde studies nie.

Studie 2: ons het die uitkomste van 99 afgekoelde babas gedokumenteer. 17 babas het gesterf en 33 het nie opgevolg nie. Van die 50 oorlewendes wat teen 1 jaar geevalueer is was 82% normal en 18% het betekenisvolle inkorting gehad. ‘n Erg abnormale aEEG, erge HIE en ‘n abnormale MRI was geassosieer met swak uitkomste. ‘n Goeie suig, matige HIE, primipariteit en ‘n normale MRI was geassosieerd met ‘n goeie uitkoms.

Studie 3: 45 babas is ingesluit in ‘n ewekansige studie wat terapeutiese hipotermie alleen vergelyk het met terapeutiese hipotermie plus morfien. Geen betekenisvolle verskille is gevind in die latere uitkomste tussen die groepe nie, maar babas in die terapeutiese hipotermie-plus-morfiengroep het minder lewerdisfunksie gehad asook ‘n laer konvulsielading in die vroeë kliniese verloop.

Studie 4: morfienkonsentrasies is gemeet in die serum teen 24,72 en 96 uur: en teen 72 uur in die serebrospinale vog. Daar was geen toksiese vlakke met die toegediende dosisse nie. Daar was ook geen verlengde verblyf of toename in ventilasie of inotrope ondersteuning soos gevind in ander studies nie.

***Gevolgtrekking***

Terapeutiese hipotermie is uitvoerbaar en veilig in hierdie instelling. Oorlewendes het goeie uitkomstes. Die kombinasie van morfien met terapeutiese hipotermie word goed verdra en mag bydra tot breinbeskerming en moet verder ondersoek word.

# ACKNOWLEDGEMENTS

My research was supported in part by the South African Medical Research Council Self-Initiated Research fund. I also received funding support from Imperial College London through Professor Mary Rutherford, from the NRF through Professor Ben Marais, and from the Paediatrics Department at Stellenbosch University through Professor Mariana Kruger.

I wish to extend my sincerest gratitude to my mentors and supervisors Professor Johan Smith from Stellenbosch University and Professor Mary Rutherford from Kings College London, who have always encouraged and supported me and continue to inspire me. I also wish to thank Professor Mariana Kruger for her ongoing support and ensuring I completed this work. I would also like to acknowledge Professor Georg Simbruner, formerly of Innsbruck University, for planting the idea of investigating morphine neuroprotection.

I am also grateful to:

The families of the infants that took part in all the studies, and the neonatal and paediatric teams that assisted with their recruitment and management.

Dr Celeste Cilliers (anaesthetist) and the radiologists (named in chapter 6) who came in after hours to enable the MR imaging to be done.

Dr Miriam Martinez Biarge who traveled from the UK to assist me with my research.

The late Professor Bongani Mayosi, who throughout my academic career always encouraged me to push myself further than my own limitations.

Lastly, I would like to thank my family:

My parents Aubrey and Nozenzele Kali, and my brother Temba Kali for always believing in me and being my greatest cheerleaders and support. BooQudeni! Xaba!

My maternal grandfather Rotoli John Xaba, who through great hardship while studying for his medical degree in Scotland[1], pioneered the way for me to be ultimately presenting this work today.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AABR | automated auditory brainstem responses |
| aEEG | amplitude integrated electroencephalography |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| ATNAT | Amiel-Tison Neurologic Assessment at Term |
| ATP | adenosine triphosphate |
| AUC | area under curve |
| BGT | basal ganglia thalami |
| BS | burst suppressed |
| BSID | Bayley Scales of Infant and Toddler Development |
| CCR5 | chemokine receptor type 5 |
| CLV | continuous low voltage |
| CNV | continuous normal voltage |
| COMT | catechol-O-methyltransferase |
| CSF | cerebrospinal fluid |
| CUS | cranial ultrasound |
| CXCL | chemokine (C-X-C motif) ligand |
| DALYs | disability life years |
| DNV/DC | discontinuous normal voltage/discontinuous |
| EPO | Erythropoietin |
| Erk | extracellular signal-regulated kinase |
| FGP | frozen gel packs |
| FMs | fidgety movements |
| FT | flat trace/inactive |
| GMs | general movements |
| HI | hypoxia ischaemia |
| HIE | hypoxic ischaemic encephalopathy |
| HIV | human immunodeficiency virus |
| IFN | Interferon |
| IL | Interleukin |
| LCMSMS | liquid chromatography with tandem mass spectrometry |

| | |
|---|---|
| LMIC | low to middle income countries |
| LOS | length of stay |
| LPS | Lipopolysaccharide |
| M-3-G | morphine-3-glucuronide |
| M-6-G | morphine-6-glucuronide |
| MAS | meconium aspiration syndrome |
| MIP | macrophage inflammatory protein |
| MOR | mu-opioid receptor |
| MRI | magnetic resonance imaging |
| MRS | magnetic resonance spectrometry |
| NE | neonatal encephalopathy |
| NEC | necrotising enterocolitis |
| NICU | neonatal intensive care unit |
| NK | natural killer |
| NMDA | N-Methyl-D-aspartate |
| NPV | negative predicitive value |
| NRBC | nucleated red blood cell counts |
| NSE | neuron-specific enolase |
| OAE | otoacoustic emissions |
| OMRM1 | opioid receptor mu 1 |
| PAF | Platelet activating factor |
| PKs | Pharmacokinetics |
| PLIC | posterior limb of internal capsule |
| PPHN | persistent pulmonary hypertension of the newborn |
| PPV | positive predictive value |
| RANTES | regulated on activation T cell expressed and secreted |
| RI | resistance index |
| TBH | Tygerberg Hospital |
| TH | therapeutic hypothermia |
| TH+M | therapeutic hypothermia plus morphine |
| TNF | tumour necrosis factor |
| UGT1A1 | uridine diphosphate-glucuronosyltransferase-1A1 |

| | |
|---|---|
| UGT2B7 | uridine diphosphate-glucuronosyltransferase-2B7 |
| VRA | visual reinforcement audiometry |
| WBC | white blood cell |
| WM | white matter |
| YLDs | years lived with disability |

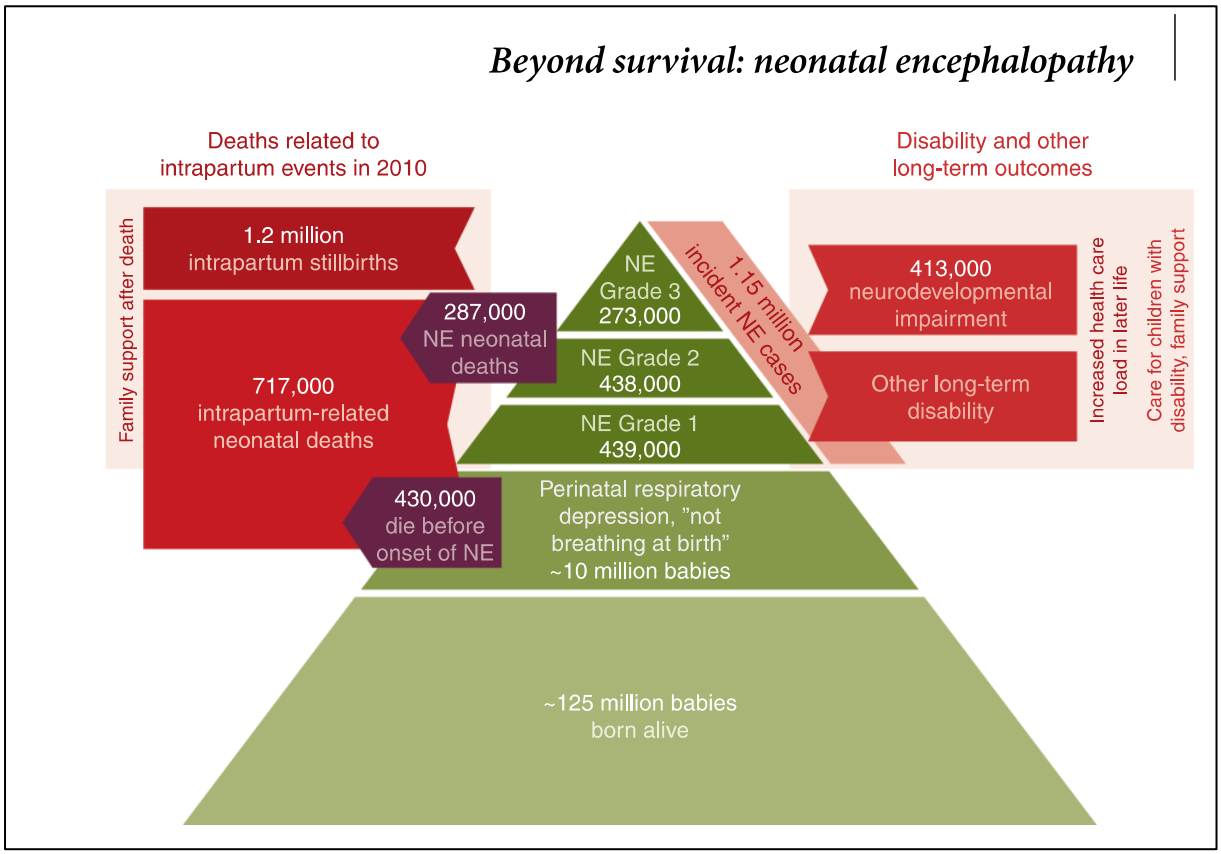
# CHAPTER 1

## Introduction

Neonatal encephalopathy (NE) is the clinical syndrome of disordered neurological function, and hypoxic ischaemic encephalopathy (HIE) is NE that follows a perinatal hypoxic ischaemic event. Strict criteria for making this diagnosis are constantly being developed but the essential components are the development of encephalopathy in a term/near term neonate soon after birth following an intrapartum event likely to cause hypoxia-ischaemia(1). The incidence varies widely throughout the world and even within the same country can vary due to differing leves of care, and also due to different definitions(2,3). It remains the leading cause of term/near term neonatal deaths in successive reports on perinatal mortality in South Africa(4,5). In excess of 400 000 infants survive with some degree of neurological impairment worldwide following NE (Figure 1), and the majority of them in middle income countries such as South Africa(6). In a study performed in our institution, perinatal insults accounted for 38% of cerebral palsy cases with hypoxia ischaemic encephalopathy a leading cause(7). Global burden of disease estimates in 2010 showed that intrapartum-related conditions result in 50 million disability adjusted life years (DALYs) and 6 million years lived with disability (YLDs)(6). These outcomes have major emotional and fincancial implications for families and health systems caring for children and adults with disabilities, and for the child's prospects for academic and economic achievements. In low resource countries where even basic care is deficient, these outcomes have even more severe consequences.

The clinical picture in HIE involves 3 stages of severity (stage 1-3 or mild, moderate and severe), which involve varying degrees of abnormalities in level of consciousness, tone, reflexes, autonomic function and seizures soon after birth. The original classification of HIE was described by Sarnat and Sarnat(8) and various simpler encephalopathy scores in use today are based on this classification. The outcome without treatment differs based on the severity of encephalopathy. Infants with mild HIE are expected to have a generally good outcome, approximately 25% of those with moderate HIE may have a poor outcome, and the majority of those with severe HIE will either die or survive with disability.

Figure 1: Intrapartum related death and disability outcomes in 2010(6)




*Pathophysiology*

It has been shown that the injury process following a hypoxic ischaemic insult evolves over time. After the initial insult there is usually some recovery of cerebral energy metabolism after resuscitation and reperfusion but this is followed, after a latent period lasting several hours, by a cascade of events that involves accumulation of excitatory neurotransmitters, reactive oxygen species, intracellular calcium and cytotoxic oedema, mitochondrial dysfunction and inflammation. This is the so-called period of secondary energy failure and results in ultimate neural cell death. It appears that the type of cell death may also differ in the different phases, with more necrotic cell death occurring in the acute phase or with severe insults and apoptotic cell death over a longer period of time(9). The phasic nature of changes in cerebral energy metabolism following hypoxic insult is demonstrated in brain MRS studies(10).

It was then found, initially in animal studies, that mild to moderate hypothermia applied during the latent phase before the onset of the secondary injury phase and for a long enough duration preserves high energy phosphates and is neuroprotective(11).

Following on from the animal and pilot human studies confirming the safety and efficacy of therapeutic hypothermia (cooling) (12,13), several large international studies were performed to examine the effects of whole body cooling (14–17); or selective head cooling with mild systemic hypothermia (18) on outcome in neonates with hypoxic ischaemic encephalopathy (HIE). Their results showed that in infants with HIE, cooling is safe and if initiated timeously (within 6 hours of birth), reduces the likelihood of death and of severe handicap in survivors up to 18 months of age.

Many systematic reviews and meta-analyses including large numbers of patients have subsequently confirmed the beneficial effects of cooling (19,20) and that both whole body and selective head cooling methods are equally efficacious (20). Importantly, they also showed increased survival with normal neurological function. These positive results have been shown to persist even into pre-school age (6-7 years) (21,22) and there is an association between favourable outcomes at 18 months and those at school-going age (7-8 years) (23). On the other hand, because the effects are quite modest, researchers have investigated whether altering the period of cooling or cooling to even lower temperatures might lead to even better outcomes. One such study was discontinued early after a futility analysis indicated a low likelihood of showing a significant benefit of longer and/or deeper cooling (24).

Current recommendations from international regulatory organizations (25) suggest that cooling should now be part of standard care for infants with HIE, and should be offered in settings where intensive care facilities are available, following the strict protocols used in the cooling trials. They, however, still recommend caution in applying this therapy in low- and middle-income countries, where resources are limited and the safety profile has not been well-established. These regions also happen to be where the greatest burden of disease is experienced(6,26), and where neuroprotective therapies are most needed.

In these settings, it is also important to be able to assess those infants that will (may) benefit from current and future neuroprotective therapies and to predict likely outcome in order to direct resources appropriately. Some of the tools that have been used/studied are described below.

*Clinical and laboratory assessment*

Raised circulating nucleated red blood cells (NRBC) numbers have been reported in response to hypoxia. They have been used to distinguish asphyxiated from non-asphyxiated infants, to predict the development and severity of HIE, brain injury on MRI and neurodevelopmental outcome (27,28). This predictive ability has been confirmed even in infants undergoing TH (29). As this is a widely available test, it could be a useful tool for selecting patients for neuroprotective therapies where facilities for blood gas sampling are not available.

An absolute requirement for receiving TH is the presence of moderate-severe encephalopathy. This can be assessed clinically or using neurophysiological tools such as amplitude integrated electroencephalogram (aEEG).

Various clinical scoring tools have been developed to assess degree of encephalopathy in different settings. The most commonly used in Sub-Saharan Africa is the Thompson HIE score (30), which is a simple scoring system based on Sarnat (8) staging that was developed in the pre-cooling era, but has been shown to still be useful both for selecting patients for therapy and for predicting those who will have a poor outcome(31,32). This can be useful in settings where no other predictive tools are available.

*Neurophysiological assessment*

aEEG was used as part of the criteria for TH in at least 2 of the large cooling trials (14,18) . This was because it is an objective method of classifying encephalopathy, and early aEEG had a high predictive value for outcome prior to the cooling era (33). The predictive ability is shifted with hypothermia, but it still has many clinical applications. Outcome prediction is best at 36-48 hours with TH (34–36), and non-recovery of background pattern at this point can assist with decisions about directing ongoing care. aEEG is also useful for diagnosing sub-clinical seizures, which can worsen outcome if untreated (37).

The role of near infrared spectroscopy (NIRS) is not clear, but some studies have reported an association between high cerebral oxygenation (rScO2 >77-90%) and poor outcome(36).

A study by Toets looking at the predictive ability of a combination of aEEG and NIRS in infants with HIE showed that aEEG had better predictive value, but NIRS did have some predictive ability. rSO2 and FTOE remained stable in those infants with normal outcome, but increased and decreased respectively after 24 hours in those with poor outcome. The increased rSO2 and decreased FTOE are thought to reflect increased cerebral oxygenation and reduced O2 utilisation consistent with secondary energy failure (38). Another study by Lemmers confirmed that the combination of aEEG and NIRS improved the predictive value of either on its own, and enabled prediction of outcome as early as 12 hours (39).

Quantitative electroencepalogram (EEG) measures have demonstrated ability to distinguish between HIE grades(40), and once further developed and automated classification simplifies interpretation of neonatal EEG this may add to the arsenal of tools for selecting patients for treatments where available.

It would therefore appear that a combination of neurophysiological tests may be more useful. A challenge for low income settings is the considerable cost of the equipment.

*Imaging*

The gold standard in neuroimaging for diagnosing type/timing of injury and predicting outcome is MRI (±MRS). However, this is not universally available and involves moving the ill infant to the site of the scanner. Cranial ultrasound (CUS) is a safe, more readily accessible method of imaging the neonatal brain but has always been considered to have a low predictive value for outcome. However, with improvements in equipment and technique it is proving to be a very valuable tool with good predictive ability for more severe abnormalities(41).

A low resistive index (≤0.55) on doppler ultrasound was previously associated with severe HIE and predicted poor outcome prior to the cooling era. With the introduction of TH many recent studies have shown that the predictive value of a low RI is altered and may be predictive before or after the cooling period (42,43). One study by Li et al found that even with TH, a very low RI of <0.46 on day 3 predicted poor outcome at 2 years with 80% accuracy (44).

Recently, different groups have validated CUS as a predictive tool for use in settings where MRI not available. Annink et al developed and validated a CUS scoring system where they assigned a composite score accounting for different areas of the brain and specific signs, and correlated it with MRI scores, histological findings in those that died and outcome at 2 years of age. Scores on CUS performed in the first week after birth had good correlation with outcome. Correlation between CUS and MRI was moderate (0.67). Histological findings in those that died were consistent with CUS findings, but the damage was more extensive(45).

Magnetic resonance imaging (MRI) and MRS in the first 2-3 weeks has been used both for timing injury, defining the extent of injury and for prognosis in infants with HIE. Most studies use scoring systems to describe the areas of the brain that are damaged, and the predictive value of MR imaging is preserved even with TH (46). MRI after TH could be used to predict outcome up to school-going age (47). A recent meta-analysis suggested that the best predictive value is obtained from MR imaging performed in the first week (<8 days) (36).

*Early post discharge assessment*

After the neonatal period, it is important to follow the infants up to assess the effects of treatments on neurodevelopment. In low resource settings where long term follow-up is frequently challenging, it is even more critical to have tools that can be used early on to select those infants that require special attention and direct the limited resources accordingly. Assessment of general movements (GMs), which are part of the normal movement repertoire of infants up to approximately 5-6 months, has been shown to be highly predictive of later neurological development. Two specific types of movements have been associated with outcome. The character of fidgety type movements (FM) is particularly useful, with absent FMs having a sensitivity and specificity of 95% each for predicting cerebral palsy (48,49).

Similarly, cramped synchronized movements at 1 and 3 months correlated very strongly with central gray matter abnormalities on early MRI and with motor outcome at 2 years in an Italian cohort (50).

The benefits of this method are its relative cost effectiveness. It requires a short video recording of the infants in the awake state, which can even be assessed remotely if there is no local expertise in GM interpretation.

In summary, using a combination of tools that are feasible for the setting is likely to yield the best predictive power for later outcome (51).

**Rationale for study:**

Whilst there is animal evidence to suggest a synergistic effect of TH with some other agents there is currently minimal data showing whether, in the human neonate, combining therapeutic hypothermia with other neuroprotective strategies enhances the beneficial effects of cooling on outcome. Some ongoing/pilot studies are examining the effects of various combinations targeting the different pathophysiological mechanisms of injury (52,53). One of the agents that may potentially enhance the neuroprotective effects of cooling due to its immune modulating properties(54), but which has not been extensively studied in this respect, is morphine. There is evidence that morphine shares some properties with cytokines, which are the main immune modulators. Opioid receptors are expressed on immune cells and opiods modulate the immune response centrally and peripherally(55).

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# CHAPTER 2

## Literature review

Neonatal encephalopathy or hypoxic ischaemic encephalopathy (HIE), which is neonatal encephalopathy associated with (presumed/documented) intra-partum events, continues to be a cause of significant neurological morbidity and mortality worldwide. The incidence has been reported as 1-8/1000 live births in some regions, to as high as 26/1000 in others (1). The true incidence is difficult to ascertain and varies widely due to a number of factors: improvements in antenatal and perinatal care in developed countries which have the lower incidence in contrast to severe resource and skills deficiencies in some developing settings; and differences in definition of HIE within and between studies(2).

When infants present with neonatal encephalopathy, there is frequently debate about the exact nature and timing of the insult. There is conflicting evidence in the literature about whether the majority of insults occur in the antenatal or intrapartum period. Some studies indicate a predominance of antenatal factors contributing to NE(3,4); while more recent studies which included neuroimaging suggest that antenatal risk factors may be contributory, but intrapartum factors are the final requirement for development of HIE(5,6). This is significant, as more recent injury may be more amenable to neuroprotective therapies.

### Mechanisms of injury in HI

Studies on animal models such as the 1-week old piglet model, relevant because of similar brain injury patterns to human newborns following hypoxia ischaemia, indicate a highly organized and topographic process of injury that targets regions of sensorimotor integration and control of movement(7).

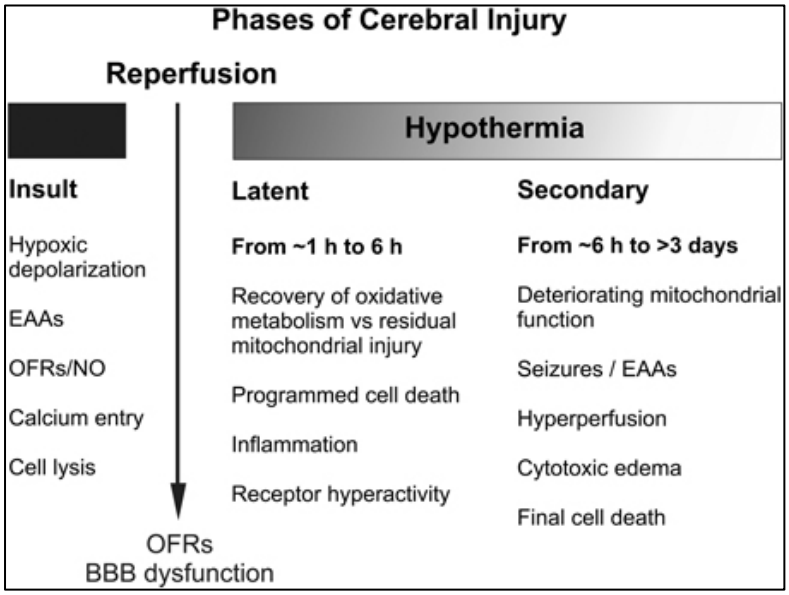
Glutamate receptor-mediated excitotoxicity plays a major role in brain damage in newborns after HI (Figure 2.1) (8,9). Glutamate is both critical to neuronal cell function and, in excess, toxic to those same cells. Glutamate binds to several receptors on neurons, including the N-methyl-D-aspartate (NMDA) receptors. Once released into the synaptic cleft, excess glutamate is removed via specific transporters found in astrocytes and astroglia and other neurons (10,11).

Altered glutamate handling and excess following cerebral ischaemia results in excitotoxicity characterized by alterations in intracellular ion concentrations, dysregulated protein phosphorylation via kinase activation and phosphatase inactivation, defects from energy depletion (adenosine triphosphate: ATP), mitochondrial failure, generation of reactive oxygen species, nitric oxide synthase (NOS) activation and prostaglandin synthesis(12,13). There is some evidence showing that reactive oxygen species (ROS) contribute significantly to cell death in cerebral ischemia. ROS activate transcription factors, including nuclear factor kappa b which in turn activates cyclooxygenase 2, inducible nitric oxide synthase, adhesion molecules and inflammatory cytokines (14). ROS cause an increase in blood-brain-barrier permeability, abnormal arteriolar reactivity, altered transport activity, enhanced neutrophil and platelet adhesion to endothelium, promote phospholipase A2 activation, platelet activation factor production (PAF) and post-ischaemic hypoperfusion (15).

Platelet-activating factor (PAF) is known to be elevated in the cerebrospinal fluid (CSF) of infants with hypoxic-ischemic encephalopathy and in rats, PAF is implicated in the progression of hypoxic-ischemic brain injury resulting from its stimulation of glutamate release(16,17).

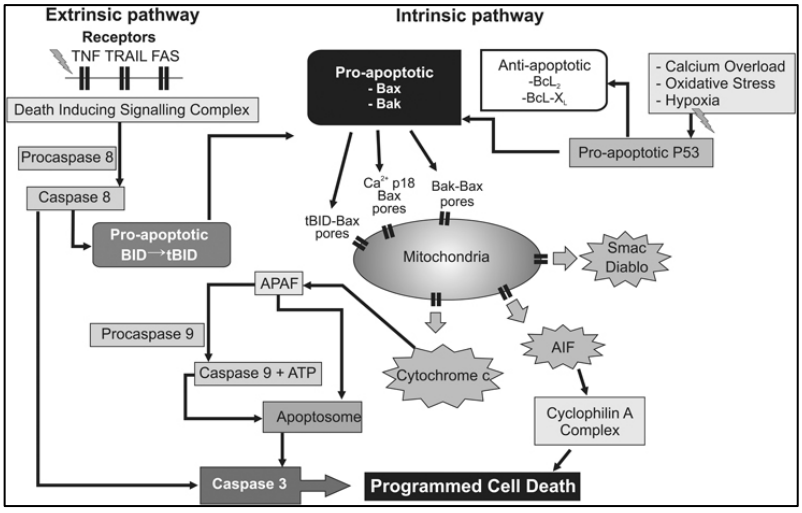
Several authors have described the central role played by the enzyme caspase 3, reactive oxygen and nitrogen species in hypoxic ischaemic injury(15,18). A significant mechanism of injury after secondary energy failure is accelerated and disordered apoptosis, which has been shown to be mediated by caspase 3 (Figure 2.2) (9).

**Figure 2.1: Schematic diagram illustrating the different pathological phases of cerebral injury after severe hypoxic-ischemia(9).**




OFRs, oxygen free radicals; BBB, blood brain barrier; EAAs, excitatory amino acids; NO, nitric oxide.

**Figure 2.2: Flow chart depicting several intracellular mechanisms associated with permeabilization of the mitochondrial membranes, leading to progressive failure of mitochondrial oxidative phosphorylation and ultimately delayed programmed cell death(9).**




AIF, apoptosis inducing factor. Apaf-1, apoptotic protease-activating factor−1; ATP, adenosine triphosphate; BAK, $Bcl_2$-antagonist/killer 1; BAX, $Bcl_2$-associated × protein; $Bcl_2$, B-cell lymphoma 2 protein family; Bcl-$X_L$, B-cell lymphoma-extra-large; BID, BH3 interacting-domain death agonist; Diablo, direct inhibitor of apoptosis binding protein with low Pi; P53, p53 tumor suppressor protein; Smac, Second mitochondria-derived activator of caspase; tBID, truncated BH3 interacting-domain death agonist; TNF, tumor necrosis factor receptor; TRAIL, TNF-related apoptosis-inducing ligand receptor.

## Hypothermic neuroprotection

Some of these described processes have been shown (mainly in animal studies) to be attenuated by whole body or head cooling. This is thought to work by modulating various processes such as glutamate release and oxidative stress, microglia activation and cytokine release, and normalizing protein synthesis(17,19). Other mechanisms by which hypothermia is thought to work are through reducing the loss of high energy phosphates(20), attenuating caspase-3 activation and hence apoptotic cell death(21), decreasing brain energy utilization and thereby maintaining ATP stores(22).

Several clinical trials on human infants have been conducted in the last 20 years to assess the effect of TH, administered as selective head cooling with mild systemic hypothermia or as whole-body cooling, on outcomes of infants with HIE. The infants were recruited using strict entry criteria, cooled commencing within 6 hours of birth for a duration of 72 hours, and then re-warmed slowly. The results of these trials showed the efficacy of TH in reducing mortality and neurodevelopmental impairment at 18 months, and even at school-going age. Some fairly recent meta-analyses involving 1200-1300 infants recruited in the majority of these studies showed significant reductions in the composite outcome of death or disability in survivors (RR 0.75, 95% CI 0.68-0.83), with a number needed to treat of 7 (23,24). There was similar efficacy of the two cooling methods, and no differences in multi-organ dysfunction and physiological effects of treatment (23). Following publication of these results, TH has been endorsed by regulatory organisations (25,26) and become standard of care in high income countries.

Timing has consistently been shown to be critical when providing TH. While the original studies indicated that cooling had to be instituted within 6 hours in order to preserve brain tissue, both animal and human evidence shows that cooling is more effective the earlier after injury it is commenced (27–30), with better motor outcomes in human infants when cooled within 3 hours of birth (30). The therapeutic window appears to be inversely proportional to the severity of the hypoxic ischaemic insult; and Sabir et al showed that in rat pups with severe insult delayed cooling up to 12 hours is even deleterious, resulting in greater brain injury than normothermia (29). In

human infants it appears that the current protocols of providing TH are the most suitable (optimal). Several different cooling strategies have been attempted without showing improved efficacy: starting cooling later (6-24 hours) and continuing for 96 hours showed no clear benefit (31); while cooling for longer duration (120 hours) and to a lower temperature (32°C) was not shown to increase the benefits and might be associated with more adverse effects (32). It is therefore imperative to optimise current practice and find ways of rapidly transferring infants to treatment centres or even cooling in transport where feasible. This, however, should be offered in settings where appropriate equipment and monitoring is available and has to be balanced against the dangers of uncontrolled passive cooling. There is increasing evidence that even in well-resourced settings with experienced staff, passive cooling in transit results in many infants having sub-therapeutic temperatures on arrival at the treatment centre and prolongs stabilisation time to target temperature (33–37). More efficient transport cooling is achieved with servo-controlled portable devices (38). This would also require less intensive monitoring by the transporting team, but this is currently limited by the cost of these devices.

*Optimising TH*

The beneficial effects of TH can be optimized by paying attention to certain parameters while providing treatment.

Hyperthermia was shown in 3 of the cooling trials to be associated with increased likelihood of death and disability (39–41). It is therefore important to avoid hyperthermia before and after cooling.

Low carbon dioxide levels affect cerebral blood flow and exacerbate brain injury, and have been associated with unfavourable outcomes in both the NICHD and CoolCap trials (39,42). Thus, it is essential to avoid hypocarbia, particularly in ventilated patients.

Resuscitation after birth should be initiated without supplemental oxygen as hyperoxia has been linked with adverse outcomes and delayed cellular recovery in animal studies (43); whereas 21% O2 is associated with decreased encephalopathy, brain injury on MRI and mortality (44–46).

Glucose control is important as a post hoc analysis of the CoolCap study showed that both hypoglycaemia and hyperglycaemia are associated with adverse outcome in infants with HIE. This was independent of degree of encephalopathy or TH, and hypoglycaemia was worse than hyperglycaemia (47).

Detection and management of seizures is another important aspect that requires attention. There is evidence that seizures in the presence of HIE are independently associated with additional brain injury and worse adverse outcomes (48,49). Seizure burden appears to be important, with total duration more than 40 minutes or more than 13minutes/hour having the highest risk of abnormal outcome at 24-28 months (50).

*Other neuroprotection*

Because TH has relatively limited beneficial effect, with approximately 40-50% of treated infants still surviving with neurodevelopmental impairment, there is ongoing research effort to investigate the use of other neuroprotective therapies in combination with TH (Figure 2.3) (51). Several candidates have shown promise, particularly in pre-clinical studies.

Erythropoietin (EPO) has been shown to have antioxidant, anti-inflammatory, anti-apoptotic and neurotropic properties; and pre-clinical trial showed improved outcomes in non-human primates after hypoxia ischaemia(45). In human neonates with HIE, multiple dose EPO in combination with TH was shown to be safe and to result in reduced brain injury on early MRI and improved motor outcomes at 1 year(52).

Melatonin, which is an endogenous hormone that regulates circadian rhythms, has been shown to be a powerful antioxidant and anti-apoptotic agent at high doses. It was shown to be safe and resulted in reduced infarct size and improved neurological outcomes in pre-clinical models (53).

Xenon, an anaesthetic gas with anti-apoptotic properties which showed promise in animal studies when combined with TH, conferred no additional benefit in human infants when added to TH for 24 hours and assessed using MRI and MRS (54).

Stem cells were shown to improve histological injury and improve neurological outcome when used alone in animal models and it has been shown that it would be

feasible to administer them after birth to infants with HIE(55). However, combining stem cells with TH in a mice model resulted in worse outcomes than either therapy on its own (56,57).

Allopurinol, a xanthine oxidase inhibitor with antioxidant and free radical scavenging activity, is another agent that is currently being studied for its neuroprotective properties. The large ALBINO trial will assess its effect on neurocognitive outcomes at 2 years when used in combination with TH after birth (58). It may also potentially be neuroprotective to the hypoxic foetus when administered to the pregnant mother (59).

2-Iminobiotin, a vitamin B7 analogue which is a selective inhibitor of neuronal and inducible nitric oxide synthase, was shown to be neuroprotective after perinatal hypoxia-ischaemia in a pre-clinical model (60). It was found to be safe, inexpensive and easy to administer in a study on newborns in a low income country (61).

These examples illustrate the need for further pre-clinical followed by large clinical trials before adjuvant therapies to TH can be introduced into clinical practice to define the optimal methods and timing of use (Table 2.1) (62).

**Figure 2.3: Model of damaging pathways and proposed mechanisms/times of action of different potential therapies(51)**

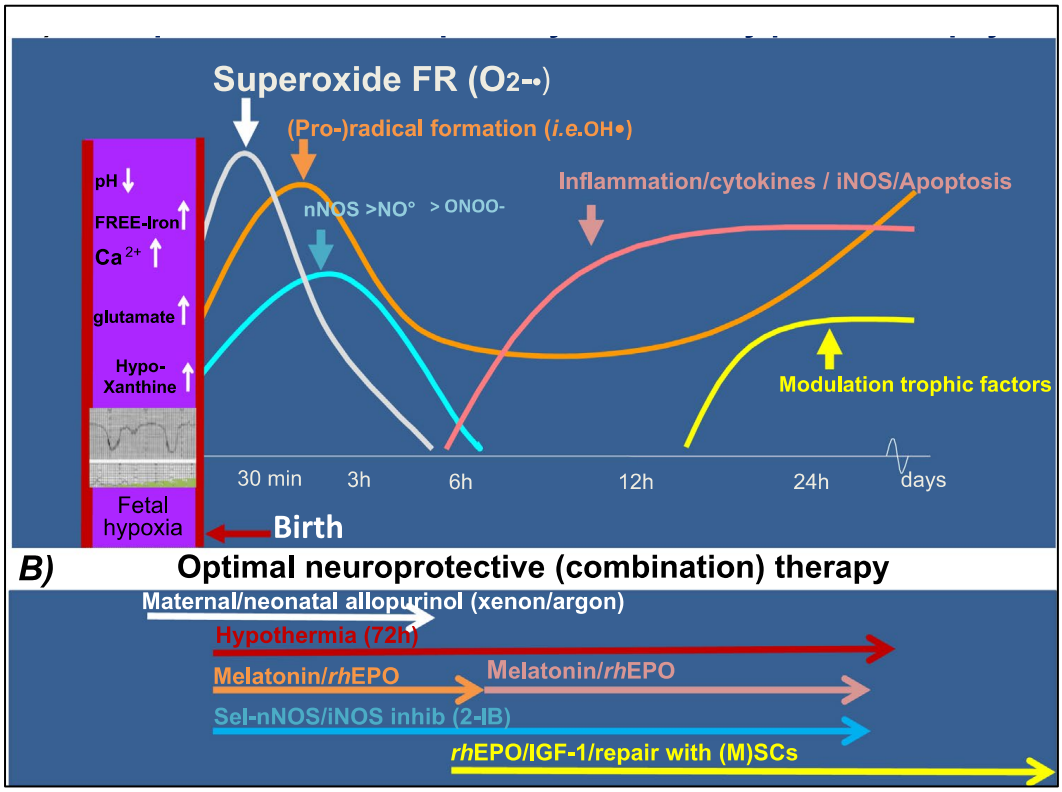


**Table 2.1: Summary of the evidence for additive neuroprotective effects with hypothermia and potential combination treatments**(62).

| Combination treatment | Species | Age | Additive effects | HT started | Other intervention started | Comment |
|---|---|---|---|---|---|---|
| **Anti-inflammatory/neuroregenerative** | | | | | | |
| Erythropoietin | Non-human primates | Full term | Yes (survival, motor, cognitive, cerebellar growth, MRI) | Immediately | 30min | HT x 72h |
| | Neonatal rat | P7 | No (sensorimotor, histopathology) | 1h | Immediately | HT x 8h |
| | Neonatal rat | P7 | Borderline (sensorimotor, histopathology) | Immediately | Immediately | HT x 3h |
| Stem cells | Neonatal rat | P7 | Yes (histology, MRI, functional) | 6h | 6h | HT 32°C x 24h |
| **Anti-oxidative/anti-inflammatory** | | | | | | |
| Melatonin | Newborn piglet | Full term | Yes (MRS, histology) | 2h | 10min | HT x 26h |
| **Anti-apoptotic** | | | | | | |
| IGF-1 | Fetal sheep | Term equivalent | No (EEG, histology) | 5.5h | 4.5h | HT x 72h |
| **Anticonculsant agents** | | | | | | |
| Xenon | Neonatal rat | P7 | Yes (histology, functional) | 4h | 4h | HT x 90min |
| | Newborn piglet | Term | Yes (neuropathology, clinical neurology) | <40min | 30min | HT x 12-24h |
| | Newborn piglet | Term | No (trend, MRS, histology) | 2h | 2h | HT x 24h |
| | Humans | Term | Yes (seizures only) | <12h | <12h | Reduced seizures |
| Phenobarbital | Neonatal rat | P7 | Yes (histology, MRI, functional) | 1-3h | 15min | HT 30°C x 3h |
| Dizocilpine | Fetal sheep | 0.7 gestation | No (EEG, histology) | 5.5h | 15min | HT x 72h |
| **Connexinhemichannel blockade** | | | | | | |
| Cx43 mimetic proteins | Fetal sheep | Term equivalent | No (EEG, histology) | 3h | 3h | HT x 72h |

*Cx43, connexin 43; EEG, electroencephalography; IGF-1, Insulin-like growth factor; MRI, magnetic resonance inaging; MRS, magnetic resonance spectroscopy; functional, neurobehavioral test.*

## Role of Morphine

### Morphine

One of the cooling studies in HIE (41) whose findings confirmed the beneficial effects of cooling showed a greater treatment effect of therapeutic hypothermia than some of the other larger studies. It showed a 32% lower rate of the combined outcome of death or severe disability (OR 0.21) in the cooled infants, and importantly a 39% lower rate of severe disability in survivors. One explanation for this was the fact that all the infants in this particular study routinely received morphine as co-treatment.

It is not clear why this hypothermia trial reported by the Neonatal Neuro Network Trial group showed better outcomes than other studies after cooling. The authors speculated that the administration of morphine, either as continuous infusion or 4-hourly bolus dosages, averaging approximately 25µg/kg/h, may have contributed (G Simbruner, personal communication).

It has previously been shown in animals that applying hypothermia without sedation may interfere with its neuroprotective effect, and this may be related to the stress of being cold and shivering(63).

The ability of opioids to attenuate brain injury has been demonstrated by Angeles and colleagues, who showed significantly less brain injury on MRI, less alteration of brain metabolites on Proton MRS as well as better long-term neurological outcomes in infants with asphyxia when treated with opioids during the first week of life, despite being subjected to multiple painful procedures, including lumbar punctures, compared to controls(64). This group used higher doses of opiates (morphine: 30 – 40 µg/kg/min) than routinely used in clinical practice. Although it was a retrospective study and morphine or fentanyl was used, the authors showed that opioid therapy did not cause harm (apart from temporary requirement of ventilatory support) in asphyxiated term neonates exposed to repetitive tissue-damaging procedures in the first 2-4 days of life and suggested that it may provide a degree of neuroprotection.

Short-term hypothermia has been shown to alter the metabolism of fentanyl in juvenile pigs(65). Roka and co-workers reported reduced morphine clearance and elevated serum morphine concentrations during moderate hypothermia and with infusion rates

exceeding 10µg/kg/h in comparison to normothermic infants(66). This group also did not describe any significant complications such as severe hypotension, although the hypothermia group did show a not statistically significant tendency to require a more extended period of ventilatory and cardiovascular support, and had a worse clinical encephalopathy score on day 4 of life. More recently, a secondary analysis of the MARBLE study(67) found that with pre-emptive morphine there was prolonged ventilation and hospital stay. This study showed neither increased neuroprotection, nor increased brain injury markers on MR spectroscopy or worse neurodevelopmental outcome in the morphine group. The relevance of elevated concentrations of morphine and/or morphine metabolites (not measured in the study) remains undetermined.

There is some evidence that morphine or opioids have an immuno-modulatory function (Table 2.2) (68–71), and it is known that inflammation plays a significant role in mediating injury following perinatal hypoxia-ischaemia(72).

A better understanding of the anti-inflammatory actions of opioids may result in important treatments for neuroinflammation secondary to perinatal hypoxic-ischaemic brain injury. Opioids act as modulators of neuronal cell death and survival and these effects appear to be independent of their analgesic properties(73). Morphine prevents peroxynitrite-induced apoptosis of primary astrocytes(74) and enhances proliferation of hippocampal progenitor neurons(75). Neuroinflammatory responses in astroglia, including chemokine expression (CXCL 10), are altered or down-regulated by opioids (Table 2.3) (68,76). While the mechanisms for the neuroprotective effect of morphine are incompletely understood, there is evidence to suggest that morphine attenuates oxidative and pro-inflammatory stress(77,78) suggesting that morphine may have an effect on neuroinflammation after a hypoxic-ischemic insult to the brain. Morphine treatment is associated with increased Erk expression and/or phosphorylation in neurons (79,80) and Erk is one messenger by which opioids transmit their neuroprotective effects.

Morphine down-regulates phagocytic cell function, particularly of peripheral mononuclear cells and polymorph nuclear cells(81). Morphine attenuates leucocyte / endothelial interactions by influencing peripheral leucocyte rolling and sticking through

stimulation of endothelial-derived nitric oxide synthase(82). Exploring the immune suppressive effects of morphine and its possible role in enhancing perinatal transmission of the HIV, it was found that morphine up-regulates the shared opioid / beta chemokine CCR5 receptor expression on neonatal cord blood monocyte-derived macrophages and inhibits endogenous production of macrophage inflammatory protein-1 beta (MIP-1β)(70). In human fetal microglia and astrocyte cultures, chemokine genes are activated with distinct patterns. With regard to microglia, lipopolisaccharide (LPS) acts as a potent stimulant for MIP-1α and MIP-1β mRNA induction, but has no effect on chemokine induction for astrocytes (83). Microglia are mobile, specialized macrophages capable of phagocytosis that support and protect neurons of the central nervous system and multiply when the brain is damaged. The bidirectional interactions between neuron and glia are important for maintaining normal neural activities and signaling mediators between these cell-types play a role in nerve regeneration. Microglia apparently migrate toward injured neuronal cell bodies to which they attach. Disturbance of this milieu through nerve cell injury results in CCR5 expression in microglia and subsequent expression of mRNA's for the ligands: MIP-1α and MIP-1β and RANTES(83). As previously mentioned, morphine inhibits CCR5 ligand MIP1β expression and the question is how morphine at this level would affect nerve cell regeneration if it is taken into consideration that suppression of inflammatory mediators in microglia is by RANTES via CCR5 and that CCR5-deficient mouse brain shows accelerated cell death (84).

The temporal relationship of expression of some cytokines such as interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF$\alpha$), and chemokines such as beta2-integrin and RANTES to that of immune cells led some authors to conclude that these molecules may have a role in the inflammatory response to insults in the immature central nervous system. Morphine was shown to suppress this response via activation of microglial p-opioid receptors(85).

Although the interplay between morphine and systemic monocyte-derived macrophage receptor activity and brain glial cell receptor expression after hypoxia-ischemia is likely to be complex, the possibility exists that morphine may play a role in decreasing the migration and flux of systemic inflammatory cells towards the region of

hypoxia-ischaemia as well as suppressing expression of pro-inflammatory chemokines such as MIP-1β, and affect RANTES (Table 2.4).

**Table 2.2: Effects of opioids on immune functions**(68)**_(summarised)**

| **Function** | **Species** |
|---|---|
| Suppression of natural killer cell activity | Mouse, rat, human in vivo |
| Suppression of cellular responses to mitogens | Mouse, rat, human ex vivo |
| Depression of antibody production | Mouse in vivo |
| Depression of T cell mediated adaptive immune responses | Mouse in vivo |
| Depression of cellularity | Mouse in vivo |
| Induction of apoptosis | Mouse in vivo, mouse in vitro, human in vitro |
| Inhibition of cell growth | Mouse in vivo, mouse in vitro, human in vitro, monkey in vivo |
| Suppression of phagocytosis | Mouse in vivo, mouse in vitro |
| Down-regulation of cytokines and other inflammatory associated mediators | Mouse in vivo, mouse in vitro, rats in vivo, human in vitro |

**Table 2.3: Opioid effects on chemokine levels**(68)**_(summarized)**

| **Chemokine ligands** | **Species** | **Opioid** | **Effect** |
|---|---|---|---|
| CCL2/MCP-1 | Human in vivo<br>Mouse in vitro<br>Human in vitro | Morphine<br>Morphine<br>DAMGO | ↓<br>↓<br>↑ |
| CCL4/MIP-1β | Human in vitro | Morphine | ↓ |
| CCL5/RANTES | Human in vitro<br>Mouse in vitro<br>Human in vitro | Morphine<br>Morphine<br>DAMGO | ↓<br>↓<br>↑ |
| CCL12/MCP-5 | Mouse in vitro | Morphine | ↓ |

| | | | |
|---|---|---|---|
| CXCL1/IL-8/KC | Human in vitro | Morphine | ↓ |
| CXCL10/IP-10 | Human in vitro | Morphine, DAMGO | ↑ |
| **Chemokine receptors** | | | |
| CCR3 | Human in vitro | Morphine | ↑ |
| CCR5 | Human in vitro | Morphine, Methadone, DAMGO | ↑ |
| CXCR4 | Human in vitro | Morphine, DAMGO | ↑ |

*DAMGO, a mu-opioid receptor selective agonist*

**Table 2.4: Morphine/opioid effects at different levels**

| Site | Mechanism | Effect | Ref |
|---|---|---|---|
| Brain opioid receptors | Sympathetic nervous system activation | ↓ NK cells, lymphocytes, cytokines (IL-2, IFN-g) | (86) |
| Central and peripheral opioid receptors | Opioid receptor expression by immune cells<br>Complex interactions with inflammatory cells (astrocytes, microglia, monocytes, macrophages) | ↓ antibody and cellular immune responses, NK cell activity, cytokine expression, phagocytic activity | (87) |
| Central and peripheral | Increased Erk expression and/or phosphorylation in neurons and immune cells<br>- main signalling pathways involved in mitogenic responses to external stimuli | Increased neurogenesis/growth promotion and reduced apoptotsis | (73) |
| Central and peripheral | Neural-immune circuit with direct and indirect action on immune cells<br>(Human and animal studies) | ↓NK cells, B cells, T cells, and phagocytic cells (polymorphonuclear leukocytes and mononuclear cells) when given in vivo | (81) |

| | | | |
|---|---|---|---|
| | | Direct ↓ effect on phagocytic cells in vitro | |
| Central | Activation of microglial μ-opioid receptors<br>- effect blocked by naloxone and β-funaltrexamine (general and MOR antagonists) | Production of and migration towards RANTES (chemokine) by human microglia | (85) |
| Peripheral (in vitro) | b-chemokine alteration | Up-regulation of CCR5 receptor expression (coreceptor for HIV entry into macrophage), significant ↓endogenous MIP-1b<br>→HIV replication in neonatal monocyte-derived macrophages (MDM) | (70) |
| Cellular and molecular level | NO anti-oxidant action | Inhibition of Rotenone-induced oxidative cell death<br>blocked IFNγ-induced expression of LMP7 (pro-inflammatory immunoproteasome catalytic subunit)<br>dose-dependent increase expression of ubiquitin (marker for protein degradation) – less oxidised proteins | (78) |

NK=natural killer. RANTES=regulated on activation, T cell expressed and secreted. MIP=macrophage inflammatory protein. MDM=monocyte-derived macrophages. MOR=m opioid receptor. NO=nitric oxide. IFN-interferon

## Biomarkers

For clinical interventions it is important to identify infants at risk for brain damage soon after birth, and within a therapeutic window. Cooling is considered to be most effective if instituted as early as possible within the six-hour therapeutic window after injury(30). Furthermore, there is a need to identify biomarkers of tissue injury that may assist in separating infants who would benefit from therapy from those who might be harmed by that same therapy(88). Some authors have suggested that it may ultimately be possible to use biomarkers to stratify infants undergoing TH into (i) those that are responding to treatment and have a good prognosis, (ii) those that are not responding and are at risk of surviving with neurodevelopmental impairment and (iii) those that are likely to die and hence need redirection or modification of further management accordingly(89,90). It is particularly important in resource-poor settings to have relatively simple tools for predicting outcome and for identifying those infants that might benefit from neuroprotective strategies.

Many serum biomarkers of hypoxic ischaemic brain injury, such as protein S-100, neuron-specific enolase (NSE) and brain-specific creatine kinase (CK-BB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), have the limitations of non-specificity or temporal pattern of release, necessitating frequent sampling. Hence, they are more useful for differentiating asphyxiated from non-asphyxiated infants(91–93). In a systematic review, Ramaswamy and co-workers identified the biomarkers CSF neuron-specific enolase (NSE), CSF interleukin 1b and serum interleukin-1b and interleukin-6 as predictors of abnormal outcomes in survivors of encephalopathy after asphyxia or cerebral infarction. They suggested that these markers of outcome be studied prospectively to validate their usefulness(88).

Another relatively simple marker of perinatal hypoxic injury that may be applicable even in resource-constrained settings is nucleated red blood cell counts (NRBC). These have been shown to increase to varying degrees following fetal hypoxia. In one study the number of NRBC in cord blood increased in response to both acute and chronic hypoxia, with the counts increasing to a greater degree after chronic hypoxia and with more severe encephalopathy(94).

This makes this test a promising candidate for selecting the most appropriate patients, even from remote areas (as this test is relatively widely available), that may benefit from neuroprotective treatment.

Preliminary unpublished data from our cohort of infants with HIE treated with TH showed that increased NRBC within 6 hours of birth was associated with poor outcome (see addendum, chapter 8). This suggests that this simple blood marker could be further explored as a tool for selecting infants that may/may not benefit from neuroprotective therapies such as TH.

**Cooling in low resource settings**

While therapeutic hypothermia (cooling) has been established as standard of care for infants with moderate to severe hypoxic encephalopathy for at least a decade in more developed (high income) countries(25,26), its use in less resourced settings is still somewhat controversial and limited by a number of factors.

One of these has been a lingering uncertainty about whether it is directly applicable or safe in these settings and will not cause more harm. This concern was driven in large part by the results of a pilot study in Uganda where there was increased neurological morbidity and mortality in the therapeutic hypothermia group(95). Some of the postulated reasons for this lack of effect and even adverse outcomes were that there may be some underlying factors that make these populations different from those in which the original cooling studies were performed. These include maternal factors such as malnutrition; higher prevalence of complicated labour and infective processes that are often impossible to confirm due to unavailability of blood tests in many settings (96); the “natural cooling” phenomenon that may occur in more severely affected infants that may dilute the treatment effect.

Notwithstanding the above concerns, the greatest need for neuroprotective therapies such as therapeutic hypothermia is in these low- and middle-income countries where the major burden of disease is, with 96% of newborns who developed intrapartum-related neonatal encephalopathy in 2010 being born in the low resourced settings(97).

By virtue of their being low resourced, introducing cooling to these areas is beset by many challenges:

- The infants may be too severely affected to benefit from therapy due to lack of skilled birth attendants and resuscitation facilities, or not being delivered at health facilities
- The available tools for assessing and selecting infants for treatment, and for providing the treatment itself and monitoring, are often very costly and assessment requires expertise
- The infrastructure needed (roads, ambulances, etc.) to ensure infants reach treatment centres within the required time frames/therapeutic window is frequently lacking. Patients sometimes have to find private transportation to get to hospital and pay for treatment, which further limits access.
- Availability of intensive care facilities and adequate monitoring, which is an essential component of providing therapeutic hypothermia, is often limited.
- Long term neurodevelopmental follow up is often challenging

Some of these limitations can be mitigated by the use of simple tools, which have been validated and shown to be useful in clinical practice. The Thompson score is a clinical score for assessing the degree of encephalopathy based on Sarnat staging(98) that was originally developed in the pre-cooling era, and shown to be easy to implement and useful in predicting outcome at 1 year(99).

In the cooling era, it has been re-validated and shown to be a still useful clinical tool. For selecting patients that could benefit from neuroprotection, Horn et al (SA) showed that a Thompson score of 7 or greater at 3-5 hours predicted an abnormal amplitude integrated EEG at 6 hours and moderate to severe encephalopathy within 72 hours of birth(100,101). Similarly, Biselele et al in the DRC showed that infants with scores of 7-15 within 6 hours of birth were eligible for neuroprotective therapies and this could be used as a selection tool(102).

For predicting short-term outcome, the Thompson score has been shown to still be a valid tool, with multiple studies showing that a high score predicts death or severe adverse outcomes even with therapeutic hypothermia. In separate studies Horn and Biselele showed a score of 16 or greater predicted death before discharge (101,102), while Thorsen et al showed a score of 12 or greater predicted death before discharge or severe seizures(103).

In order to assess the status of TH in LMIC, surveys have been conducted in some MIC:

Joolay et al surveyed public and private neonatologists and paediatricians in South Africa in 2010 about their opinions and practices of TH. They found that 42% of respondents offered TH and 9% transferred to other centres for cooling. 24% did not offer, or plan to offer TH in near future. However, 98% of respondents felt that TH should be standard of care in tertiary units (104).

Chandrasekaran et al conducted a similar survey of Indian neonatal units a few years later. They found a similar proportion of practitioners (51%) that offered TH as standard of care, 44% who wanted to but had no resources and 5% who wanted further rigorous evidence before starting. Many who did offer TH did so outside the established international protocols. 53% of them used locally improvised methods (105).

Cooling devices

It is evident that LMIC are not homogenous, and some areas are even less well-resourced than others. To address the issue of expensive devices, many innovative methods of cooling have been or are being studied in low income settings to provide both SHC and WBC at low cost. These include frozen ice caps, frozen gel packs (FGP), water bottles, water cooling caps, servo-controlled fans, phase changing materials (PCM), laminar flow devices, clay pot cooling devices and even ambient temperature (106). Some of the challenges experienced with these methods have been excessive shivering, excessive temperature fluctuations and possible over-cooling in absence of adequate nurse-to-patient ratios. Two studies that evaluated the PCM method in India found that it was safe and feasible, but was only effective in low ambient temperature (<28°C) and required intensive nursing monitoring to achieve target temperatures (107,108). When FGP were compared to PCM, there was a shorter induction time but even greater temperature fluctuations requiring more nursing input with FGP (109). The ideal cooling device in these settings is one that is servo-controlled but affordable, can run even without electricity, and requires minimal nursing input. Two such devices have been studied in LMIC countries and found to be effective, safe and relatively inexpensive: a laminar flow device in Brazil (110) and a

Tecotherm-HELIX device which is a simplified version of an existing device that was tested in India (111). Some unexpected findings in one of these feasibility trials were the high incidences of gastric bleeding, and significant tachycardia. These findings were not common in the studies from high income countries. These infants were also smaller, with mean birthweight 2900g. These highlighted the need for further study to understand the underlying pathophysiological processes and intrinsic factors that may be different in these populations, while offering TH in a safe controlled manner.

A few studies, mostly using gel packs, looked at short term outcomes after TH in LMIC and found improvements in markers of oxidant stress, neurological state at discharge, mortality and developmental impairment at 6 months(112–114). A Brazilian study that did have longer term outcomes confirmed the utility of MRI for predicting outcome in developing countries (115).

Several systematic reviews and meta-analyses have been conducted in the past few years in an attempt to answer the question of suitability of TH for low resource settings. Pauliah et al reviewed data involving 567 infants from 4 countries (116). They found that TH (both WBC and SHC) had no significant effect on mortality (RR 0.74; 95% CI 0.44 to 1.25). They highlighted some factors that may have accounted for the apparent lack of treatment effect. The majority (63%) of patients had mild-moderate HIE, most (88%) were not ventilated and few were sedated. The degree of NE may have diluted the treatment effect, and highlights the difficulties in assessing an evolving disease process particularly without access to objective assessment tools. Other contributing factors may have been patient-related such as obstructed labour, intrauterine growth restriction, maternal nutritional status, infections, and their response to treatment. However, concerns about the presumed higher prevalence of perinatal infection and that TH may not be as protective in inflammation-sensitised brains may prove not to be always warranted. Studies on Uganda (96) and India (112) showed a prevalence of <10% bacteraemia in encephalopathic neonates, which is similar to that shown in the studies from developed countries and would make TH not any less likely to be effective in LMIC countries. Environmental factors such as inadequate monitoring resulting in temperature fluctuations and excessive oxygen administration, inadequate sedation, lack of ventilation facilities, and possible inefficacy of the low technology devices used were also considered. They also could not report on outcomes as many

of the studies had no outcome data. This again highlights the challenges of conducting studies in these settings, and MRI (where available) could play an important role in profiling the disease process as well as assessing the effects of neuroprotective therapies (117). Galvao et al in the same year performed a meta regression analysis involving 1889 infants in different settings, which included 662 infants in low resource settings. This study showed a significant reduction in mortality (RR = 0.77; 95% CI: 0.65–0.92) with TH, which was not influenced by the GDP per capita of the setting where applied (118).

Another systematic review and meta-analysis assessed the effects of low technology TH (LTTH) in settings where intensive care and ventilation facilities were available, and included 2 studies from LMIC (none from low income countries). They showed that LTTH had similar results to TH administered with high technology devices when applied in an intensive care environment (119). They suggested that LTTH should be offered as standard of care in low resource settings where high technology cooling equipment is not available, but facilities for ventilation and monitoring possible.

Ethics/economics

Other concerns about introducing TH to LMIC settings have been about converting high mortality into disability, which would be an even greater burden in low income settings. This concern has largely been dispelled in high income countries as data from cooling trials showed reductions in both mortality and disability in survivors, and there is at least some data showing similar findings in lower income settings albeit without longer term outcomes. Infants cooled in these settings also tended to have more moderate encephalopathy (116), and may thus derive the most benefit from being cooled. It is hoped that a large ongoing RCT (HELIX) being conducted in 3 LMIC (India, Bangladesh and Sri Lanka) that will enrol 408 infants will provide much of the required information about TH in LMIC. This study will have information on perinatal infection, gene expression, early MRI findings and long term outcome (120).

**The aims of the current work are therefore:**

- To document the safety and applicability of therapeutic hypothermia (cooling) in a middle-income setting, where facilities for monitoring and ventilation are

available, and when applied within a strict protocol similar to the ones used in the clinical trials.

- To document the early (1 year) outcomes of infants treated in this setting.
- To determine the effects of combining cooling with morphine on outcome.

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# CHAPTER 3

## Methodology

In this chapter, I discuss the methods used in conducting the study.

### Encephalopathy score

A modification of the TOBY study entry criteria was used to select patients for therapeutic hypothermia (TH) in the initial cohort. The two main changes were:

Amplitude integrated electroencephalography (aEEG) was not an essential criterion, and was used only when available. The other was the inclusion of the Thompson encephalopathy score of ≥10 in order to simplify the clinical assessment particularly at referring centres.

This is a simple clinical scoring system based on the Sarnat and Sarnat classification of hypoxic ischaemic encephalopathy (HIE), that was developed in the pre-cooling era to enable the prediction (mainly in the developing world) of neurodevelopmental outcome at 1 year of age without the need for specialist paediatric/neurology training or expensive technology (1). It has only 9 clinical items that are scored (0-3) and the maximum score is 22. The peak score and duration of abnormality was highly predictive of outcome. The combination of a peak score of >15 and persisting abnormal score on day 7 had a high positive predictive value (PPV) of 92%, and a negative predictive value (NPV) of 100% for abnormal outcome at 1 year. Infants who had a peak score less than 10 and were normal by day 7 had a normal outcome (see Figure 3.1). This was used to categorise infants into those at high risk and in need of close follow up, or those that could be discharged early from follow up.

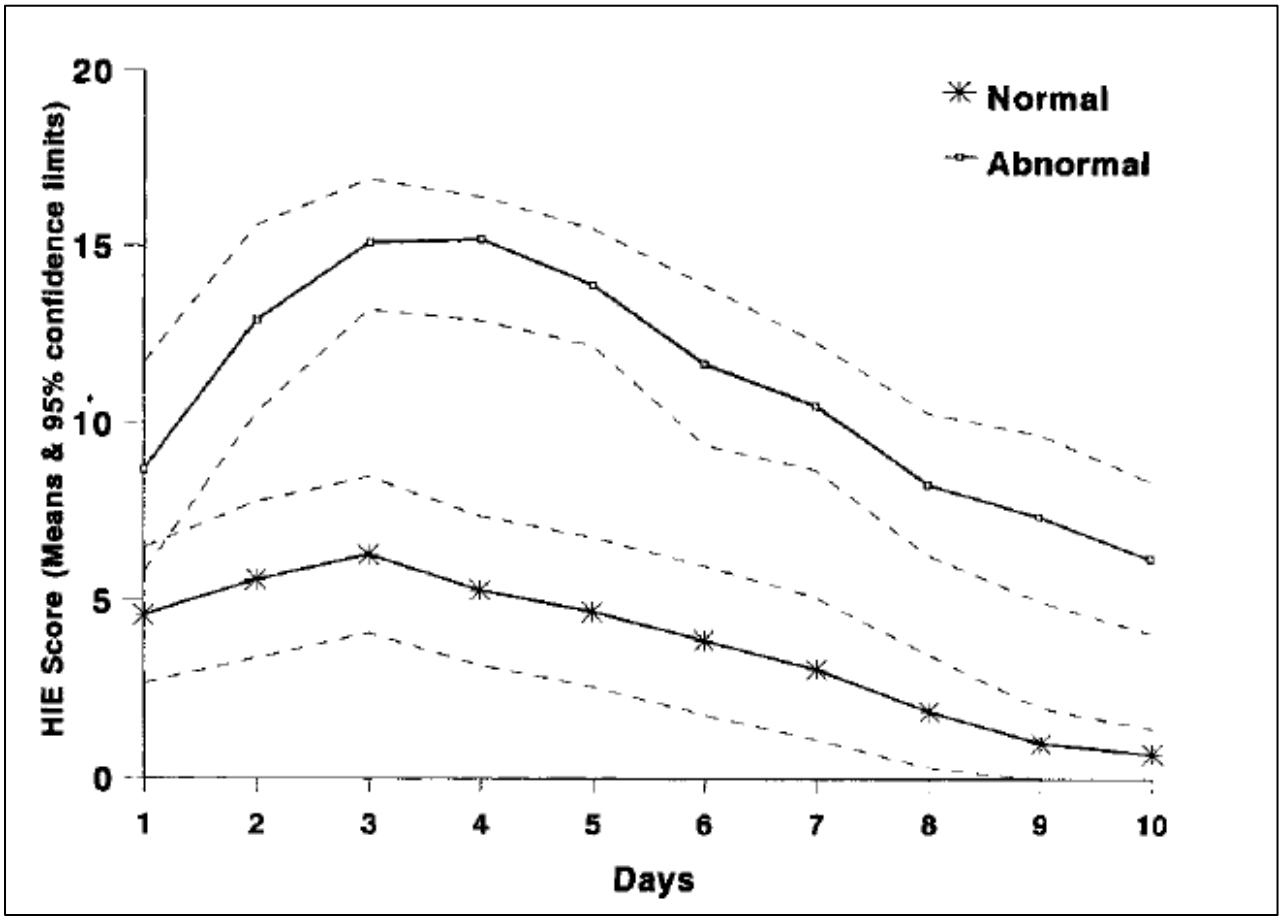
In the cooling era, it was re-validated and it was found that a Thompson HIE score of ≥7 at 3-5 hours predicted an abnormal aEEG at 6 hours with a sensitivity and specificity of 90% and 92% respectively, and could be used to select infants for referral for neuroprotective treatments such as TH (2).

Based on these findings, we adapted our criteria for TH and in the prospective RCT comparing TH and TH+M a threshold Thompson score of ≥7 was used to recruit patients.

We also used the Thompson HIE score as originally used to monitor clinical condition daily, and classified encephalopathy grade based on the peak score in the first week: mild ≤ 10, moderate 11–14 and severe ≥15. We used this classification to calculate the predictive values of a high score ≥15 (indicating severe encephalopathy) for death or neurodevelopmental impairment at 1 year of age in the context of TH.

In the prospective cohort, infants were classified according to initial HIE score prior to initiation of TH: moderate 7-14, severe ≥15. This was based on the findings by Horn et al that an early score of ≥7 predicted an abnormal 6 hour aEEG that would qualify infants for TH and moderate-severe encephalopathy within 72 hours with high accuracy (3).

**Figure 3.1: Thompson score trends in normal/abnormal outcome**(1)



**Amplitude integrated electroencephalography (aEEG)/neurophysiological monitoring**

Amplitude integrated electroencephalography (aEEG) is EEG that has been filtered, rectified, smoothed, compressed in time and is presented on a semi-logarithmic voltage scale. It was initially used to identify neonates at risk of brain injury and poor neurological outcome after birth asphyxia (4). It can be classified in two ways (see Figure 3.2 below):

1. pattern recognition technique with 5 main background patterns
    - Continuous normal voltage (CNV)
    - Discontinuous normal voltage (DNV/DC)
    - Burst suppressed
    - Continuous low voltage (CLV)
    - Flat trace/inactive (FT)
2. voltage criteria with 3 amplitude categories (5)
    - normal: upper margin of band of aEEG activity >10 mV and the lower margin >5 mV
    - moderately abnormal: upper margin of band of aEEG activity >10 mV and the lower margin <5 mV
    - suppressed: upper margin of the band of aEEG activity <10 mV and lower margin <5 mV, may be accompanied by bursts of high-voltage activity (“burst suppression”)

Seizure activity is suspected when there is a sudden increase in voltage, together with narrowing of the band of electrical activity. This then confirmed by viewing the raw EEG that is recorded simultaneously.

The advantages of aEEG are that it is more practical for use in the neonatal intensive care (NICU). It can be applied at any time of the day, without depending on availability of technicians. It provides continuous monitoring of trends of brain electrical activity. Neonatal staff can be skilled in application and interpretation with minimal training. The voltage classification system is easiest to teach/learn, and was found to have high inter-observer correlation (5). However, it preferable to use a combination of the 2

classification systems as the voltage may be altered artefactually by electrical interference such as electrocardiogram or ventilator equipment.

Earlier monitors used one channel with bi-parietal electrodes that monitored general electrical activity and trends, but could not localize pathology. Newer models usually have 2 channels, allowing for monitoring of both hemispheres of the brain.

In the study by Hellstrom-Westas, it was found that aEEG background pattern in the first 6 hours after birth predicted outcome at 18-24 months with 92% accuracy (4). This was useful as other tools such as US, CT or MRI only become predictive later on in neonatal course, and may not be helpful in selecting candidates for neuroprotective therapies that need to be instituted soon after birth. It was on this basis that abnormal aEEG formed part of the entry criteria of 2 of the large TH trials for HIE (6,7).

In our unit, 2-3 aEEG devices were available (for 12 bed NICU & HC) at various times during the conduct of the studies, which meant that aEEG was not always available for all infants that required it. We did not discontinue monitoring of infants if the trace was still abnormal, but on occasion monitoring was discontinued before the end of TH if the trace was normal and equipment was needed for another infant.

As discussed above, when available a 30-minute aEEG recording was used in our studies to support the diagnosis of moderate-severe encephalopathy, especially when there was some uncertainty about severity. aEEG patterns that were eligible for selection for TH were (Figure 3.2): discontinuous normal voltage (DNV), burst suppression (BS), continuous low voltage (CLV), and flat trace (FT) and any seizure activity.

Once TH commenced, our infants were monitored continuously during the cooling and re-warming period where feasible, and occasionally for longer periods if aEEG remained abnormal.

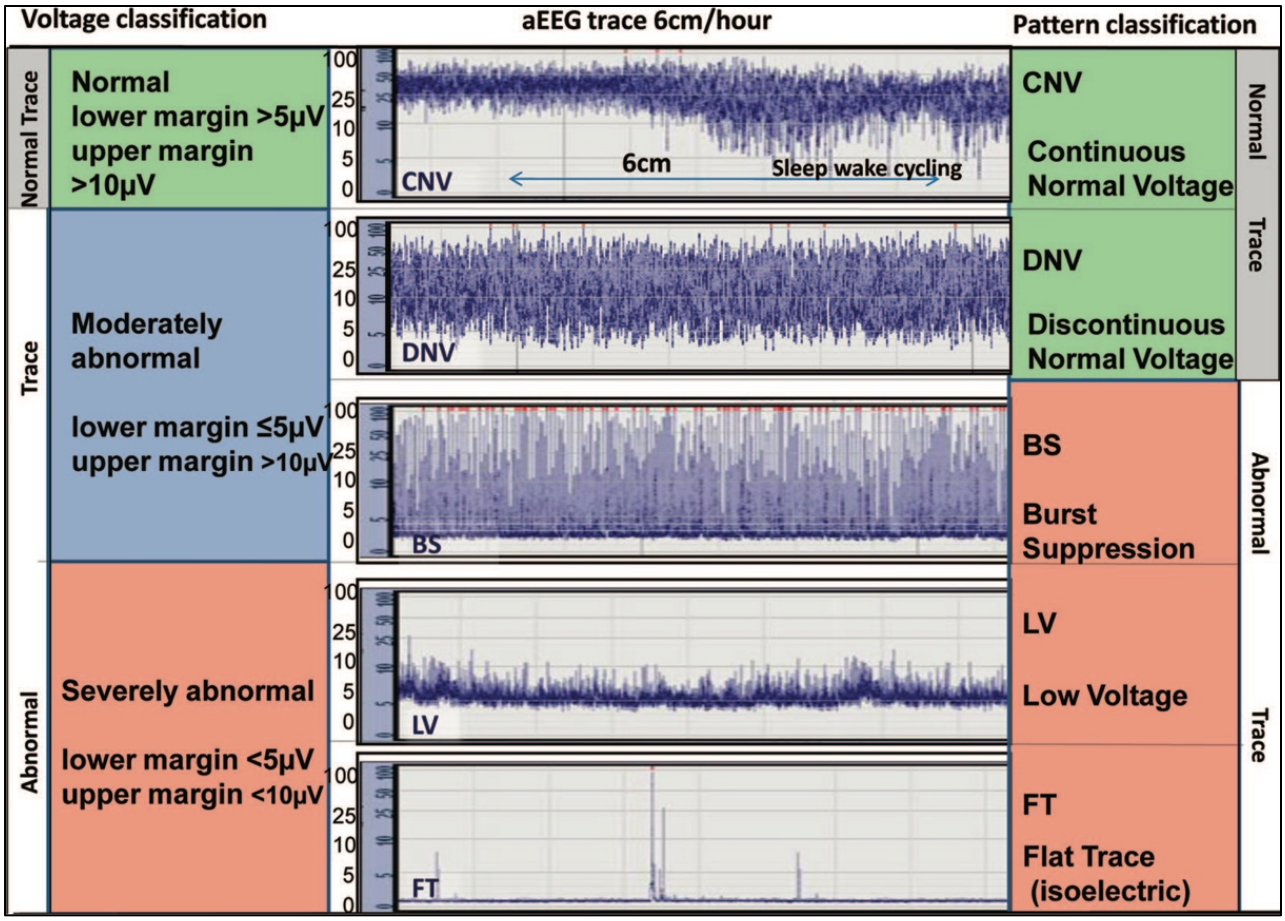
The recordings were assessed for duration of abnormal pattern, presence or timing of appearance of sleep wake cycling, symmetry, and presence of and burden of seizures.

High seizure burden was defined as having >3 seizures/hour, or a single seizure lasting ≥30minutes. When there was suspicion of ongoing/sub-clinical seizures after

the period of continuous monitoring, formal EEG was performed in some infants. Recognition of seizures and in particular a high seizure burden was important in light of the reported association with additional brain injury in the setting of HIE, although this may be modulated to some extent by TH (8,9).

Time taken to recovery of background pattern (in conjunction with other clinical parameters) was used to counsel parents about prognosis, and to make decisions about ongoing intensive care. Severely abnormal background (CLV/FT) persisting up to/beyond 48 hours was regarded as a poor prognostic sign(10,11).

**Figure 3.2: aEEG background classification**(10)



## Imaging

### Cranial ultrasound

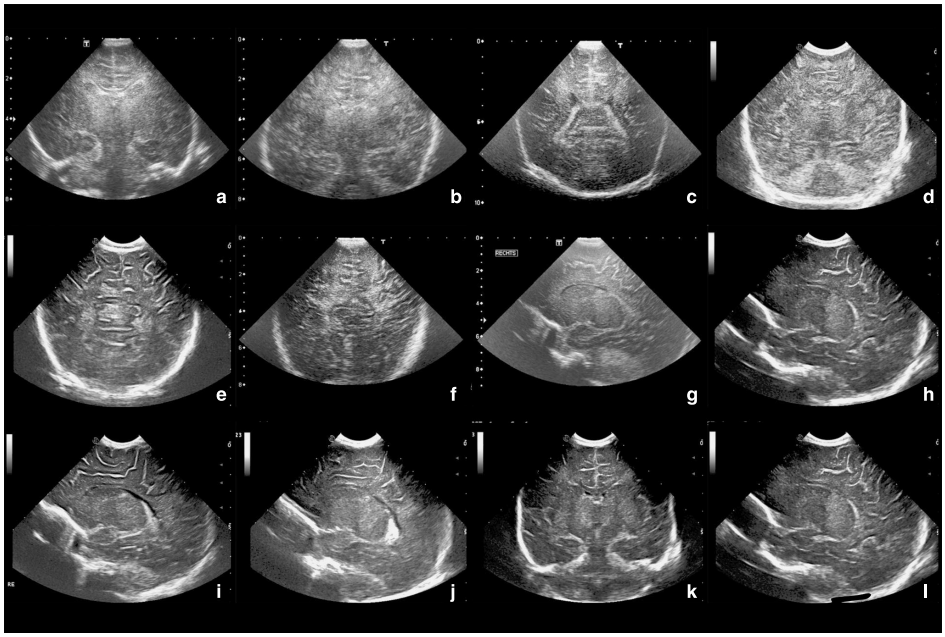
Cranial ultrasound (CUS) is the most frequently used, safe and accessible method of imaging the neonatal brain. It is mobile, relatively inexpensive, does not require movement of the ill infant and can be used in the NICU. In the context of neonatal encephalopathy (NE), it is used to screen for congenital abnormalities, for serial monitoring of brain pathology and to assist with prognostic information. Its limitations are that it is not as sensitive as MRI, certain areas of the brain are not easily visualized (convexities, posterior fossa), and some abnormalities (eg. white matter or myelination) may be subtle and easily missed. Quality of imaging is also influenced by the experience/skill of the sonographer, views obtained and equipment utilized.

Common findings in HIE include(12,13):

- diffuse increased echogenicity of the brain parenchyma, slit-like ventricles, obliteration of the extracerebral fluid spaces and the interhemispheric fissure (cerebral oedema)
- echogenicity in periventricular white matter
- echogenicity in the basal ganglia or thalami (may become prominent over time)
- cortical highlighting (injury to cortex and subcortical white matter)
- global brain injury - deep grey matter, cortical grey matter and white matter
- haemorrhage – intraventricular, intraparenchymal, convexities
- low resistance index (RI)

Using improved scanning protocols, it has been demonstrated that CUS can be used in settings where MRI is not available/accessible (see Figure 3.3) and has reasonable predictive accuracy (PPV 79-92%; NPV 79-96%) for outcome at 2 years of age (13). Major abnormalities on CUS have also been shown to predict neonatal mortality in a low resource setting (12).

**Figure 3.3: Common ultrasound findings in HIE**(13)




a Moderate cerebral oedema (1 point), b severe cerebral oedema (2 points), c moderate periventricular white matter (1 point), d severe periventricular white matter (2 points), e moderate subcortical white matter (1 point), f severe subcortical white matter (2 points), g moderate thalamus (1 point), h severe thalamus (2 points), i moderate putamen (1 point), j severe putamen (2 points), k “four-column sign” which means that both left and right thalamus and putamen are visible at the coronal view as four columns (1 point), l visibility of the PLIC (1 point).

All infants had a minimum of one, and up to three, ultrasound scans during the NICU admission. The images were assessed by experienced neonatologists (2 reviewers in retrospective cohort; single reviewer in prospective cohort) using a local consensus classification. Timing of imaging was recorded and the following findings were documented:

- presence of cysts within ventricles
- cerebral oedema
- abnormalities in basal ganglia and thalami (BGT), white matter (WM), cortex
- intra-ventricular haemorrhage;
- infarction
- other abnormalities
- the resistance index (RI) values on Doppler studies, if performed
  A RI <0.55 was considered significantly abnormal

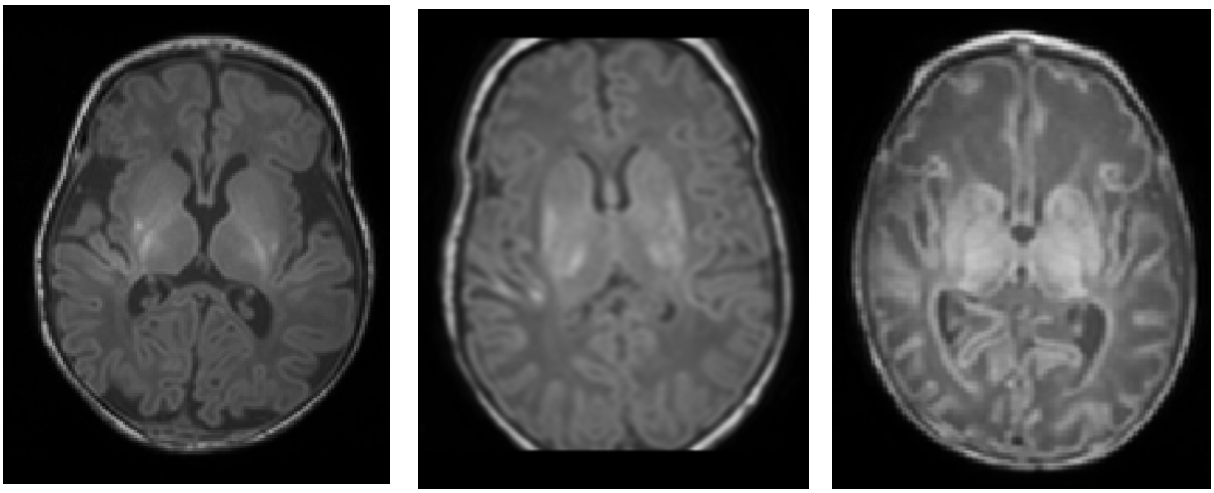
MRI

Magnetic resonance imaging (MRI) in the first weeks after birth is considered the gold standard for evaluation of the brain after hypoxic ischaemic injury. It is useful for assessing the timing, site and severity of injury, and MRI plus magnetic resonance spectroscopy (MRS) have been shown to be the best imaging predictors of long-term outcome. It can predict death and cerebral palsy; but also has the ability to provide quite specific information for carers regarding outcome, such as ability to walk or feed independently, likelihood of attending normal school, etc(14).

Different classification systems (14,15) have been used to describe injury, which usually assess the following (Figures 3.4-3.7):

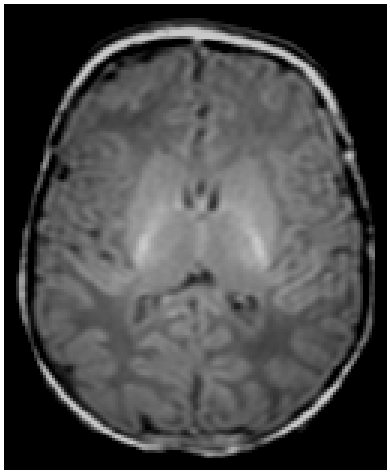
- anatomical development
- evidence of subacute/established injury
- unusual patterns of injury
- abnormal signal intensities in: BGT, WM, posterior limb of internal capsule (PLIC), cortex, brainstem, cerebellum

**Fig 3.4: Scoring system for BGT injury**

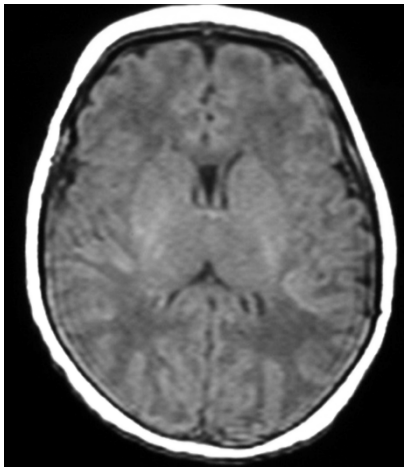


Mild BGT | Moderate BGT | Severe BGT

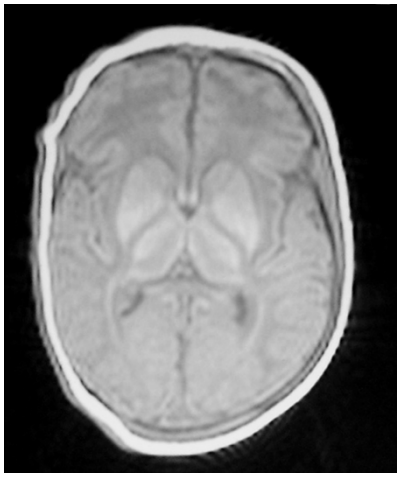
## Fig 3.5: Scoring system for the posterior limb of the internal capsule



Normal SI in the PLIC

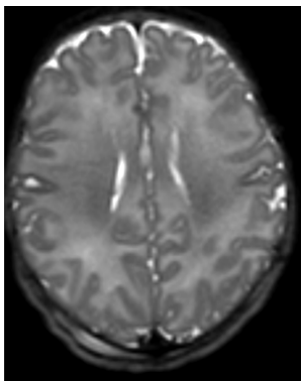


Equivocal PLIC
Asymmetrical and slightly reduced signal intensity in the PLIC

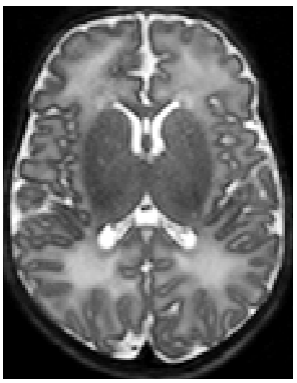


Abnormal PLIC
Absent signal intensity in the PLIC

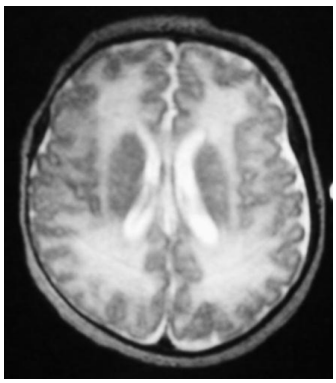
## Fig 3.6: Scoring system for white matter injury



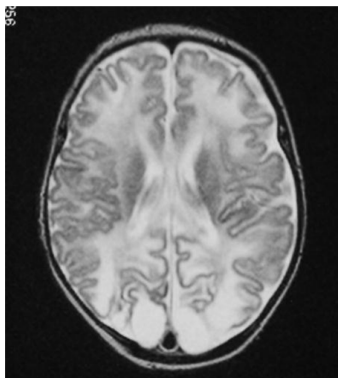
Normal WM



Mild WM

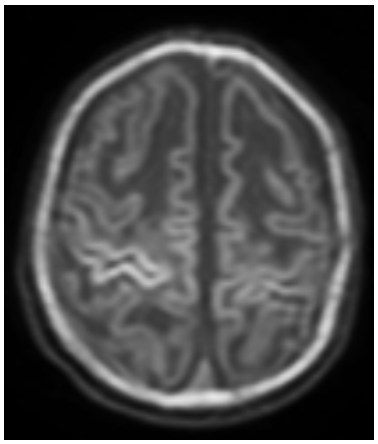


Moderate WM

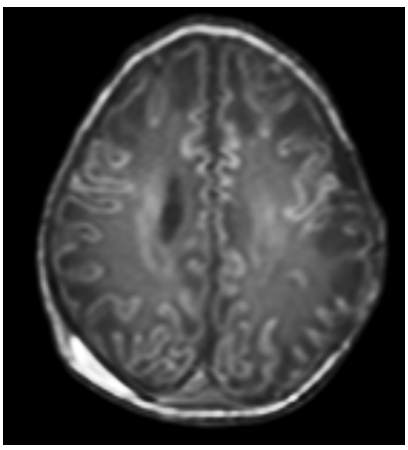


Severe WM

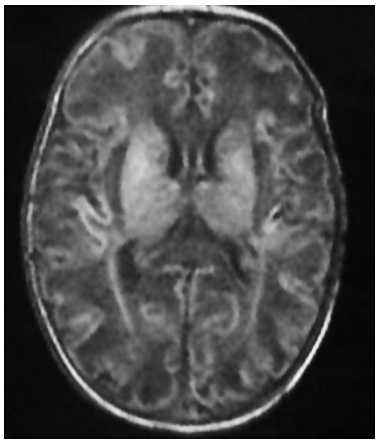
## Fig 3.7: Scoring system for cortical injury



Mild: 1-2 sites involved



Moderate: 3 sites involved



The predictive ability of MRI was found to be preserved even in the context of TH, with moderate-severe abnormalities in the BGT, severe WM lesions, abnormal PLIC having a PPV of 84% for adverse outcome at 18 months (16).

There are several limitations to the use of MRI for the indications listed above. A major challenge in most except the most well-resourced settings is limited access. In our setting, there is one scanner servicing a large tertiary hospital, which severely limited access for our infants. A further limitation was the requirement for imaging infants under general anaesthesia in our hospital. Another challenge of MRI is the need to move infants who may still be very ill to the scanner.

In our studies, due to the limited access to the scanner, MR imaging could not be performed on all infants. In the retrospective cohort, infants were initially routinely referred for MR imaging if they had received TH and were imaged when a slot was available. This protocol was later changed, and infants were referred only if there was an atypical clinical history, severe cranial ultrasound abnormalities or suggestion of a congenital or metabolic disorder. In the prospective cohort, all infants were referred for imaging and aimed to be scanned within 3 weeks of birth. In both cohorts, there was a wide range of imaging times with some infants returning for scan after discharge. Infants were imaged under general anaesthesia, which is routine at this hospital, and T1, T2 and diffusion weighted sequences were acquired. The MRI images were assessed by an experienced neuro-radiologist who was blinded to the early clinical course and later outcomes. The appearances of the BGT, posterior limb of the internal capsule, white matter, cortex and brainstem were recorded and classified as previously described (14).

**Neurological condition at discharge**

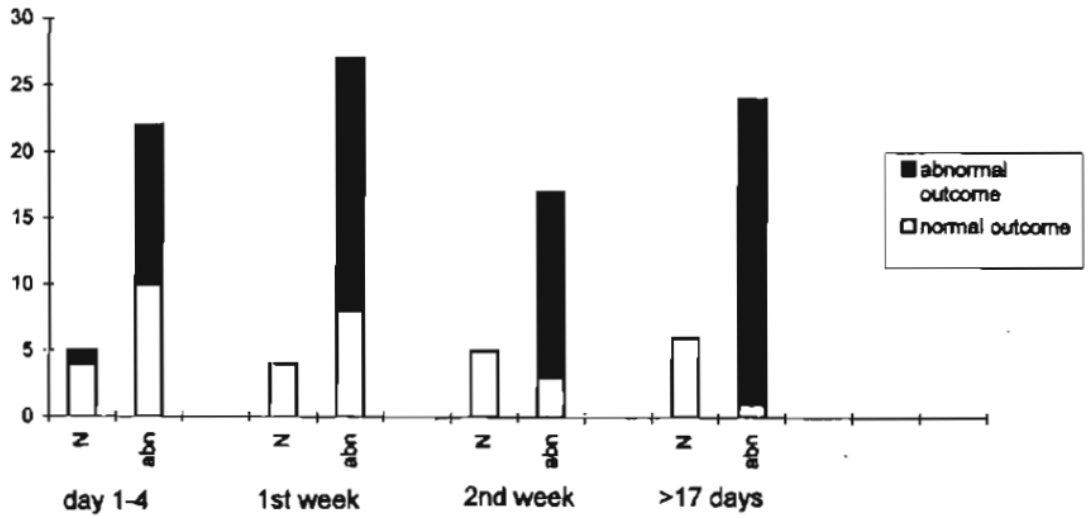
There have been studies showing that clinical neurological examination (which included assessment of tone, movement, reflexes, feeding ability) after the first week or at the time of discharge can be predictive of outcome (17,18). They showed that abnormal findings were more predictive the later in the neonatal course the examination was done (Figure 3.8). A normal examination was predictive of normal outcome at any time. Time taken to establish feeding was found to be a useful marker. These findings could be useful in settings where no imaging or neurophysiological

tools for predicting outcome are available. The studies mentioned above, however, used detailed standardized neurological assessments which were not feasible in our setting.

In our studies, we elected to evaluate 3 clinical parameters at the time of discharge and relate them to outcome:

- having a good suck (feeding independently by cup or breast)
- being still encephalopathic (abnormal level of consciousness)
- abnormal tone

**Figure 3.8: Normal and abnormal outcome in infants grouped according to the age at which the neurological examination was performed**(17)**.**




**Outcome assessment**

After discharge from hospital, infants in both cohorts were followed up at 3 months and 12 months, and up to 18 months in the prospective cohort. At these visits they had the following assessments:

- a basic neurological examination with test positions and angles as described by Amiel-Tison (19)
- the Bayley Scales of Infant and Toddler Development - Third Edition screening test (Bayley-III Screening Test) in the retrospective cohort

- the full Bayley Scales of Infant and Toddler Development -Third Edition (BSID-III) in the prospective cohort
- hearing assessments using otoacoustic emissions (OAE) and automated auditory brainstem responses (AABR) in the first three months
- visual reinforcement audiometry (VRA) at one year of age

The Amiel-Tison Neurologic Assessment at Term (ATNAT) was developed as a clinical neurological tool to examine neonates at term and shown to predict outcome (19). The test positions and angles described in the ATNAT are, however, frequently used in routine practice in follow up assessments of high risk infants even after the neonatal period, and enable detection of tone abnormalities.

The Bayley-III Screening Test can be administered in a shorter time (15-25 minutes) to determine whether an infant is developing normally or whether further comprehensive testing is needed, and can therefore be used in routine clinical practice. Both developmental tests can be administered to infants from 1-42 months, and assess 5 domains of development: cognitive, expressive and receptive language, fine and gross motor function. Composite scores are then assigned for cognitive, language and motor development. Mean scores of 100 (±15) were considered normal, and scores <70 on any of the sub-scales were considered delayed, and those <85 were considered at-risk. Although developed in children from a more developed setting, the BSID-III has recently been validated in a cohort of inner city South African children and found to be appropriate for use in this setting (20).

Hearing impairment is reported frequently (9-10%) in infants with HIE treated with TH(21). Therefore, all infants are routinely screened for hearing loss.

OAEs are used as a routine screening test for hearing loss in neonates. They assess cochlear/outer hair cell function, and can assist with confirming the type of hearing loss.

AABRs are used to determine presence and type of hearing loss, and to estimate hearing levels for individual frequencies in each ear. They are used in newborns and infants who are incapable of providing accurate information for behavioural tests

VRA is audiometry that has been adapted to assess hearing sensitivity in young children of 6 months to 3 years, where regular audiometry cannot be performed. The audiometer is reinforced with visual cues to allow the child to respond to sounds by turning the head.

General movements (GMs) are part of the movement repertoire of normally developing young infants in the first 5-6 months. When evaluated in infants with HIE, it was found that absence of fidgety movements at 3 months had a high accuracy (87%) for prediciting outcome at 18 months (22); and presence of cramped synchronised movements at 1 and 3 months correlated very strongly with early MRI central gray matter abnormalities and with motor outcome (r = 0.83) at 2 years (23).
At the 3 month visit, the infants in the prospective cohort had video recordings of their GMs made. Analysis of these recordings has not been completed.

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# CHAPTER 4

## Management of therapeutic hypothermia for neonatal hypoxic ischaemic encephalopathy in a tertiary centre in South Africa

This chapter was published in Archives of Disease in Childhood - Fetal and Neonatal Edition (Impact factor 3.258).

Kali GTJ, Martinez-Biarge M, Van Zyl J, Smith J, Rutherford M. Arch Dis Child Fetal Neonatal Ed 2015;100: F519–F523.
http://dx.doi.org/10.1136/archdischild-2015-308398

# Management of therapeutic hypothermia for neonatal hypoxic ischaemic encephalopathy in a tertiary centre in South Africa

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▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/archdischild-2015-308398).

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Received 8 February 2015
Revised 9 June 2015
Accepted 10 June 2015
Published Online First 30 June 2015

## ABSTRACT

**Aim** Therapeutic hypothermia (TH), shown in developed countries to improve outcome in infants with hypoxic-ischaemic encephalopathy (HIE), was introduced into standard care at Tygerberg Children's Hospital in 2008. We aimed to describe the management and characteristics of infants treated with TH at this tertiary centre as well as the logistical challenges encountered.

**Methods** Infants admitted for TH between 2008 and 2011 were included. They fulfilled TOBY study entry criteria and were cooled using a whole-body cooling system. A retrospective analysis of the cooling process and clinical findings was made using data collected during treatment.

**Results** 100 infants with mild (32%), moderate (45%) and severe (23%) HIE were treated over 3 years. Mean time to admission was 4.87 (±1.63) hours, median time from delivery to target temperature was 7.5 h (range 2.5–15.5 h). Mean temperature on admission was 35.5°C (±1.5°C). Overall, rectal temperature was within target temperature for 82.8% of the time. Complications noted were clinically suspected/proven infection (45%), abnormal coagulation tests (48%), thrombocytopenia (34%), need for inotropic support (17%), hypoglycaemia (4%) and hyperglycaemia (10%). Rate of follow-up at 1 year among survivors was 57%. Infants not attending 1-year follow-up were more likely to have HIV-infected mothers, but there were no other demographic or clinical differences when compared with those who attended follow-up.

**Conclusions** Cooling is feasible in a resource-limited setting, within a strict protocol. With close monitoring, the known and common complications occur as frequently as in less resource-limited settings. Surrogate markers of later outcome need to be explored where follow-up is problematic.

### What is already known on this topic

- Therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy (HIE) has been shown to be beneficial in reducing mortality and later developmental impairment.
- The majority of studies showing this benefit were done in developed countries.
- It is still a largely considered experimental therapy in developing countries.

### What this study adds

- Therapeutic hypothermia for neonatal HIE is feasible in resource-limited settings within strict protocols.
- Monitoring is essential as complications do occur.
- Long-term follow-up is a challenge.

## INTRODUCTION

Hypoxic-ischaemic encephalopathy (HIE) resulting from perinatal asphyxia is the leading cause of death in term-born infants in South Africa.[1] Therapeutic hypothermia (TH) is the only treatment that has been proven in multinational studies[2–6] to improve outcome in these infants.

In 2008, TH was introduced into standard care at Tygerberg Children's Hospital, a tertiary public hospital with an eight-bedded neonatal intensive care unit (NICU) and nurse:patient ratio of 1:2 or 1:3. However, most of the large studies showing conclusive evidence of the benefits of TH in infants with moderate or severe HIE have been carried out in developed countries, with possibly different populations and resources compared with our local setting. There are few studies from emerging or developing countries and those that have been published had small study populations and/or used varying low technology methods of cooling.[7 8] One of these trials[8] (done in a very low-resourced region) had a high mortality rate in the cooled group, which was of concern. It has been suggested by different authors and regulatory organisations[9 10] that cooling in these settings remains experimental and should only be considered in intensive care units under strictly controlled conditions.

Our aim therefore was to ascertain whether TH was directly applicable and safe in our setting and to document the complications and technical and logistical issues encountered before and after initiating TH.

## METHODS

Infants admitted consecutively to Tygerberg Children's Hospital (TCH) for TH between November 2008 and November 2011 were included. The selection criteria were based on the TOBY study entry criteria.[2] These included infants of 36 weeks gestation or greater (assessed on the best available of early ultrasound, Ballard score or foot length (unpublished TCH data)) and with a

**To cite:** Kali GTJ, Martinez-Biarge M, Van Zyl J, *et al*. *Arch Dis Child Fetal Neonatal Ed* 2015;**100**: F519–F523.

birth weight of at least 1.8 kg. The infants needed to fulfil at least one of the following: need for resuscitation for ≥10 min, Apgar score ≤7 at 10 min, pH ≤7 or base deficit ≥16 on cord gas or infant blood within an hour of birth. In addition they had to have seizures or moderate to severe encephalopathy on clinical grounds, or a Thompson HIE score of at least 10.[11] Where amplitude-integrated electroencephalogram (aEEG) was available and time permitted, a short 30 min recording was used to confirm the presence of moderate or severe encephalopathy.

Infants received whole body cooling for 72 h to a rectal temperature of 33°C –34°C, using the Tecotherm TSmed 200 N system. A retrospective analysis, with ethical approval from Stellenbosch University Human Research Ethics Committee (N10/05/157), of the cooling process and clinical findings during cooling was made using information from contemporaneous data collection proformas and from medical notes.

## RESULTS

One hundred infants were treated with TH over 3 years. Perinatal characteristics of this cohort are summarised in table 1.

Classified using the Thompson score,[11] 32% had mild, 45% moderate and 23% severe HIE; four infants were not classified because of insufficient records (table 2).

Eleven infants died in the neonatal period before discharge.

### Cooling process

Mean time to admission to NICU was 4.87±1.63 h (median 5, range 1.5–8.5 h); median time from delivery to target temperature was 7.5 h (range 2.5–15.5 h) (figure 1). Twenty-two infants were older than 6 h, and 8 older than 6.5 h at admission. Eight of the infants admitted after 6 h were born at the cooling centre.

Mean temperature on admission was 35.5±1.5°C (figure 2). Five infants had very low temperatures on admission: 29°C in one and 32°C in four. In four infants, the temperature on admission was high: 38°C in two and 39°C in two.

Average time within the target temperature range was 19.3 ±3.5 h on day 1, 19±3.7 h on day 2 and 21.4±3.5 h on day 3 (figure 3). Overall, rectal temperature was within target temperature for 82.8% of the time.

**Table 1** Perinatal characteristics

| | N=100 |
|---|---|
| Maternal characteristics | |
| Maternal age, median (range), years | 23.5 (15–46) |
| Primiparity, n (%) | 58/93 (62.3) |
| HIV positive, n (%) | 19/89 (21.3) |
| Infant characteristics | |
| Male, n (%) | 59 (59) |
| Inborn, n (%) | 33 (33) |
| Gestational age, median (range), weeks | 39 (35–43) |
| Birth weight, median (range), grams | 3060 (1960–5190) |
| Emergency Caesarean section, n (%) | 21/98 (21.4) |
| Sentinel event, n (%) (UR, abruption, cord prolapse, acute exsanguination) | 8/98 (8.1) |
| Shoulder dystocia, n (%) | 5/98 (5.1) |
| Apgar at 1 min, median (range) | 2 (0–6) |
| Apgar at 5 min, median (range) | 4.5 (0–8) |
| Apgar at 10 min, median (range) | 6 (1–10) |
| Apgar at 5 min <5, n (%) | 47/97 (48.5) |
| pH within the first hour, mean±SD | 7.03±0.15 |
| pH within the first hour, median (range) | 7.02 (6.61–7.4) |
| pH within the first hour <7.00, n (%) | 35/81 (43) |

UR, uterine rupture.

**Table 2** Clinical course and short-term outcomes

| | n (%) |
|---|---|
| HIE score* | |
| Grade 1 | 31/96 (32.3) |
| Grade 2 | 43/96 (44.7) |
| Grade 3 | 22/96 (22.9) |
| Abnormal coagulation | 37/77 (48.1) |
| Thrombocytopenia | 26/77 (33.8) |
| Inotropic support | 13/77 (16.9) |
| Suspected and confirmed infection | 45 (45) |
| Time to full oral feeds (days) | |
| Mean (SD) | 7.58 (±3.47) |
| Median (range) | 7 (3–18) |
| Strong suck at discharge | 49/66 (74.2) |
| Abnormal neurology at discharge | 11/68 (16.2) |
| Neonatal deaths | 11 (11) |

*Thompson *et al*.[11] HIE 1: ≤10, HIE 2: 11–14, HIE 3: ≥15.
HIE, hypoxic-ischaemic encephalopathy.

### Additional clinical findings

Forty-five per cent of the infants were treated for clinically suspected infection; blood and/or cerebrospinal fluid culture was positive in 8% (table 2). Coagulation test results were abnormal in 48% (14% required treatment), and 34% had thrombocytopenia. None had a clinically significant bleeding disorder. Seventeen per cent received inotropes for blood pressure support. There was no difference in complications between HIV-exposed and HIV-unexposed infants.

Other neonatal complications were transient renal (14%) and liver (18%) dysfunction, hypoglycaemia (three infants) and hyperglycaemia (eight infants, of whom three required insulin treatment).

### Outcomes

#### Short term

Eleven infants died in the neonatal period; 10 were considered to be so severely affected that care was redirected with withdrawal of intensive care and in five, care was redirected before cooling was completed. These decisions were based on a combination of HIE scores,[11] severe aEEG abnormalities and cranial ultrasound findings known to be associated with poor outcome. The eleventh patient died from Acinetobacter sepsis.

#### Long term

There was a large attrition bias with 37 infants lost to follow-up, resulting in longer-term outcome data available for 52 infants only.

Three infants died after the neonatal period and two of them from causes unrelated to asphyxia—one from an AIDS-related illness, one from gastroenteritis. The third infant's cause of death is uncertain.

Neurodevelopmental assessments between 5 and 12 months of age (median age: 12 months) were available for the remaining 49 infants. Forty-three (87.7%) were normal, or had minor abnormalities including speech and hearing impediments. Six were severely abnormal when assessed at a median age of 12 (range 12–13) months. 10 infants with initial minor abnormalities had persisting abnormalities when reassessed at 15–32 months. Four

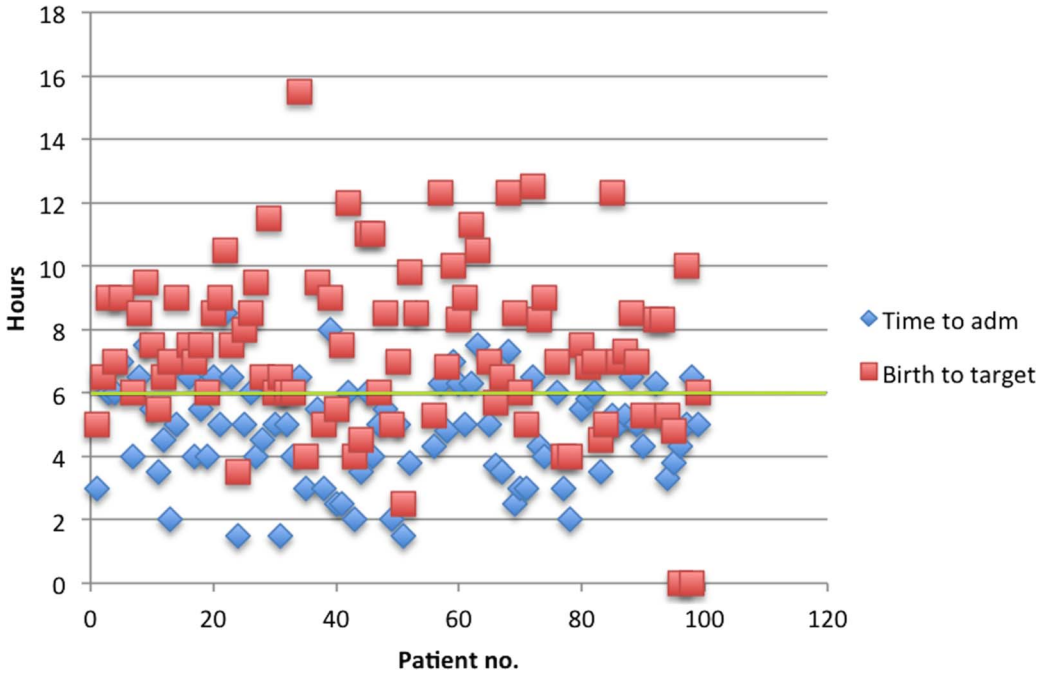


**Figure 1** Times to admission and target temperature.

had speech delay (18–32 months), three had developmental delay (18–20 months), one had mild fine motor delay (23 months) and two had autistic features (18 months). One infant with aqueduct stenosis died postoperatively at 15 months. Two of these 10 infants with further follow-up were HIV exposed: one had developmental delay at 18 months and the other had speech and mild fine motor delay at 23 months of age.

More inborn than outborn infants (50% vs 23.3%; p=0.045) had a poor outcome.

Of the five infants with excessively low admission temperatures, two were normal at 1 year, one died and two had unknown outcomes (including one with a temperature of 29°C and one with a normal MRI at 3 months).

Three of the four infants (75%) with high temperatures on admission had poor outcome: two died, one has cerebral palsy; the remaining infant's outcome is unknown.

Of the 22 infants cooled after 6 h, 10 had good outcome, 8 had poor outcome (6 died, 2 have severe cerebral palsy), and in 4 the outcome was unknown. There was no significantly increased proportion of infants cooled after 6 h in the group with poor outcome (8/20, 40%) compared with the group with favourable outcome (10/43, 23.3%; p=0.232). There was also no difference in poor outcome between inborn (3/20, 15%) and outborn infants (5/20, 25%; p=1.000) in those cooled after 6 h.

Apart from a greater proportion of HIV exposure among infants who did not attend follow-up (24.3% vs 15.9%, p=0.003), there were no significant differences in perinatal characteristics, early clinical picture (encephalopathy grade, aEEG and cranial ultrasound doppler abnormalities, and complications) and neurological condition and ability to feed at discharge, between the infants with known outcomes and those lost to follow-up (see online supplementary table). In those who were scanned (routine MRI was only available until April 2011), there were also no significant differences in the rate of abnormal MRI findings (19.2% vs 20% in known vs unknown outcome, p=1.000).

## DISCUSSION

This is the first large study to report on the practical application of TH in a developing world setting that also has information on long-term follow-up. We have found many similarities to the findings of studies from developed countries and also some

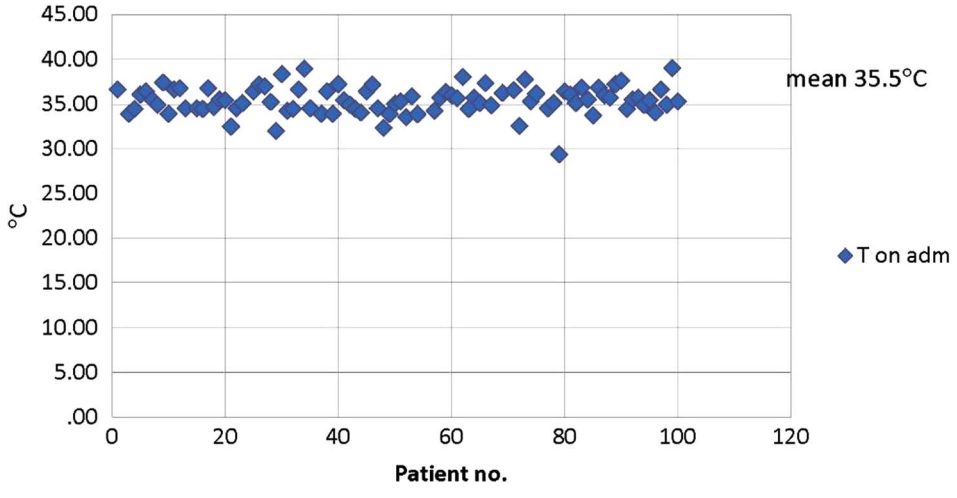


**Figure 2** Temperature on admission.

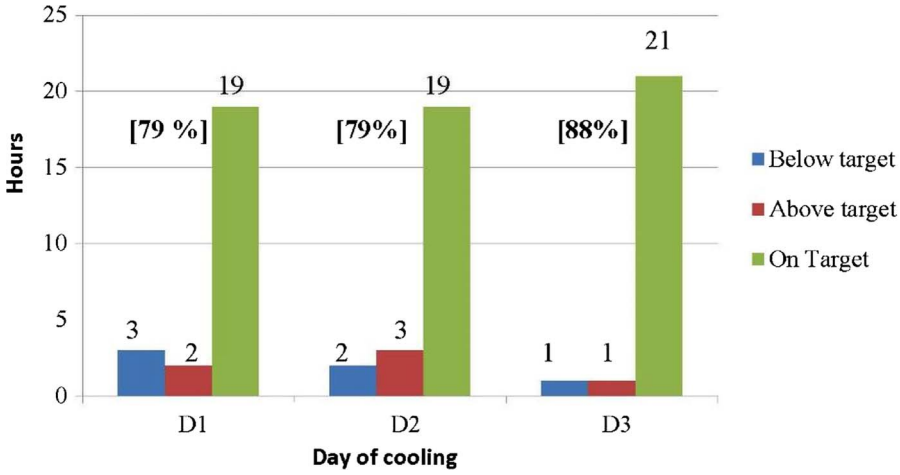


**Figure 3** Hours within and outside target temperature range.

factors that are unique to this type of setting. Our findings that the majority (77%) of cooled infants had mild or moderate encephalopathy (mild 32%, moderate 45%—table 2) are similar to those from a meta-analysis of cooling in low-income and middle-income countries.[12] This may be a reflection of the evolution of the disease in these populations or may indicate a need for more rigorous application of cooling criteria particularly where aEEG is not routinely used for selecting patients. The fact that two infants of 35 weeks gestation were cooled despite the criteria stipulating a gestation of ≥36 weeks suggests that the latter is more likely. However, this may also be related to estimation of gestational age by Ballard scoring, which may not be accurate, and clinicians' inclination to give them the benefit of doubt. In contrast to developed country settings,[13] there were also fewer ventilated patients and a greater proportion treated for infection. The low number of ventilated patients was despite cooling being applied in an NICU setting with ventilation facilities and is therefore a true reflection of the infants' degree of illness and not of resource limitations.

Effort was made to get infants to the cooling centre within 6 h of birth in order to start TH. However, since there is a limited dedicated neonatal transport service in the region and a wide geographical drainage area, cooling was still offered if the infants were received within 8 h. This decision was based on one animal study[14] showing effectiveness of cooling, although diminished with increasing time, up to 8.5 h after injury. After noting that it was largely possible to commence cooling within the recommended time in this population, the 6 h cut-off was enforced more strictly. The observed 4.87 h to commencement of cooling was similar to the 4.5 h noted in international studies.[15] The minimum time to commence cooling was 1.5 h. More importantly, 15 infants had reached the target temperature range by 6 h and 5 of them in 4 h or less. This is relevant because there was a trend towards better outcomes in infants cooled within 4 h of birth in one of the large cooling studies.[16]

Although there was no significant difference in outcome between inborn and outborn infants in those cooled after 6 h, this however did not take into account the four infants with no outcome data. Of great concern were the eight infants born in the cooling centre who commenced cooling after 6 h. This was likely due to delayed recognition of the infants' clinical condition and indications for cooling and illustrates the need for ongoing education.

Local ambulance services are not equipped with rectal probes or low reading thermometers, and staff are not trained to monitor and respond to changes in cooled infant temperatures appropriately during transit. Hence, the advice given to referring units and transport services is to maintain the infants' skin temperature at 36°C until arrival at the cooling centre. The average admission temperature of 35.5°C was similar to that found in the neo.nEuro.network Trial.[6] Fifty-five per cent of the infants had a temperature below 36°C on arrival, and alarmingly 5% had very low temperature of 32°C or less. This could be due to referring units' enthusiasm to start cooling within the therapeutic window, or a reflection of the illness severity, as it has been shown that severely encephalopathic infants have a greater propensity to drop their temperatures.[17] It does, however, highlight the dangers of uncontrolled cooling during transport. Although there have been some studies showing no changes in physiological data or increases in complications in infants cooled to temperatures as low as 31°C–32°C,[16 18] other authors do caution about the dangers of excessive cooling even if being monitored.[17 19] There is also concern that the usual response to overcooling is to rewarm the infant to target range resulting in fluctuating brain temperatures, the implications of which are unknown and might actually compromise the protective effects of hypothermia. In this cohort, of the five infants with excessively low admission temperatures two were normal at 1 year, one died and two had unknown outcomes (including one with a temperature of 29°C and one with a temperature of 32.4°C who had a normal brain MRI at 3 months).

It has been shown in the large hypothermia trials[3 5 6] that temperatures of ≥38°C are associated with unfavourable outcomes. In this cohort, 75% (3/4) of the infants with very high temperatures on admission had a poor outcome: two died, one has cerebral palsy, and the remaining infant's outcome is unknown.

Once cooling was started, the overall maintenance of temperature within target range of 83% was not significantly different from the 81% previously reported in other settings using a similar cooling method.[20] The same study demonstrated that manually-controlled cooling systems are associated with greater temperature variations when compared with servo-controlled systems. These variations, particularly in the initiation phase of cooling and the time taken to reach target temperature (figure 1), were noted in the current study and are likely to be increased in a setting where there is inadequate staffing.

The majority of laboratory abnormalities and complications were transient and recovered after the cooling period, as described in the literature,[21 22] except in four infants with other complications such as necrotising enterocolitis (NEC) and septicaemia. One infant developed NEC and had thrombocytopenia and coagulation abnormalities after the cooling period. Three

other infants had abnormal platelet and coagulation studies associated with infection, one of whom started during cooling and subsequently died after withdrawal of care due to severe infection on day 6. Importantly, there was no significantly increased proportion of infants with infection in those with poor outcome.

The role of HIV exposure was a concern in this setting with a high HIV prevalence. Mothers and infants would have been on the current national Prevention of Mother-to-Child Transmission (PMTCT) programme (which changed during the study period), depending on when they presented for care and mother's disease stage. We found more HIV-exposed infants in the group lost to follow-up; however, information on maternal and infant treatment was not systematically collected in this study. This is a limitation as this information might have given some indication of whether infants at higher risk of perinatal transmission (no or inadequate prophylaxis) had a different clinical presentation, complications such as NEC, and outcome. An association has been reported between NEC and maternal HIV infection and antiretroviral drugs.[23 24] Despite this, we were able to ascertain that apart from younger maternal age and more primiparous mothers in the unexposed group, there were no significant differences between HIV-exposed and HIV-unexposed infants regarding perinatal characteristics, severity of illness, complications and MRI abnormalities. The one infant diagnosed with NEC was not exposed to HIV.

## CONCLUSIONS

This study has shown that it is feasible to apply TH safely in resource-limited conditions, using similar technology and protocols to those used in the large published trials. It is also safe in a population with high HIV exposure and often poor availability of antenatal information. However, it should only be applied where adequate monitoring and support are possible as the well-described complications of cooling do occur, although not more commonly than in published trials. The extreme temperature fluctuations before and in the early phase of cooling are concerning, and suggest that automated systems of cooling might be more suited to this environment, to ensure better maintenance of temperatures within target range, but cost is a limiting factor.

There is a need for ongoing training in order to ensure early recognition and correct assessment and management of infants with HIE who will benefit from early cooling. This training needs to ultimately include referring, transport and treating teams.

Where follow-up is problematic, surrogate markers of outcome such as neurological examination at discharge should be explored.

**Acknowledgements** We are grateful to the neonatal medical and nursing staff at TCH who looked after the patients and assisted in collecting the data.

**Contributors** GTJK is the main author, as well as corresponding author of the manuscript. The other authors contributed as follows: MM-B—assisted with data collection and analysis and with reviewing and editing the manuscript; JVZ—did the follow-up neurodevelopmental assessments on all the infants; JS—assisted with conceptualising the study and reviewing the manuscript; MR—assisted with reviewing and editing the manuscript.

**Funding** The study was funded in part by the Stellenbosch University Research Travel Grant.

**Competing interests** None declared.

**Ethics approval** Stellenbosch University Human Research Ethics Committee (N10/05/157).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** A more detailed description of the clinical course and outcomes of the infants is described in another paper in preparation for publication, entitled: "Therapeutic Hypothermia For Neonatal Hypoxic-Ischaemic Encephalopathy At A Referral Hospital In A Middle Income Country".

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# Management of therapeutic hypothermia for neonatal hypoxic ischaemic encephalopathy in a tertiary centre in South Africa

Gugulabatembunamahlubi Tenjiwe Jabulile Kali, Miriam Martinez-Biarge, Jeanetta Van Zyl, Johan Smith and Mary Rutherford

*Arch Dis Child Fetal Neonatal Ed* 2015 100: F519-F523 originally published online June 30, 2015
doi: 10.1136/archdischild-2015-308398

# CHAPTER 5

## Therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy had favourable outcomes at a referral hospital in a middle-income country

This chapter was published in Acta Paediatrica (Impact factor 2.265).

Kali GT, Martinez-Biarge M, Van Zyl J, Smith J, Rutherford M. Therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy had favourable outcomes at a referral hospital in a middle-income country. Acta Paediatr. 2016 Jul;105(7):806-15. doi: 10.1111/apa.13392. Epub 2016 Apr 22. PMID: 26945474.

https://pubmed.ncbi.nlm.nih.gov/26945474/

ACTA PÆDIATRICA
NURTURING THE CHILD

Acta Pædiatrica ISSN 0803-5253

REGULAR ARTICLE

# Therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy had favourable outcomes at a referral hospital in a middle-income country

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**Keywords**
Middle-income country, Neonatal encephalopathy, Outcome, Predictors, Therapeutic hypothermia

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**Received**
11 September 2015; revised 3 December 2015; accepted 2 March 2016.

DOI:10.1111/apa.13392

## ABSTRACT

**Aim:** This South African study documented the survival and neurodevelopmental outcomes of infants with hypoxic-ischaemic encephalopathy (HIE) after introducing cooling to a neonatal intensive care unit and identified early markers for neurodevelopmental outcome.

**Methods:** We retrospectively reviewed infants that received cooling according to the Total Body Hypothermia trial protocol from 2008 to 2011. Infants were screened with the Bayley Scales of Infant and Toddler Development, Third Edition, at one year of age and underwent neurological and hearing assessments.

**Results:** Data on 99 infants with HIE showed that 45% of cases were moderate, 23% severe and 32% mild. An abnormal amplitude integrated electro-encephalogram (aEEG) background was documented in 45 cases within 24 hours. Magnetic resonance imaging (MRI) scans were consistent with HIE in all but one case. We reviewed 50 traceable survivors at one year. Development was significantly impaired in nine and 41 were normal or mildly impaired. A severely abnormal aEEG background, severe HIE and an abnormal MRI were associated with death and severe impairment. A good suck, mild HIE, primiparity and normal MRI were associated with good outcomes.

**Conclusion:** Most infants with HIE survived without major impairment. Previously described predictors of neurodevelopmental outcome were good surrogate markers in this population.

## INTRODUCTION

Birth asphyxia results in significant morbidity and is the third most common (23%) global cause of death in the neonatal period (1). Although it has not been well documented in sub-Saharan Africa, two studies (2,3) reported an incidence of hypoxic-ischaemic encephalopathy (HIE) resulting from birth asphyxia that ranged between 3.8 and 8.3 per 1000 live births in two different regions in South Africa.

Therapeutic hypothermia (TH) to treat HIE infants was introduced into standard care at Tygerberg Children's Hospital in Cape Town, South Africa, in 2008 following the publication of positive results from international studies (4,5). Although there were concerns about safety and applicability arising from small studies using low technology methods from developing countries (6,7), we recently demonstrated that it is feasible and safe to apply TH in resource-limited settings using similar technology and protocols as in developed countries, provided there is adequate monitoring and support. In our cohort of 100 neonates

**Abbreviations**
aEEG, Amplitude integrated electro-encephalogram; BGT, Basal ganglia thalami; EEG, Electro-encephalogram; HIE, Hypoxic-ischaemic encephalopathy; HIV, Human immunodeficiency virus; MRI, Magnetic resonance imaging; NICU, Neonatal intensive care unit; NPV, Negative predictive value; PPV, Positive predictive value; TH, Therapeutic hypothermia; TOBY, Total body hypothermia trial.

**Key notes**
- This South African study documented the survival and neurodevelopmental outcomes of 99 infants with hypoxic-ischaemic encephalopathy (HIE) after introducing cooling to a neonatal intensive care unit.
- Many infants survived without major impairment.
- Severe hypoxic-ischaemic encephalopathy, severe amplitude integrated electro-encephalogram background and abnormal magnetic resonance imaging (MRI) findings were associated with poor outcome, while mild HIE, primiparity, good suck at discharge and normal MRI were associated with favourable outcome.

treated over a three-year period, complications from cooling occurred at a similar rate as in published trials (8).

This study aimed to document the clinical course and outcomes of infants treated with TH at Tygerberg Children's Hospital and to assess which neurological investigations could be used as surrogate markers for neurodevelopmental outcomes at one year of age.

## METHODS

### Setting

Tygerberg Children's Hospital is a tertiary public referral hospital in Cape Town, South Africa, with 124 neonatal beds and 6103 annual deliveries. It serves the eastern metropolitan region of Cape Town, as well as a large rural Western Cape catchment area with 46 150 annual deliveries in 2011.

### Patient selection and management

We included all infants admitted for therapeutic hypothermia to the neonatal intensive care unit (NICU), from Tygerberg Children's Hospital and its referring centres, between November 2008 and November 2011. These infants constituted 8.2% of total NICU admissions for the study period. Their suitability for TH was determined according to the Total Body Hypothermia (TOBY) study-based criteria (9): a gestational age of ≥36 weeks and birth weight ≥1.8 kg; the need for resuscitation for ≥10 minutes and, or, an Apgar score ≤7 at 10 minutes and, or, a pH of ≤7 or base deficit ≥16 on cord gas or infant blood within an hour of birth. In addition, these clinical features had to be present: seizures or moderate to severe encephalopathy or a Thompson score ≥10 (10).

Infants were cooled for 72 hours to a rectal temperature of 33–34°C using the Tecotherm TSmed 200 N (Inspiration Healthcare Ltd, Leicestershire, UK) whole-body system.

All infants had cranial ultrasound imaging performed at least once during their NICU admission and had amplitude integrated electro-encephalogram (aEEG) monitoring where available during cooling, using the single channel Olympic CFM 6000 (Natus Medical Incorporated, San Carlos, CA, USA) or 2-channel Brainz BRM2 (Natus Medical Incorporated) devices. Some infants had full electro-encephalogram (EEG) examinations, at the attending clinicians' discretion.

Between November 2008 and April 2011, all cooled infants were routinely referred for magnetic resonance imaging (MRI) and were imaged when a slot was available. After this time, due to limited access to the scanner, infants were only referred for an MRI if there was an atypical clinical history, severe cranial ultrasound abnormalities or suggestion of a congenital or metabolic disorder. All infants were imaged under general anaesthesia, which was a routine practice at the hospital, using a Siemens Magnetom Symphony 1.5 Tesla magnet (Siemens Healthcare, Erlangen, Germany) with T1, T2 and diffusion weighted sequences.

Neurodevelopmental assessments were performed at three months and at one year by an experienced developmental paediatrician using the Bayley Scales of Infant and Toddler Development, Third Edition screening test (Bayley-III) and a basic neurological examination that included the test positions and angles described by Amiel-Tison (11). We also performed hearing assessments, in the form of oto-acoustic emissions and automated auditory brainstem responses within the first three months and visual reinforcement audiometry at one year. Non-attending caregivers were contacted by telephone and asked whether the child was still alive and if the infant had died this was included in the results.

### Data collection and analysis

Clinical information was collated from contemporaneous data collection proformas and medical notes. The infant's HIE severity was classified based on the peak Thompson score in the first week: mild ≤ 10, moderate 11–14 and severe ≥15 (10). Cranial ultrasound and aEEG recordings were individually reviewed by two experienced neonatologists.

Cranial ultrasound images were assessed using a consensus classification system for: day imaged; evidence of cysts within ventricles (sub-ependymal, choroid plexus); cerebral oedema; abnormalities in basal ganglia and thalami (BGT), white matter, cortex; intra-ventricular haemorrhage; infarction and other abnormalities. The results of Doppler studies, if performed, and the value of the resistance index were documented.

The aEEG recordings were reviewed using a pre-agreed combination of voltage and pattern recognition classifications. Recordings were divided into 12-hour time periods and analysed for predominant background pattern, presence of normal sleep–wake cycling as well as the presence and burden of seizure activity. High seizure burden was ascribed when there were more than three seizures within an hour or a single seizure lasted more than 30 minutes.

The number of hours it took to recover from abnormal background patterns were noted, as well as the time to develop sleep–wake cycling and the total duration of recordings.

Poor quality data that could not be fully interpreted were excluded from further analysis. EEG recordings were analysed and reported by paediatric neurologists. They also documented the age when they were performed, the background pattern and presence of seizure activity.

The MRI scans were assessed by an experienced neuroradiologist who was blinded to the clinical course and outcomes. The appearances of the BGT, posterior limb of the internal capsule, white matter and cortex were recorded and classified as previously published (12).

The study was approved by the Human Research Ethics Committee of Stellenbosch University (Ref. No. N10/05/157).

### Statistical analysis

GraphPad software (GraphPad Software Incorporated, La Jolla, CA, USA) was used for statistical analysis. Fisher's exact two-tailed test was used to compare categorical data and the t-test was used to compare means of continuous

data. The Bonferroni method was used to correct for multiple comparisons. StatPlus was used to test for the normality of continuous variables.

## RESULTS

Data were available on 99 of the 100 treated infants and the main demographic and perinatal data are shown in Table 1.

### Neonatal clinical course

Using the Thompson score, encephalopathy was classified as mild in 32% of infants, moderate in 45% and severe in 23%. There were three infants who were not classified due to missing data. Table 2 shows the main clinical course and complications.

Of the 48% infants with abnormal coagulation, 14% were treated with vitamin K and fresh frozen plasma, although none had severe bleeding.

Most infants (91%) received some sedative or anti-convulsant medication: 45% received just phenobarbitone and the remainder received phenobarbitone plus one or two other anti-convulsants – midazolam and lignocaine infusions – for refractory seizures.

The majority of infants received total parenteral nutrition during cooling and commenced enteral feeding after this. The average time taken to full enteral feeds was 7.5 (±3.5) days, with 65% of the infants receiving cup feeds – the routine method for feeding infants who are not breastfeeding but have a safe swallowing mechanism in this institution – 26% receiving breastfeeding and 9% receiving tube feeding.

The median age at discharge (66%) or transfer to another facility (34%) was nine (range 5–46) days. By this time, 88% of infants were receiving some breastfeeding, 8.7% were on cup feeding and 3% were receiving tube feeding.

Some infants still had abnormal levels of consciousness (16%) and tone abnormalities (27%) at discharge or transfer, including one who was still tube-fed when he died on day 30.

**Table 1** Perinatal characteristics

| | n = 99 |
|---|---|
| Maternal characteristics | |
| Maternal age, median (range), years | 23.5 (15–46) |
| Primiparity, n (%) | 58/93 (62.3) |
| HIV positive, n (%) | 19/89 (21.3) |
| Infant characteristics | |
| Male, n (%) | 59 (59) |
| Inborn, n (%) | 33 (33) |
| Gestational age, median (range) weeks | 39 (35–43) |
| Birthweight, median (range) grams | 3060 (1960–5190) |
| Emergency Caesarean section, n (%) | 21/98 (21.4) |
| Sentinel event*, n (%) | 8/98 (8.1) |
| Shoulder dystocia, n (%) | 5/98 (5.1) |
| Apgar at one minute, median (range) | 2 (0–6) |
| Apgar at five minutes, median (range) | 4.5 (0–8) |
| Apgar at 10 minutes, median (range) | 6 (1–10) |
| Apgar at five minutes <5, n (%) | 47/97 (48.5) |
| pH within the first hour, mean ± SD | 7.03 ± 0.15 |
| pH within the first hour, median (range) | 7.02 (6.61–7.4) |
| pH within the first hour <7.00, n (%) | 35/81 (43) |

*Sentinel event = uterine rupture, abruptio placentae, cord prolapse, acute exsanguination.

**Table 2** Clinical course and complications

| | n (%) |
|---|---|
| HIE grade* | |
| 1 | 31/97 (31.9) |
| 2 | 44/97 (45.3) |
| 3 | 22/97 (22.6) |
| Ventilated | 34 (34) |
| Duration, days and mean (SD) | 1.2 (±1.9) |
| MAS/PPHN | 7 (7) |
| Surfactant lavage | 3 (3) |
| Abnormal coagulation | 37/77 (48.1) |
| Thrombocytopaenia | 26/77 (33.8) |
| Inotropic support | 13/77 (16.9) |
| Infection (suspected and confirmed) | 45 (45) |
| Culture-positive (blood or CSF) | 8 (8) |
| NEC | 1 (1) |
| Renal dysfunction | 11/77 (14) |
| Liver dysfunction | 14/77 (18) |
| Hypoglycaemia | 3 (3) |
| Hyperglycaemia | 8 (8) |
| Insulin | 3 (3) |
| Strong suck at discharge | 49/66 (74.2) |
| Encephalopathic at discharge | 11/68 (16.2) |
| Abnormal tone at discharge | 17/62 (27.4) |
| Neonatal deaths | 14 (14%) |

*HIE 1: ≤10, HIE 2: 11–14, HIE 3: ≥15 [Thompson et al. (10)].
MAS = Meconium aspiration syndrome; NEC = Necrotising enterocolitis; PPHN = Persistent pulmonary hypertension of the newborn.

### Monitoring and imaging

Of the 78 aEEG recordings that were available, four were unsuitable for assessment (Table 3). The median duration of recordings was 73 hours (range 5–168). Within the first 24 hours, 36 showed infants showed severely abnormal background patterns and nine showed moderately abnormal background patterns, with 18 recovering within 48 hours of birth but 13 showing no recovery during the period of recording. Only eight infants had normal sleep–wake cycling during the time of recording. Some seizure activity was seen in 72% of recordings and 46% (26/56) of them had a high seizure burden.

Formal EEGs were performed in 30 infants at a median age of five days (range 1–12). Severely abnormal background patterns were seen in 22, with seizure activity in three. Of these infants, 11 had not had previous aEEG recordings and eight had displayed similar abnormalities in earlier aEEGs.

At least one cranial ultrasound was performed in 81 infants (Table 4) and the median age at the first scan was one day (range 1–5). The predominant findings are

**Table 3** Neurophysiological monitoring

| | |
|---|---|
| aEEG | n = 74 |
| Background in first 24 hours | |
| Normal/mildly abnormal | 29 |
| Moderately abnormal | 9 |
| Severely abnormal | 36 |
| Recovery* | |
| <48 hours | 18 |
| 48–72 hours | 3 |
| No recovery | 13 |
| Seizures | 56 |
| High seizure burden | 26 |
| Sleep–wake cycling (total) | 8 |
| <24 hours | 3 |
| 24–48 hours | 3 |
| >48 hours | 1 |
| >72 hours | 1 |
| EEG | n = 30 |
| Age performed in days, median (range) | 5 (1–12) |
| Normal/CNV | 2 |
| DNV | 1 |
| DLV | 3 |
| Generalised low voltage (2 with occasional bursts) | 20 |
| Burst suppression | 2 |
| Seizures | 3 |

*11 infants not monitored full 72 hours, recovery could not be assessed.
CNV = Continuous normal voltage, DLV = Discontinuous low voltage; DNV = Discontinuous normal voltage.

**Table 4** Imaging

| | |
|---|---|
| Cranial ultrasounds – number, day imaged and abnormalities | |
| 1 scan – median day 1 (1–5) | 81 |
| 2 scans – median day 4 (2–11) | 29 |
| 3 scans – median day 4 (3–4) | 5 |
| Cysts (subependymal/choroid plexus) | 5 |
| Cerebral oedema | 28 |
| BGT | 24 |
| WM/cortex | 43 |
| IVH | 6 |
| Infarct | 1 |
| Other | 4 |
| Doppler, total n (1st, 2nd, 3rd) | 91 (67, 22, 2) |
| RI < 0.55, total n (1st, 2nd, 3rd) | 8 (5, 3, 0) |
| MRI brain – abnormalities | n = 35 |
| Age at scan in days, median (range) | 115 (4–150) |
| BGT + PLIC | 6 |
| Thalamus only | 1 |
| White matter | 7 |
| Severe with overt infarction | 4 |
| Mild-moderate | 3 |
| Reduced WM volume | 4 |
| WM + PLIC and/or BGT | 5 |
| WM + cortex | 4 |
| Haemorrhage or thrombosis | 2 |
| Cerebellum | 2 |

BGT = Basal ganglia thalami; IVH = Intraventricular haemorrhage; PLIC = Posterior limb of internal capsule; RI = Resistance index; WM = White matter.

described in Table 4. The range of BGT abnormalities included hypodense caudate nucleus, mixed echogenicity in the BGT, increased echogenicity in the thalamus and some striatal vasculopathy. There were 91 Doppler studies carried out at a median age of one day (range 1–5) and eight had a significantly abnormal resistance index of <0.55.

Brain MRI was performed on 35 infants born during the routine referral period until April 2011, at a median age of 115 days (range 4–150) and seven infants were scanned within six weeks of age. As shown in Table 4, findings associated with a good outcome were documented in 29 infants and those associated with a poor outcome in six.

One infant with mild ventricular dilatation at five months died at 15 months after surgery for aqueduct stenosis. There were no other congenital anomalies or other disorders diagnosed using MRI scans.

## Outcomes

Outcome information was available on 67 infants and 33 infants were lost to follow-up. There were no significant differences in perinatal characteristics, early clinical conditions and neurological status at discharge between the infants with known and unknown outcomes, except for a significantly increased number of infants exposed to the human immunodeficiency virus (HIV) who did not attend the follow-up. One of these infants was infected and was on antiretroviral treatment at three months. There was also no significant difference in MRI findings between infants with known or unknown outcomes ($p = 1.000$). There was no outcome information on 10 (28.6%) of the 35 infants who had undergone MRI scans. Findings associated with a good outcome were recorded in eight of the 10 infants and with a poor outcome in two.

Of the 17 infant deaths, 14 occurred in the neonatal period and 13 of these followed the withdrawal of intensive care because of the severity of their illness, based on a combination of clinical, aEEG and cranial ultrasound findings. The fourteenth infant with moderate HIE died from gram-negative septicaemia. Of the three who died later, one died from acquired immune deficiency syndrome, another from gastroenteritis and the cause of death of the third infant was uncertain.

Of the 50 infants assessed at a median age of 12 months (range 5–12), 41 (82%) had normal neurodevelopment or had minor abnormalities, such as speech or hearing impairment, and nine (18%) had severe neurodevelopmental impairment. Further assessments were planned on 10 infants with minor abnormalities at one year and three had normal results at the age of three, three had minor speech delays at 24–35 months, one had autistic features at 18 months and three did not attend further follow ups.

An infant that had normal results at 12 months had autistic spectrum disorder at a further follow-up and another with aqueduct stenosis died after surgery at 15 months. HIV exposure was documented in two infants who were later followed up. One initially had a negative HIV result, as measured by polymerase chain reaction, and walked late at 18 months. The other had mild speech delay,

which was normal at three years, and was later confirmed to be negative for HIV.

### Predictors of outcomes

At discharge at a median age of nine days (range 5–46), 49 infants had a strong coordinated suck that was sufficient to feed adequately. Outcomes were available in 33 of them and 29 (87.9%) had a good outcome. Of the four (12.1%) infants with a good suck but a poor outcome, two had MRI abnormalities – BGT, posterior limb of internal capsule, white matter, cortex, pons and sinus thrombosis – and one had aEEG findings associated with a poor outcome. Cerebral palsy was diagnosed in three survivors. The fourth infant did not have any early poor prognostic indicators and the cause of death was unclear.

The outcome was known in 10 of the 19 infants with a poor or absent suck at discharge at a median age of 12 days (range 5–46). The outcome was poor in four (40%) and good in six (60%). The MRI results, carried out between seven and 145 days, were normal in three infants with a poor suck but good outcome and three had no MRIs but had abnormal aEEG/EEGs at two to six days.

Factors that were significantly associated with poor outcome were HIE grade three (p = 0.0001), severe aEEG

**Table 5** Comparison of outcomes

| | Poor outcome (n = 26) (Died/severe neurodevelopmental abnormality) n (%) | Favourable outcome (n = 41) (Normal/mild abnormality) n (%) | p |
|---|---|---|---|
| Maternal data | | | |
| Age | | | |
| Mean (SD) | 26.95 (7.91) | 24.95 (7.31) | 0.333 |
| Median (range) | 27 (17–43) | 22 (17–46) | |
| Primigravida – n (%) | 9 (25) | 30 (73.2) | **0.003** |
| HIV-infected – n (%) | 5 (19.2) | 5 (12.2) | 0.493 |
| Delivery at TBH – n (%) | 10 (38.5) | 10 (24.4) | 0.277 |
| EMCS | 6 (23.1) | 6 (14.6) | 0.515 |
| Infant data | | | |
| Birth weight (g) | | | |
| Mean (SD) | 3093 (546) | 3174 (678) | 0.670 |
| Median (range) | 3045 (2100–4657) | 3070 (1960–5190) | |
| Female | 15 (57.7) | 12 (29.3) | **0.025** |
| Gestation* – n (%) | | | |
| Pre-term | 4 (15.4) | 6 (14.6) | 1.000 |
| Term | 18 (69.2) | 26 (63.4) | 0.793 |
| Post-term | 4 (15.4) | 8 (19.5) | 0.753 |
| 10 minute Apgar <7 – n (%) | 13 (50) | 15 (36.6) | 0.317 |
| ph† <7 – n (%) | 13 (50) | 11 (26.8) | 0.070 |
| HIE‡ | | | |
| Grade 1 | 4 (15.4) | 21 (51.2) | **0.004** |
| Grade 2 | 8 (30.8) | 16 (39.0) | 0.604 |
| Grade 3 | 14 (53.8) | 4 (9.8) | **0.0001** |
| Abnormal Doppler on CrUS (RI < 0.55) | 2/19 (10.5) | 3/46 (6.5) | 0.625 |
| aEEG Severe abnormality | 17 (65.4) | 6 (14.6) | **0.0001** |
| Recovery <48 hours (from severe background) | 3/17 (17.6) | 4/6 (66.7) | **0.045** |
| High seizure burden§ | 11 (42.3) | 8 (19.5) | 0.055 |
| Abnormal MRI | 4/5 (80) | 1/20 (5) | **0.002** |
| Abnormal coagulation | 9 (34.6) | 12 (29.3) | 0.788 |
| Thrombocytopaenia | 9 (34.6) | 6 (14.6) | 0.074 |
| Inotropic support | 8 (30.8) | 2 (4.9) | **0.010** |
| Suspected and confirmed infection | 15 (57.7) | 14 (34.1) | 0.078 |
| Good suck | 4 (15.4) | 29 (70.7) | **0.0001** |
| Encephalopathic at discharge/transfer | 5 (19.2) | 0 (0) | **0.007** |
| Abnormal tone at discharge/transfer | 8 (30.8) | 2 (4.9) | **0.010** |

*Pre-term: <37 weeks, term: 37–40 weeks, post-term >40 weeks.
†pH within one hour of birth.
‡HIE 1: ≤10, HIE 2: 11–14, HIE 3: ≥15 [Thompson et al. (10)].
§Electrical seizures on aEEG.
Denominators = total assessed.
p-values of variables where there was a significant difference in outcomes in bold.

background pattern (p = 0.0001), abnormal MRI findings (p = 0.002), being encephalopathic at discharge (0.007). Other factors that were associated with poor outcome to a lesser degree were requiring inotropic support (p = 0.010), abnormal tone at discharge (p = 0.010), being female (p = 0.025) and no recovery from severe aEEG within 48 hours (p = 0.045). Having a good suck at discharge (p = 0.0001), primiparity (p = 0.003) and mild HIE (p = 0.004) were associated with a favourable outcome (Table 5). After correcting for multiple comparisons, only HIE grade three, severe aEEG background and an abnormal MRI were significantly associated with poor outcome.

A normal MRI had a positive predictive value (PPV) of 95%, with a 95% confidence interval (CI) of 76.18–99.88 for good outcome. Severe aEEG and poor suck at discharge, at a median age of nine days, had a PPV of 75% (95% CI 20.34–95.88) for poor outcome.

## DISCUSSION

We have described a cohort of HIE neonates cooled as part of their standard clinical care in a middle-income setting in South Africa. We have previously shown that cooling is feasible and safe in such a setting (8). The perinatal details of our cohort were similar to published cohorts (13,14) with respect to gestational age, birth weight, proportions of male infants and out-born infants. The differences were the younger mothers (23 vs 30 years) and a lower Caesarean section rate in this cohort (21% vs 56.5–69%) (4,14). The Caesarean section rate in HIE infants was similar to the general rate of 22.6% at this hospital. There was no increased incidence of complications associated with either cooling or HIE (15) when compared to published data (Table 6).

Of the whole cohort, 17% infants died, 41% showed normal development or had mild abnormalities and 9% had severe impairment at one year of age. Of the 10 infants whose development was not entirely normal at one year of age, three showed normal development, three had minor speech abnormalities, one was severely abnormal on the autistic spectrum and three did not attend further follow up. Another infant that showed normal development at 12 months was later diagnosed as autistic.

The severity of encephalopathy was assessed using the peak and duration of the abnormal Thompson HIE score (10), which is routinely used in this institution. Thompson reported a PPV of 92% and negative predictive value (NPV) of 82% for poor outcome at one year, with a peak HIE score of 15, indicating severe HIE, in the pre-cooling period. Using the same classification for severity, we also found a correlation between severe HIE and poor outcome, namely death or severe neurodevelopmental abnormality at one year, although with lower predictive values – 73.3% for PPV and 81.3% for NPV – which might be accounted for by the incomplete outcome data and the previously described effect of TH on clinical evaluation (16).

Severe aEEG background activity and no recovery by 48 hours were associated with poor outcome. Artefacts were found in one-third of the available recordings, making interpretation difficult. This is higher than the 12%

**Table 6** Comparison to TOBY study cooled infants*

| | TCH (N = 65)† | TOBY (N = 163) | p |
|---|---|---|---|
| Gestational age, weeks | 39 | 40.3 | |
| Median (range) | (35–42) | (39.1–41.3) | |
| Birthweight, grams | 3070 | 3450 | |
| Median (range) | (2300–5040) | (2957–3853) | |
| Male, n (%) | 36 (55.4) | 101 (62) | 0.373 |
| Apgar at 10 minute, median (range) | 6 (1–10) | 4 (2–5) | |
| Clinical course and complications, n (%) | | | |
| Meconium aspiration/PPHN | 6 (9.2) | 16/163 (10) | 1.000 |
| Hypotension | 13/51 (25.5) | 126/163 (77) | **0.0001** |
| Abnormal coagulation | 24/51 (47.1) | 67/163 (41) | 0.517 |
| Thrombocytopaenia | 19/51 (37.3) | 94/163 (58) | **0.016** |
| Culture-positive sepsis | 5 (7.7) | 20/163 (12) | 0.360 |
| Necrotising enterocolitis | 1 (1.5) | 1/163 (<1) | 0.490 |
| Age at discharge, days and median (range) | 11 (5–46) | 12 (8–18) | |
| Outcomes, n (%) | | | |
| Normal/mild abnormality | 20/28 (71.4) | 71/163 (44) | **0.008** |
| Severe motor/developmental abnormality | 8/28 (28.6) | 32/120 (27) | 0.817 |
| Died | 15/43 (34.9) | 42/163 (26) | 0.253 |
| Combined death and severe neurodevelopmental abnormality | 23/43 (58.1) | 74/163 (45) | 0.392 |

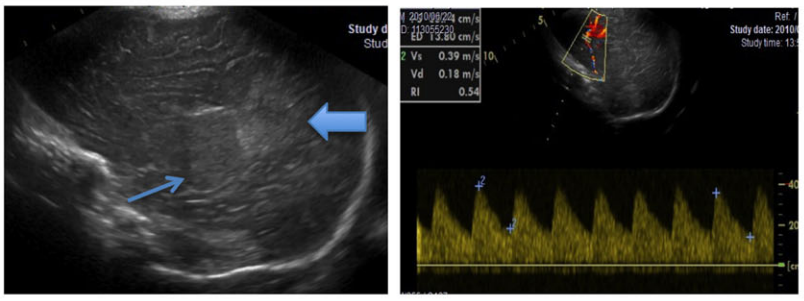
*TCH infants assessed at 12 months with Bayley 111 screen.
†Only mod-severe HIE.
Denominators = numbers assessed.
PPHN = Persistent pulmonary hypertension of the newborn.
p-values of variables that were significantly different between the studies in bold.

previously reported (17). Some were avoidable and highlighted a need for on-going training in the correct application of aEEG and the need to review recordings in real-time to check for quality.

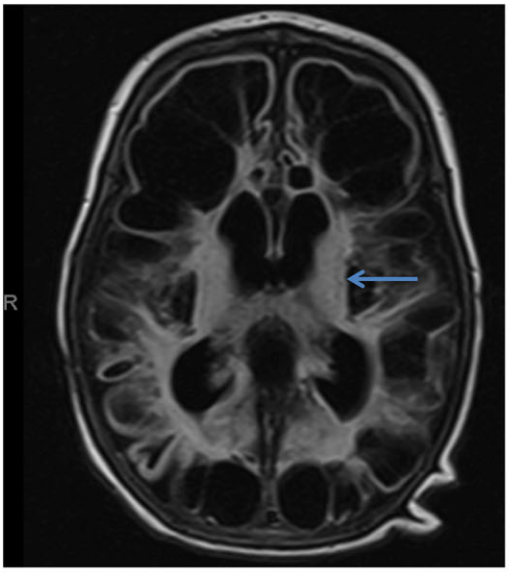
It has been shown that early inability to feed orally following HIE is associated with poor outcome (18). In this cohort, 88% of those who had established adequate feeding by discharge had a good outcome, but 60% of infants with poor suck at discharge had good outcomes at one year. This might be explained by the overall early discharge dates and assessment at 14 days, which was only available in three infants, may be more appropriate.

In total, 111 abnormal findings consistent with previously described findings in term infants following perinatal asphyxia (19) were noted on cranial ultrasound (Table 4). None had signs that suggested congenital malformations or infection. There was no association with any specific cranial ultrasound findings and outcomes. Several factors may account for this. Most infants only had one scan, the majority on day one, and in general these were only performed using just the anterior fontanelle window. It has been shown that the usefulness of ultrasound in predicting outcome can be optimised by using newer machines with better image resolution, using additional

**Early and late imaging of same infant**



CrUS on day 4. Abnormal echodensity in basal ganglia thalamus region with hypolucent internal capsule, forming the "hamburger sign" (thin arrow). There is also abnormal echodensity in periventricular white matter (thick arrow), and some cortical highlighting.
Adjacent image shows abnormal Doppler trace from same study, with a low RI of 0.54



MRI at 3 months: T1 weighted image in the axial plane showing extensive cystic infarction throughout both hemispheres. There is only a thin residual rim of cortex. The basal ganglia and thalami appear atrophied at this high level (arrow). The ventricles are dilated.
*Outcome: Spastic quadriplegia, blind, epilepsy at 1 year.*

**Figure 1** Early and late imaging of same infant.

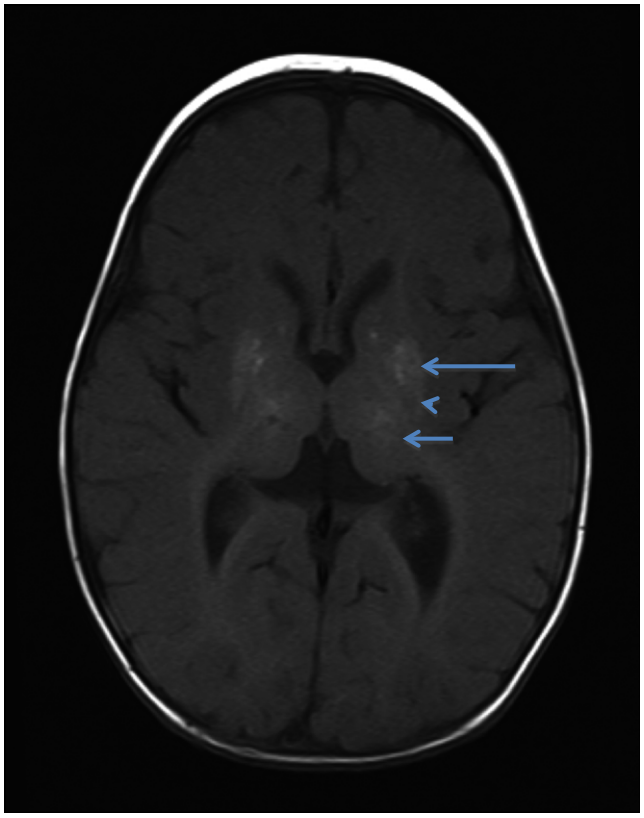
acoustic windows, improving the skills of operators and scanning serially in first two weeks (19,20). In one study, ultrasound had similar predictive values to MRI scans carried out in close temporal proximity and reliably predicted significant motor outcomes in HIE (21). However, performing serial cranial ultrasound as a matter of routine is unlikely to be feasible in this setting. It is possible that the findings that were inconsistent with the MRI scans and later outcomes were transient abnormalities that would have resolved if subsequent cranial ultrasound scans had been performed. This suggests that it might be more worthwhile to delay the scans to later in the first week. Only severe cranial ultrasound abnormalities have been shown to predict outcome (Fig. 1).

In this cohort, MRI was not performed at a consistent time after birth, but it was found to have a good predictive value. Both cranial ultrasound and MRI scans were performed in 25 infants and there was no good correlation between the two imaging modalities. The frequently large time interval between techniques may account for this (20). Cerebral palsy was diagnosed in two-thirds of infants with abnormal features shown on MRI scans at 3.0–4.5 months (Figs 1 and 2), together with the outcome information that was available. This study confirmed that MRI was a good surrogate marker for later outcome. Although it is not universally accessible in low-resource settings, it could be used as a surrogate marker to assess interventions in a setting such as our hospital. To limit demand on limited scanning time, it may be valuable to target those infants with abnormal neurological exams at discharge for further imaging with cranial ultrasound and, or, MRI scans.

The maternal HIV infection rate was similar to that in the local population during the study period (22) and it is not clear what impact HIV exposure had on later outcome. There were more HIV-exposed infants in the group that were lost to follow-up. However, HIV exposure did not appear to influence outcome in those infants where outcome data were available. Information about the mothers' treatment was not documented.

When we compared the information available on infants with moderate to severe HIE to the TOBY study, our cohort had more infants with no abnormalities or minor abnormalities at follow-up (Table 6) and did not have more complications. These comparisons need to be interpreted in light of the fact that a large proportion of our cohort (33%) had no follow-up data and the strict aEEG entry criteria and clinical assessment used in the TOBY trial. Our study infants were also assessed earlier, at the age of 12 months, using Bayley-III screening rather than the full Bayley scales.

Similar numbers with necrotising enterocolitis (NEC) (1%) and meconium aspiration syndrome/persistent pulmonary hypertension of the newborn (7%) were found as in a UK cooling registry (15). The low number with NEC is significant in this population with 21% HIV exposure, as there has been a reported association between maternal HIV infection and increased risk of NEC (23). There were



MRI at 4.5 months: T1 weighted imaging in the axial plane showing bilateral abnormal increased signal intensity in the lentiform nuclei. (long arrow) There is additional bilateral increased signal in the thalami (short arrow) and reduced signal from myelin within the internal capsule (arrowhead). *Outcome: Spastic quadriplegia, epilepsy at 1 year.*

**Figure 2** MRI at 4.5 months. MRI = Magnetic resonance imaging.

two infants who were exposed to HIV, but were not infected, who had persisting abnormalities after one year. This highlights an interesting confounding factor for future studies, as there is some evidence that HIV exposure may cause subtle neurodevelopmental impairments in less developed countries (24).

This study had several limitations. Because of its retrospective nature, some demographic, maternal and neonatal data, such as HIV stage of disease and treatment and feeding ability in the late neonatal period, were not available for analysis. Cranial ultrasounds were not sequentially performed and details about the timing and evolution of findings were not systematically recorded. Limited access to MRI scans resulted in a wide range of imaging times and inadequate staff training on aEEG meant that 30% of recordings could not be interpreted. The follow-up rate was suboptimal and this restricted the analysis between prognostic factors and outcome. Different strategies to improve this will be attempted in the future, including tracking through attendance at other clinics and accessing the Home Affairs Department deaths registry.

A strength of our study was that it is one of the larger studies of TH in this setting and it showed that if TH was applied within a strict protocol, as recommended by international regulatory organisations (25), it can be safe, even in less well-resourced settings. Other studies in similar settings have used different cooling methods, had small numbers and may not have always had access to adequate monitoring facilities (6,7). No other studies including MRI and follow-up data have been published to date in such a setting.

Some factors associated with outcome, such as severity of encephalopathy and aEEG abnormalities, were similar to those found in one of the large cooling trials (26). Future studies should aim to use the previously described tools, such as a threshold Thompson HIE score of 16 (27) to select suitable infants and predictors of outcome, such as aEEG at 48 hours and MRI scans (28,29), to assess further interventions in combination with cooling.

## CONCLUSION

We have shown that the majority of cooled survivors in our setting did not develop major impairments. Continual training on cooling criteria and the use of cranial ultrasound and aEEG is required. MRI is a good predictor of neurodevelopmental outcome and could be used as a surrogate biomarker in new prospective studies. However, a more feasible strategy in these settings might be to select infants with abnormal neurological function at discharge for further imaging with cranial ultrasound or MRI, to identify those who are likely to develop neurodevelopmental impairment.

## ACKNOWLEDGEMENTS

The authors thank the nursing and medical staff who cared for the infants and assisted with data collection.

## CONFLICTS OF INTEREST

None to declare.

## FUNDING

Funding support was received from the Stellenbosch University Research Travel Grant.

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# CHAPTER 6

## Outcomes of infants with hypoxic ischaemic encephalopathy treated with cooling or cooling plus morphine at a tertiary hospital in South Africa: a randomised controlled trial.

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**Keywords:** newborn, encephalopathy, hypothermia, morphine, outcome

**Abstract**

*Background*

Therapeutic hypothermia (TH) improves outcomes in infants with hypoxic ischaemic encephalopathy (HIE) and is now standard of care in many settings. However, the beneficial effects are limited. One previous study, the neo.nEURO.network Trial in which all the infants received morphine co-treatment, showed a greater effect of TH compared to other studies.

*Objectives*

We aimed to determine whether combining TH with morphine would improve intact survival at 18 months in infants with HIE compared to TH alone.

*Methods*

We prospectively randomized 48 infants admitted to Tygerberg Hospital for cooling between 2012 and 2016 to receive TH-only (TH) or TH-plus-morphine (TH+M). Cooling was administered for 72 hours according to the local protocol. The morphine group additionally received the drug at 25μg/kg/hr during TH . Serial morphine concentrations were measured in the serum and cerebrospinal fluid. Neurodevelopmental assessments were performed until 18 months.

*Results*

45 infants were included. Median maternal age was 23 (17-42) years, gestational age 38 (36-42) weeks, birthweight 3140 (2150-4745) g. 8.9% infants were HIV exposed; 46.7% were inborn. Median time to initiation of TH was 4.3 hours. There were no differences in the baseline characteristics between the groups. Group TH had a higher

seizure burden, 13[59.1%] vs 3[13%] (p=0.002) and liver dysfunction, 8[36.4%] vs 2[8.7%] (p=0.035) compared to TH+M. No differences were noted in ventilatory or inotropic support, length of admission, neurological condition at discharge nor severe MRI abnormalities.

There was an absolute risk reduction of 28% in the composite outcome of death or neurodevelopmental impairment in the therapeutic hypothermia-plus-morphine group which was not significant (p=0.087). The other measured outcomes also showed no significant differences: normal outcome at 18 months (17[89.5%] TH+M vs 9[72%] TH, p=0.286); number of deaths (5[22.7%] TH vs 2[8.7%] TH+M, p=0.243) and abnormal neurodevelopment (3[25%] TH vs 2[10.5%] TH+M, p=0.350).

*Conclusion*

In this cohort, therapeutic hypothermia-plus-morphine was associated with some improved early clinical markers (seizure burden and liver dysfunction), without increased complications or duration of hospital stay. While there were differences in later outcome, these were not statistically significant.

Trial registration - www.pactr.org; Identifier: PACTR202006515915908

**Introduction**

Worldwide, intrapartum complications resulting in hypoxic ischaemic encephalopathy (HIE) remain a leading cause of neonatal deaths (1). The only treatment that has been shown to improve outcomes in infants with HIE (2), and that is now standard of care in many settings, is therapeutic hypothermia (cooling). We have previously shown that cooling in our setting is safe and the majority of survivors were normal or had minimal impairments at 1 year of age (3,4). However, the beneficial effects of cooling compared

to normothermia are limited, with many infants worldwide still having poor long-term outcomes. There is consequently a need to test combination therapies that may complement the effects of cooling. One previous study, the neo.nEURO.network Trial, showed a greater treatment effect of cooling compared to some of the other large studies. A possible explanation was that all the infants in this study received morphine sedation at a relatively high dose as part of their treatment. There is some evidence that morphine may have immune-modulatory functions which we postulate may influence some of the inflammatory pathways that are involved in the injury process following a hypoxic ischaemic insult, and thus limit damage (5,6). It may also be related to a reduced stress response to hypothermia as it has been shown in animal studies that the beneficial effect of cooling is negated in the absence of sedation(7).

We therefore sought to explore whether morphine conferred additional neuroprotective properties to cooling alone in neonates with HIE.

**Objectives**

- To describe and compare the clinical course of infants treated with therapeutic hypothermia plus morphine (TH+M) and with therapeutic hypothermia alone (TH).
- To determine whether infants treated with TH+M had better short-term and long-term outcomes when compared to TH alone.

**Ethical approval**

Ethical approval to conduct the study was obtained from the Human Research Ethics Committee of Stellenbosch University (N10/05/157), and also from the management of Tygerberg Hospital (TBH).

Written informed consent was obtained from the parents of all the included infants.

## Methods

### *Setting*

The study was conducted from March 2012 to June 2016 in the neonatal intensive care unit (NICU) of Tygerberg Hospital. This is a tertiary hospital with a total 124 neonatal beds (8 intensive care, 4 high care), which also admits referrals from a large rural Western Cape drainage area. Total annual deliveries at the hospital ranged from 7855-8008 during the study period. The NICU admits approximately 500 infants/year and has offered a cooling service for neonates presenting with HIE as standard care since 2008. The service cools up to 100 neonates annually. 46% (45/97) of cooled infants in 2016 were inborn.

Infants with gestational age ≥36 weeks and birth weight ≥1.8kg were selected for TH according to criteria based on the TOBY study as previously described (4,8), including an initial Thompson HIE score of at least 7 (9).

### *Patient selection and management*

This was a non-blinded randomised controlled trial. All infants referred to the NICU for therapeutic hypothermia (TH) with a suspected diagnosis of HIE who were >1.8kg and had a getstaional age >36 weeks were eligible for the study. They were recruited and allocated to a treatment arm according to random computer generated numbers, to receive either TH alone or TH+M at a dose of 25μg/kg/hr for the duration of the cooling period (72 hours). Infants not in the morphine group were sedated with phenobarbitone, benzodiazepines or clonidine when needed.

We excluded infants with these criteria: presence of congenital abnormalities or abnormalities requiring surgery within the first three days, admission outside of 6 hours of birth, intensive care not offered because of severity of encephalopathy.

Infants were managed according to the standard TBH cooling guidelines (based on TOBY protocol) for 72 hours, and re-warmed at a rate of not greater than 0.5°C/hr. Cooling systems used were Tecotherm TSmed 200 N or the Blanketrol III.

Serial samples were collected for measuring morphine concentrations in serum and cerebrospinal fluid as well as inflammatory cytokines and chemokines, at pre-specified time points. Analysis of this data is the subject of another paper.

All the infants had at least one ultrasound scan of the brain performed during the admission.

MR imaging was acquired where possible. This was done under general anaesthesia (routine at this hospital) using a Siemens Magneton Symphony 1.5 Tesla magnet (Siemens Healthcare, Erlangen, Germany) with T1, T2 and diffusion weighted sequences.

After discharge, infants were followed up in a high risk clinic by a single experienced neurodevelopmental specialist from 3 months up to 18 months of age, who was blind to treatment arm. The examinations included a basic neurological examination with test positions and angles as described by Amiel-Tison (10), and assessment using the Bayley Scales of Infant and Toddler Development-Third Edition (BSID-III). Hearing was also assessed using otoacoustic emissions and automated auditory brainstem responses in the first three months, and visual reinforcement audiometry at one year.

General movements at 12-15 weeks were recorded (11,12), and analsysis of this data is still ongoing.

*Study measurements and procedures*

Clinical information was collated from routinely used cooling data collection forms and the electronic record of medical notes, and transcribed into a secure electronic database.

Severity of initial encephalopathy was assessed based on Thompson score: moderate 7-14, severe ≥15 (9).

Cranial ultrasound scans were assessed by a single reviewer and the following findings were recorded: day imaged; evidence of cysts within ventricles (sub-ependymal, choroid plexus); cerebral oedema; abnormalities in basal ganglia and thalami (BGT), white matter, cortex; intra-ventricular haemorrhage; infarction and other abnormalities. The results of Doppler studies, and day acquired, indicating the value of the resistance index were documented.

MRI images were assessed by an experienced neuro-radiologist who was blinded to the early clinical course and later outcomes. The appearances of the BGT, posterior limb of the internal capsule, white matter and cortex were recorded and classified as previously published (13).

At follow up assessments, infants were considered severely impaired if they had cerebral palsy, were deaf or blind, or had significant developmental delay as defined by a composite score of <70 (<2SD below mean) in any of the BSID-III domains (cognitive, language and motor).

*Statistical analysis*

GraphPad software (GraphPad Software Incorporated, La Jolla, CA, USA) and Stata 15 (College Station, Texas USA.) were used for statistical analysis. We summarized continuous variables using mean (standard deviation) or median (range) depending on the distribution, and categorical variables using count (percent). We used Chi-squared test or Fisher's exact test to test associations between categorical variables and the t test or Wilcoxon rank-sum test to compare continuous variables between groups. We performed a logistic regression to determine the odds of a normal outcome following TH+M treatment. We set statistical significance at $p< 0.05$.

**Results**

*Clinical course*

Forty-eight infants were recruited to the study. Three were subsequently excluded; 1 had trigonocephaly and dysmorphic features and 2 only commenced cooling after 7 hours of age (Figure 6.1).

Of the remaining 45 infants, 22 received TH alone and 23 received TH+M.

Baseline characteristics and clinical condition of all study infants are depicted in Table 6.1.

Median maternal age was 23 (range 17-42) years. Twenty eight (62%) of them were primiparous, and 4 (8.9%) were HIV positive.

Sixty four percent (29) of the infants were male; 46.7% (21) were inborn.

There were no significant differences in the baseline characteristics of the two groups of infants (see Table 6.1). Time to admission for cooling and to target temperature were similar between the groups.

Thirty eight (84%) of the infants had moderate and seven (16%) severe HIE.

The clinical course of all study infants is depicted in Table 6.2.

Twenty seven infants required ventilation for a mean duration of 5.5(±2.7) days. The TH+M group had no significantly increased duration of ventilation (p=0.206).

Of the 24(53%) infants with coagulation abnormality and 19(42%) with thrombocytopaenia, three had clinical bleeding requiring treatment (one in TH+M group and two in TH group, p=0.608).

Within the two groups similar proportions of infants (3/23[13%] TH+M vs 2/22[9.1%] TH; p=1.000) required more than one inotrope.

The TH+M group had significantly less infants with liver dysfunction (ALT>100 IU/L) and with a high seizure burden (defined by the number of anti-seizure medications used).

There were no significant differences in time taken to establish feeding or duration of admission between the groups; nor in neurological condition at time of discharge as evidenced by level of consciousness (p=0.746) or tone abnormalities (p=1.000).

*Imaging*

80 cranial ultrasound scans were performed on the infants, and all infants had a minimum of 1 scan (Table 6.3). Up to 3 scans on each infant were performed at median

ages of 2 (1-8), 5 (2-12) and 8 (7-27) days for the first, second and third scans respectively.

71 doppler studies were performed and significantly abnormal flow velocities (RI≤0.55) were found in 16 studies on 14 infants, the majority (12/16) of them on days 2-3.

25/45 (55.6%) infants (24 survivors) had MRI imaging, at a median age of 15 (range 7-256) days (Table 6.3). Of these infants, 20 (80%) had findings associated with a normal outcome or minor abnormalities, and 5 (20%) had findings associated with abnormal outcome.

Imaging findings did not differ between treatment groups.

There was, however, a difference in imaging findings when classified according to outcome at 18 months (see Table 6.4). All 3 infants who had severe BGT abnormalities on US, and 2/3 of those with severe WM abnormalities had poor outcome. These severe changes appeared on later imaging between days 6-27, with severe BGT abnormalities being most significant.

On MRI, the 2 infants who had severely abnormal findings and normal outcome had abnormalities in white matter and cortex only. One of them had a relatively small focal right-sided infarct both on ultrasound at 7 days and MRI at 3 months of age, not involving the corticospinal tracts (Figure 6.1). The other infant with severe findings was still normal at 3 years of age. The remaining 3 infants with severely abnormal findings had abnormal outcome in keeping with the MRI findings (Figures 6.2 & 6.3).

Of the 20 infants with normal MRI imaging or minor abnormalities, 17 (85%) had normal outcome at 18 months; one infant had a sudden deterioration around 1 year of

age due to acute hydrocephalus and had an abnormal outcome most likely secondary to this; the outcome of 2 infants was not known.

*Outcomes*

Primary outcome - 18 months

Outcome information at 18 months was available for 38 (84.4%) of the 45 infants, 7 (15.6%) were lost to follow up (Table 6.5). Seven infants had died, one of them after the neonatal period. It was possible to perform neurodevelopmental assessments on 31/38 (81.6%) surviving infants at this time point. Twenty-six (83.9%) had normal neurodevelopment or minor abnormalities, and 5 (16.1%) had abnormal neurodevelopment. Overall, 12 infants had an adverse outcome (31.6%) at 18 months.

Of the twenty-six infants who were normal or had minor abnormalities such as residual Erb's palsy or strabismus at 18 months, 17/19 (89.5%) were in the TH+M group and 9/12 (75%) in the TH group; p=0.286. We found statistically insignificant increased odds of normal outcome following TH+M treatment, OR 3.78 [95% CI, 0.89-16.05; p=0.072]. This was unchanged after imputing for missing outcome data (Table 6.5).

Of the twelve infants that had the composite outcome of death or severe neurodevelopmental impairment, 4/21 (19.0%) were in the TH+M group and 8/17 (47.1%) were in the TH group; p= 0.087.

Of the five (16.1%) infants who had severely abnormal neurodevelopment, 2/19 (10.5%) were in the TH+M and 3/12 (25%) were in TH group; p=0.350. One infant in the severely abnormal group also developed bilateral sensorineural hearing loss, and required hearing aids.

Seven (15.6%) infants had died by 18 months, 2/23 (8.7%) in the TH+M group and 5/22 (22.7%) in the TH group; p=0.243. 6 died during the neonatal period and 1 thereafter (from a suspected respiratory infection). At a review at 3 months, this infant was already very abnormal with developmental delay and spastic quadriplegia. He subsequently defaulted further follow up appointments before he died. Of the 6 neonatal deaths, 1 died within 24 hours from severe encephalopathy, 4 had care redirected due to severity of illness and one had methicillin resistant staphylococcus aureus (MRSA) septicaemia.

35 infants had some longer term follow up and were seen at either 12 or 18 months. Four were not seen at either of these 2 time points. Of these 4 infants, one was normal at 3 months of age; another had tone and reflex abnormalities at 3 months, but was thought to be normal at 9 months.

There were no significant differences between the infants whose outcome was known and those with unknown outcome (S1 Table).

Primary outcome - 12 months

Six (13.3%) infants had died during the neonatal period, 5 of them from severe HIE and one from infection. Thirty-four (87.2%) of the 39 survivors presented at the 12 month appointment; 5 (12.8%) did not attend follow up appointments. However, 2 of the infants that attended could not be assessed: one had developed acute hydrocephalus and was referred for a ventriculo-peritoneal shunt; the other was very uncooperative.

Of the 32 infants that were assessed, 27 (84.4%) had normal neurodevelopment or minor abnormalities and 5 (15.6%) were categorised as having severely abnormal neurodevelopment.

There were no significant differences in outcome at this time point between the TH and TH+M groups.

**Discussion**

In this randomised controlled trial comparing treatment of infants with HIE with TH alone and with TH plus Morphine, we found differences in intact survival to 18 months of age but these did not reach statistical significance. There was no significant difference in the composite outcome of death or severe neurodevelopmental impairment. Mortality and severe disability were also not significantly reduced when assessed separately. We found improvements in some short-term clinical outcomes and did not find increased adverse events in the TH+M treated group.

During the initial admission the TH+M treated infants had some improved clinical markers, with significantly less liver dysfunction and a lower seizure burden. A possible explanation might be that there was a lower proportion of infants with severe HIE in the TH+M group, but this difference was not statistically different and infants with severe HIE constituted a small fraction of the whole cohort (15.6%).

The adverse events that could be anticipated with such a high dosage of morphine that have been described in other papers (14,15) were not observed in this study. Unlike in the paper by Liow et al (15), there was no significant increase in hypotension requiring inotropic support, requirement and duration of ventilation (see Table 6.2) and

no increased length of stay in the morphine group despite a consistently higher dose administered for 72 hours.

Other clinical findings that have been shown to be surrogate indicators of extent of brain injury and to predict outcome (16), such as ability to establish feeds and neurological condition at time of discharge, were not different between the two groups.

Our findings that the majority of those infants who had severe BGT and WM abnormalities on later US imaging had poor outcomes were similar to what has been reported in literature, that the more significant findings associated with poor outcome may appear later in the postnatal course or become more prominent with time (17,18). Using a score similar to that used by Tann et al in a Ugandan study (19), we found that having ≥2 significant US abnormalities had a PPV of 67% and a NPV of 71.4% for poor outcome. Using severe BGT abnormalities alone, the PPV was 99.9% and NPV 74.3%.

Abnormal flow velocities with low resistance index (RI) <0.55 on doppler studies were found in 55% of infants with poor outcome vs 20% with favourable outcome, p=0.056. This is consistent with recent literature which has shown that an abnormally low RI, which was previously known to predict poor outcome in the pre-cooling era, may not be as predictive during cooling. A few studies reported that a low RI (<0.55 or <0.6) predicted poor outcome before or after the cooling period, but not during cooling (20,21). In our study, an RI <0.55 during TH had a PPV of 55% and NPV of 78% for poor outcome, which was similar to the findings of Elstad et al who reported a PPV of 60% and a NPV of 78% (22).

MRI imaging findings, in keeping with previously published studies (13,16,23), correlated well with outcome. 17/19 (89.5%) of infants with a good outcome that were imaged had normal studies or minor abnormalities; whereas ¾ (75%) of those with poor outcome had significant abnormalities consistent with the outcome on imaging. In the one infant who had a poor outcome and minor abnormalities on initial MRI, the outcome was thought to be unrelated to HIE and his subsequent MRI imaging was severely abnormal. MRI had a sensitivity of 90% and a positive predictive value of 95% for normal outcome.

Although the study by Liow did not show any benefit of routine morphine sedation during TH, they also did not show any additional brain injury on MRI and MRS.

There was an important difference between our study and the Liow study (the only study we've found looking specifically at this question). Although much larger, theirs was a secondary analysis of a multicentre study. Morphine sedation was administered as part of routine care at individual clinicians' discretion and for differing unspecified indications. Consequently, there was a wide range of dosages and no standard duration of administration. In our study the treatment was prospectively and randomly allocated, and the dosage and duration of administration was standardised.

Our data are more consistent with the study by Angeles et al (24), which suggested that in infants with neonatal encephalopathy that were subjected to the repeated stressful procedures that are frequently performed in NICUs, opiate treatment did not cause more morbidity and might even confer some neuroprotection when assessed with MR spectroscopy.

We defined abnormal outcome as cerebral palsy, blindness, deafness or a score of <70 (<2SD below mean) on any Bayley III composite score. There are some concerns

in the literature that using a cut-off of <70 to define severe neurodevelopmental delay might result in underestimating the prevalence of developmental delay. However, a recent study done in a similar population showed that using this cut-off was appropriate (25). Looking at our data, only 2 infants who were considered normal by these criteria would have fallen into the at-risk category (any Bayley III composite score <85/<1SD below mean). One infant (in the TH group) had a cognitive score of 80, but was very uncooperative and there had been only one opportunity to test him. The other one (in the TH+M group) had a motor score of 82. He was a later walker, which accounted for the low score but he had no upper motor neuron signs or other problems.

It is not clear why there was a trend towards more infants in the TH+M group surviving with a normal outcome, which is contrary to what was recently published regarding morphine sedation with cooling (15). The fact that there was less liver dysfunction and a lower seizure burden in the TH+M group, may be indicative of some immune modulating effects related to morphine treatment. These are currently being explored by the authors. Another consideration is the fact that seizures are thought to contribute to neurological injury in the setting of HIE (26), and evidence from animal studies suggesting that anti-seizure medications themselves may cause apoptotic neurodegeneration (27). This may have a role in those infants experiencing fewer seizures and anti-seizure medication tending to have a more favourable outcome.

Globally, it appears that it has been accepted that current protocols for TH (duration & depth of cooling) are optimal (28). What is required is that units focus on applying TH protocols timeously and rigorously, and to test combination therapies that may augment the TH effect. While doing this we need to explore mechanisms by which

they may act (many overlap), to ensure they are complementary and do not negate each other (29).

In our study, neither of the groups were commenced on cooling within 3-4 hours, which has been shown to improve motor outcomes (30). Despite aiming for this window period to commence TH, we were constrained by competing needs and the lack of a specialised paediatric/neonatal transport team at the time of recruitment. It is conceivable that earlier cooling might have shown a better effect of TH+M.

*Limitations*

A major limitation of the study is that we recuited 60% of the number of patients that would have been required to be adequately powered. This may have accounted for the lack of statistical significance in the outcomes we measured.

Data on outcome was missing for 16% of patients, who did not attend follow up assessments as planned. This is a common problem in our system with very mobile communities, and various strategies are attempted in order to maximise follow up information such as accessing the national death registry for mortality data and monitoring attendance at other clinics.

This is, however, an improvement on the loss to follow up rate of 33% we previously published in this setting (4). It is hoped there can be further improvements in future studies, with the progressive introduction of unified systems of patient data management in the province.

Although all the patients had real time aEEG recordings which were used as part of their clinical assessment and used in conjunction with the Thompson HIE score to grade severity of encephalopathy and diagnose seizures, we have not described the

findings in our results due to loss of captured data (becoming corrupted during attempt to download) which meant the recordings could not be rigorously classified and reported.

Another possible limitation is that we did not correct for the possible effects of the other sedative medications on outcome as it would be difficult to tease out which effects are the result of the seizures or of the treatment. However, a majority of infants in both groups received some sedation other than morphine, and there was no significant difference between the groups (Table 6.2).

*Strengths*

Although the study did not show the desired outcome, it is the only study we have found to date looking in a controlled fashion/systematically at the effects on neurodevelopmental outcome of combining morphine sedation with TH. The lack of a statistically significant positive effect may be due to the relatively small numbers, and could still be clinically important. Our findings of a trend towards more survival with normal outcome at 18 months are similar to those from the TOBY study (8), which despite showing no significant effect on the primary outcome of death or severe impairment found increased survival with favourable neurological outcomes following TH treatment. Further work is ongoing to assess blood and CSF levels of morphine and inflammatory markers, and explore these interactions.

**Conclusion**

In this cohort, cooling-plus-morphine was associated with some improved early clinical markers and was not associated with increased complications or prolonged hospital stay, but did not significantly reduce mortality or disability at 18 months.

High dose morphine can therefore not be recommended for routine use in infants with HIE that are being cooled at this stage. Potential benefits need to be confirmed in larger adequately powered studies whilst exploring mechanisms of possible additional neuroprotection.

**Acknowledgements**

We are grateful to the TBH neonatal medical and nursing staff, who provided ongoing care to the patients and also assisted with collecting data.

We are also extremely grateful to the anaesthetist Dr Celeste Cilliers and anaesthetic nurses, and the following radiographers who assisted with MR imaging outside their normal working hours: Ms TL Thomas, Ms L Johnson, Ms AN Jansche Van Rensburg, Ms MC Nasson.

**Funding**

The study was funded in part by the South African Medical Research Council.

**Competing interests**

None

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**Table 6.1: Baseline characteristics of participants by study treatment arm**

| Characteristics | Hypothermia + Morphine (Group 1) N=23 | Hypothermia only (Group 2) N=22 | P-value |
|---|---|---|---|
| Maternal age, mean (SD), years | 26.1 (6.2) | 23.3 (6.1) | 0.133 |
| Primiparity, n (%) | 13 (57) | 15 (68) | 0.420 |
| Inborn, n (%) | 11 (48) | 10 (46) | 0.873 |
| Gestational age, mean (SD), weeks | 38.9 (1.5) | 38.3 (1.5) | 0.350 |
| Birthweight, mean (SD), grams | 3263 (646) | 3172 (466) | 0.592 |
| Male, n (%) | 15 (65) | 14 (64) | 0.761 |
| Head circumference, mean (SD), cm | 35.1 (1.9) | 35.7 (1.9) | 0.360 |
| Sentinel event*, n (%) | 7 (30) | 6 (27) | 0.815 |
| Apgar at 5 minutes <5, n (%) | 7 (30) | 8 (36) | 0.673 |
| pH within 1st hour <7, n (%) | 11 (48) | 12 (55) | 0.652 |
| Time to admission, h mean (SD) | 4.4 (1.3) | 4 (1.6) | 0.362 |
| Time to target (33.5°C), h mean (SD) | 7.1 (2.8) | 6.13 (2.3) | 0.204 |
| HIE 2 #, n (%) | 21 (91) | 17 (77) | 0.194 |
| HIE 3#, n (%) | 2 (9) | 5 (23) | 0.243 |

*sentinel event: cord prolapse, abruptio placentae, uterine rupture, eclampsia

#HIE 2: Thompson score 7-14; HIE 3: Thompson score ≥1

**Table 6.2: Neonatal course of infants by treatment group**

| **Clinical course and complications** | **Hypothermia + Morphine (Group 1) N=23** | **Hypothermia only (Group 2) N=22** | **P-value** |
|---|---|---|---|
| Ventilated, n (%) | 16 (70) | 11 (50) | 0.231 |
| Duration of ventilation, days<br>Mean (SD) | 4.4 (4.0) | 2.9 (3.7) | 0.206 |
| MAS/PPHN, n (%) | 5 (22) | 4 (18) | 1.000 |
| Inotropic support, n (%) | 6 (26) | 4 (18) | 0.722 |
| Coagulation abnormality, n (%) | 11 (48) | 13 (59) | 0.554 |
| Thrombocytopaenia, n (%) | 11 (48) | 8 (36) | 0.550 |
| Culture-positive infection, n (%) | 4 (17) | 1 (5) | 0.346 |
| NEC, n (%) | 1 (4) | 1 (5) | 1.000 |
| Renal dysfunction, n (%) * | 5 (22) | 8 (36) | 0.337 |
| Liver dysfunction, n (%) * | 2 (9) | 8 (36) | **0.035** |
| ALT, median (range) | 28 (11-319) | 37 (13-452) | 0.119 |
| Hypoglycaemia, n (%) # | 9 (39) | 8 (36) | 1.000 |
| Hyperglycaemia, n (%) | 4 (17) | 7 (32) | 0.314 |
| Any sedatives, n (%) | 17 (74) | 20 (91) | 0.242 |
| Significant seizure burden, n (%) $ | 3 (13) | 13 (59) | **0.002** |
| LOS, days - mean (SD) | 12.7 (5.9) | 11.9 (6.9) | 0.699 |

MAS=meconium aspiration syndrome. PPHN=persistent pulmonary hypertension of the newborn. NEC=necrotising enterocolitis. ALT=alanine transaminase. LOS=length of stay.

* Early transient

# Both early transient & requiring treatment (only 1 required treatment, in morphine grp)

$ needing 2 or more anti-epileptic drugs

**Table 6.3: Imaging findings in the two groups of infants**

| | **Hypothermia + Morphine** | **Hypothermia only** | **P-value** |
|---|---|---|---|
| **CUS (n)** | **N=40** | **N=40** | |
| • BGT | 29 | 27 | 0.808 |
| • White matter/cortex | 34 | 33 | 1.000 |
| • Infarct | 1 | 1 | 1.000 |
| • Doppler abnormal (RI=/<0.55) | 10 | 6 | 0.402 |
| **MRI (n)** | **N=15** | **N=10** | |
| • Moderate-severe BGT | 1 | 2 | 0.544 |
| • BGT + PLIC | 0 | 2 | 0.150 |
| • White matter ± cortex | | | |
| ▪ Severe with overt infarction | 2 | 3 | 0.358 |
| ▪ Mild-moderate | 4 | 2 | 1.000 |
| • *WM + BGT ± PLIC | 1 | 2 | 0.544 |
| • Haemorrhage or thrombosis | 9 | 4 | 0.428 |
| • Cerebellum (mild) | 3 | 3 | 0.653 |
| • Brainstem | 0 | 2 | 0.150 |

CUS=cranial ultrasound scan. BGT=basal ganglia thalami. IVH=intraventricular haemorrhage. RI=resistance index. PLIC=posterior limb of internal capsule. WM=white matter

*WM + BGT – mild in TH+M group

**Table 6.4: HIE grade and imaging findings by outcome**

| Clinical/radiological feature | Favorable outcome (N=26) n (%) | Poor outcome (N=12) n (%) | P-value |
|---|---|---|---|
| HIE 2 | 25 (96.2) | 6 (50) | 0.002 |
| HIE 3 | 1 (3.8) | 6 (50) | 0.002 |
| **CUS abnormalities:** | | | |
| Severe BGT | 0 (0) | 3 (25) | 0.026 |
| Severe WM-cortex | 1 (3.8) | 2 (16.7) | 0.230 |
| RI </= 0.55 | 5/25 (20) | 6/11 (54.5) | 0.056 |
| **MRI abnormalities:** | **(N=19)** | **(N=4)** | |
| Severe WM + cortex | 2 (10.5) | 3 (75) | 0.021 |
| Severe WM + moderate-severe BGT + PLIC | 0 (0) | 2 (50) | 0.024 |
| Moderate-severe BGT + severe WM + cortex | 0 (0) | 3 (75) | 0.014 |
| Brainstem | 0 (0) | 2 (50) | 0.024 |

HIE=hypoxic ischaemic encephalopathy. HIE 2=moderate encephalopathy; HIE 3=severe encephalopathy. CUS=cranial ultrasound scan. BGT=basal ganglia thalami. WM=white matter. PLIC=posterior limb of internal capsule

**Table 6.5: Outcome at 18 months by treatment group**

| **Later outcome (18 months)** | **Hypothermia + Morphine (Group 1) n=23** | **Hypothermia only (Group 2) n=22** | **P-value** | **OR* (95%CI)** | **P-value** |
|---|---|---|---|---|---|
| Normal, n (%) | 17/19 (89.5) | 9/12 (75) | 0.286 | 3.78 (0.89-16.05) – non-imputed<br>3.05 (0.75 – 12.30) - imputed | 0.072<br><br>0.118 |
| Composite outcome – death/abnormal neurodevelopment, n (%) | 4/21 (19.0) | 8/17 (47.1) | 0.087 | | |
| Lost to follow up, n (%) | 2/21 (9.5) | 5/17 (29.4) | 0.207 | | |
| Abnormal, n (%) | 2/19 (10.5) | 3/12 (25) | 0.350 | | |
| Death, n (%) | 2/23 (8.7) | 5/22 (22.7) | 0.243 | | |

OR: odds ratio, imputed for missing data; CI: confidence interval.

**Figure 6.1: Consort flow diagram of recruited and enrolled infants**

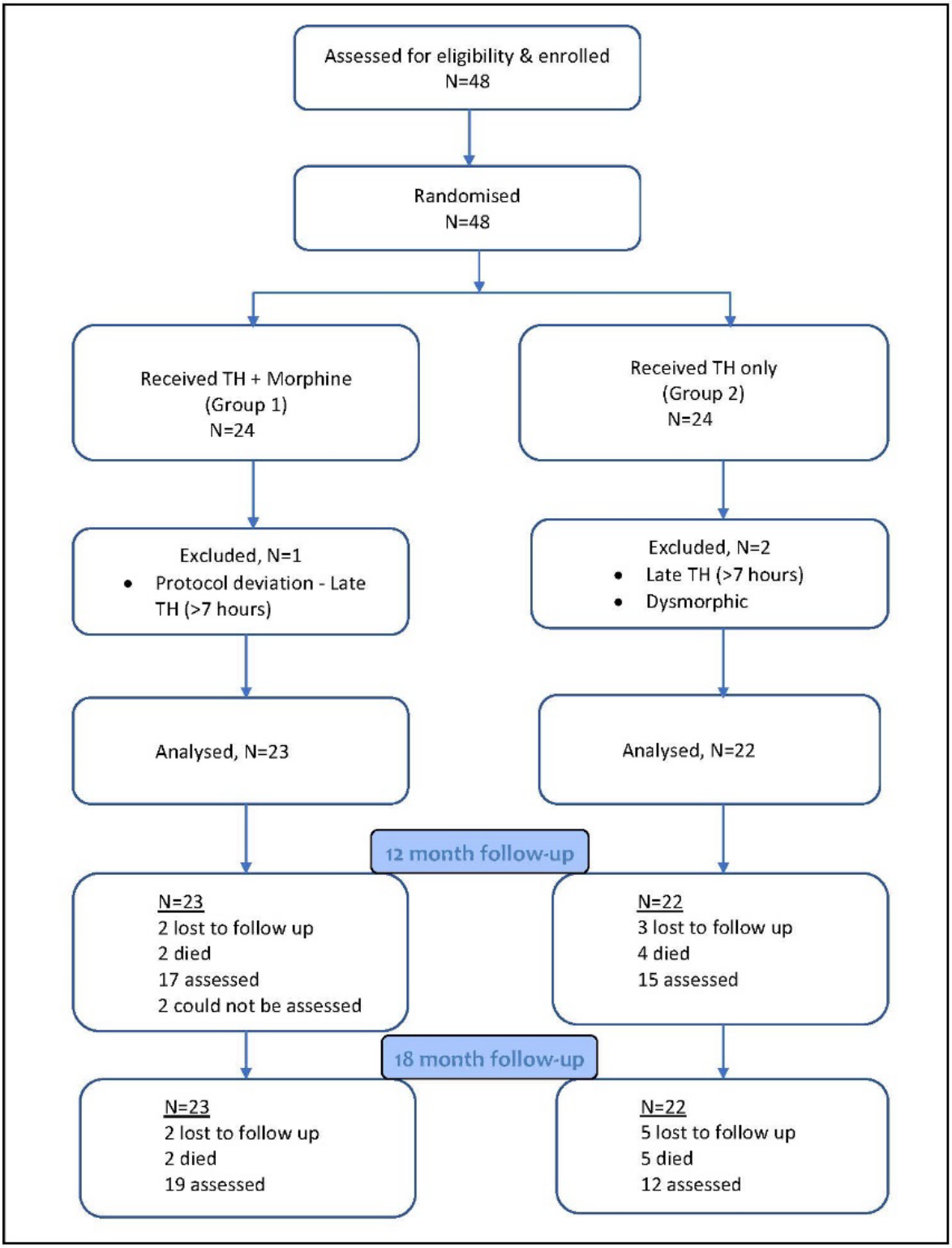


**Figure 6.2: MRI image showing a small focal infarction in the territory of the right middle cerebral artery but not involving the corticospinal tracts**

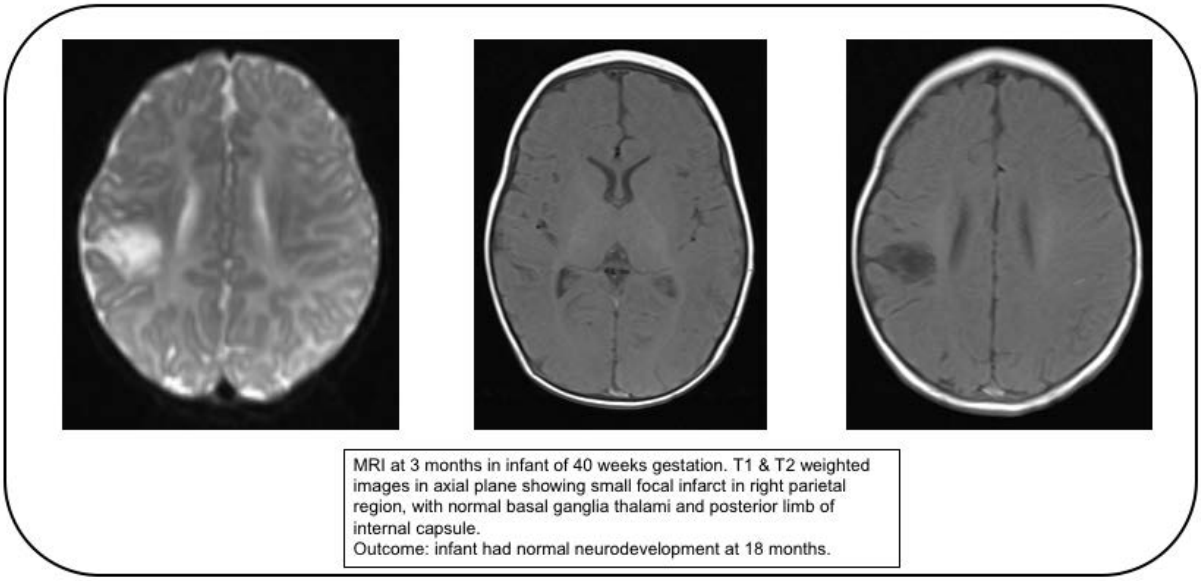



**Figure 6.3: MRI image showing injury predominantly in white matter and cortex**

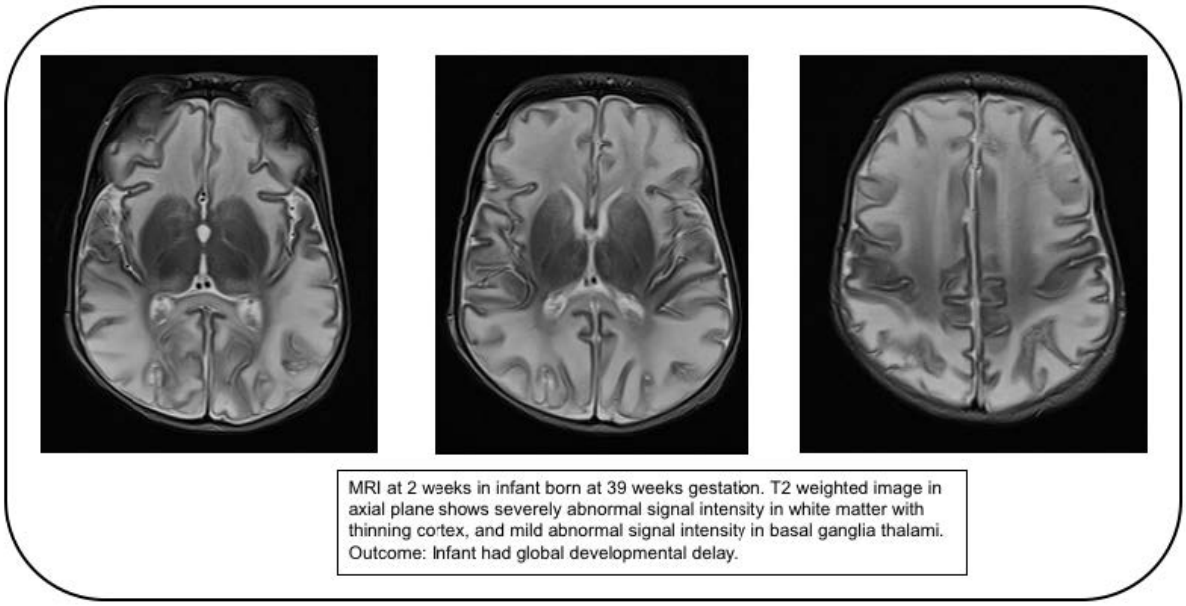
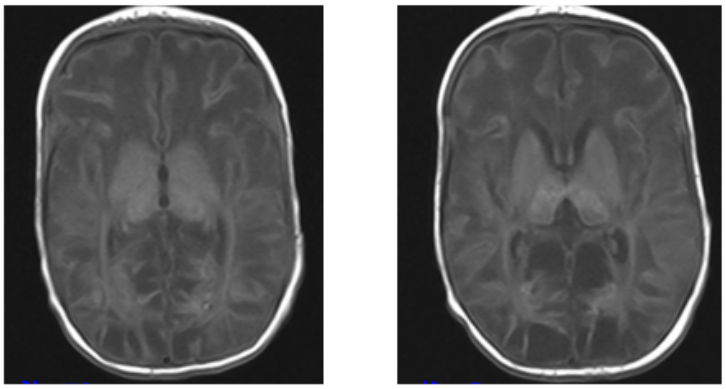


**Figure 6.4: MRI images showing injury in most areas of the brain**



MRI on day 17 in infant of 38 weeks gestation. T1 weighted images in axial plane showing severely abnormal signal intensity in basal ganglia thalami and posterior limb of internal capsule, as well as very abnormal white matter and cortex with cystic infarction.
Outcome: Severe spastic quadriplegic cerebral palsy. Died before 18 months.

# CHAPTER 7

## Morphine concentrations in serum and cerebrospinal fluid of infants with hypoxic ischemic encephalopathy treated with therapeutic hypothermia.

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**Funding**

The study was funded in part by the South African Medical Research Council

**Conflict of interest**

The authors have no conflict of interest to declare.

**Acknowledgements**

The authors are grateful to the parents of the participants in the study. We are also grateful to the TBH neonatal medical and nursing staff, who provided ongoing care to the patients and assisted with the study processes.

Word count (body): 3304

Abstract: 185

Tables/Figures: 7 (5+2)

References: 25

**Data sharing**

The data underlying the results of this study are available upon request because it contains potentially sensitive information. Interested researchers may contact the corresponding author via email at kali@sun.ac.za.

**Abstract**

*Background*

Sedation with agents such as morphine is frequently recommended for infants undergoing therapeutic hypothermia for hypoxic ischemic encephalopathy, despite concerns about the paucity of pharmacokinetic data on morphine and possible toxicity in these infants.

*Methods*

We conducted a randomised controlled trial to compare outcomes in 45 infants treated with therapeutic hypothermia or therapeutic-hypothermia-plus-morphine (at a dose of 25μg/kg/h) between 2012-2016. For this nested study, we analysed serial serum (at

24, 72, and 96 hours) and cerebrospinal fluid (72 hours) concentrations of morphine and its metabolites (morphine-3-glucuronide and morphine-6-glucuronide) in the patients that were treated with therapeutic-hypothermia-plus-morphine in the first 96 hours after delivery using LC-MSMS.

*Results*

We did not find toxic concentrations at this constant morphine infusion rate. Serum concentrations of morphine and metabolites were below 20 μg/L at all time points. Mean CSF morphine, morphine-3-glucuronide and morphine-6-glucuronide concentrations at 72 hours were 5.19, 3.68 and 0.61μg/L respectively. There were no increased complications such as increased length of stay, need for ventilation or inotropic support as found in other studies.

*Conclusion*

We found that morphine infusion at 25μg/kg/h was tolerated well in this cohort.

Trial registration - www.pactr.org; Identifier: PACTR202006515915908

**Keywords**

morphine, neonates, encephalopathy, hypothermia

## Introduction

Infants with hypoxic ischemic encephalopathy (HIE) undergoing treatment with therapeutic hypothermia frequently receive sedative agents. This is because a majority of protocols for therapeutic hypothermia recommend sedation during therapy, based on some animal evidence that the neuroprotective effects of therapeutic hypothermia may be diminished or even negated if applied without sedation[1]. There is also evidence that encephalopathic infants admitted to neonatal intensive care unit (NICU) are often subjected to multiple traumatic and stressful procedures that may

add to brain injury as demonstrated on magnetic resonance imaging (MRI) and MR spectroscopy, and this may be ameliorated by sedating them with opioids[2].

Although morphine is a commonly used agent for sedation during therapeutic hypothermia, there are a number of concerns with using this drug. One important concern is that there is not an abundance of data about its pharmacokinetics during therapeutic hypothermia in this vulnerable population particularly in cerebrospinal fluid, and that it may accumulate to toxic levels. It is known that drug metabolism is altered in these patients, both from altered blood flow to the organs that metabolise drugs due to the disease process and from the effects of cooling[3,4]. Old evidence from animal studies indicated significantly increased morphine concentrations in plasma and cerebrospinal fluid, and resultant side effects during hypothermia[5]. It has thus been suggested that ongoing cooling studies should include drug monitoring [6].

A published study that analysed morphine concentrations in one site of one of the major therapeutic hypothermia trials, showed higher cumulative concentrations in the therapeutic hypothermia group, and increasing concentrations as the treatment continued. They also showed more frequent instances of toxic concentrations (>300ng/mL or μg/L) in the therapeutic hypothermia group and when infusion rates exceeded 10μg/kg/h[7].

Morphine exerts its analgesic and sedative effects by activating μ and κ opioid receptors. Its major metabolic pathway is via glucuronidation in the liver by the UGT2B7 enzyme to morphine-3-glucuronide (M-3-G) and morphine-6-glucuronide (M-6-G). The metabolites are then cleared by the kidneys. M-6-G has been shown to be a strong μ-receptor agonist with an even higher affinity than morphine, and is responsible for a substantial part of the analgesic effect after morphine

administration[8,9]. Any impairment in liver or renal function may therefore result in accumulation of morphine and/or its metabolites and exaggerated effects.

A more recent study of morphine pharmacokinetics (PKs) in cooled infants showed that morphine clearance was affected by both therapeutic hypothermia (reduced) and postnatal age, increasing significantly after re-warming resulting in steady state never being reached in the first 5 days. Morphine metabolites, on the other hand, accumulated during the therapeutic hypothermia period but were not as affected by postnatal age and so did reach steady state once normothermia was established and clearance increased[10].

Pacifici conducted a review of morphine PKs in neonates not undergoing therapeutic hypothermia and found that clearance varied with gestational age and birthweight, with wide variations particularly in the first few days – a likely reflection of changes in hepatic blood flow and immaturity of conjugating systems[9].

**Objectives**

In infants with HIE treated with therapeutic hypothermia (TH) alone and with therapeutic-hypothermia-plus-morphine (TH+M) we aimed to document serum and cerebrospinal fluid *morphine* concentrations in the first 96 hours after birth, and to document serum and cerebrospinal fluid concentrations of *morphine metabolites* (morphine-3-glucuronide and morphine-6-glucuronide) in the first 96 hours after birth in the TH+M group.

We also aimed to describe the clinical course and document the clinical tolerability of the current dosage in infants undergoing TH?

## Methods

### *Ethics*

Ethical approval to conduct the study was obtained from the Stellenbosch University Human Research Ethics Committee (N10/05/157), and from the management of Tygerberg Hospital (TBH).

Written informed consent was obtained from the parents of all the included infants.

### *Setting*

The study was conducted in the neonatal intensive care unit of TBH, a tertiary referral unit in Cape Town (South Africa) between March 2012 and June 2016. The service has 8 intensive care beds with ventilation facilities and 4 high care beds, and a total of 124 neonatal beds.

### *Study design and procedures*

Forty five infants admitted for TH were included. These were infants who met TOBY[11] study-based entry criteria for TH (at least 36 weeks gestational age, with evidence of perinatal compromise, and clinical and/or electrophysiological evidence of moderate to severe encephalopathy). The infants were selected using random computer-generated numbers to receive either TH alone or TH+M at a morphine dose of 25 μg/kg/h (given as morphine sulphate) from the onset of hypothermia for the duration of the cooling period (72 hours). Infants that did not meet the criteria for therapeutic hypothermia were excluded.

All the infants were commenced on TH within 6 hours of birth, and cooled to a rectal temperature of 33.5°C for a duration of 72 hours. They were then rewarmed slowly at a rate not exceeding 0.5°C/h.

Infants that required additional sedative drugs for any indication received phenobarbitone, benzodiazepines or clonidine at attending clinicians' discretion.

All infants had routine monitoring of hematological (full blood count, coagulation) and biochemical (renal function and liver enzymes) parameters, as well as routine intensive care monitoring of HR, BP, O2 saturations and blood gases as indicated.

In addition, the infants in the TH+M group had serial blood and cerebrospinal fluid (CSF) samples taken for measurements of morphine and metabolite (M-3-G and M-6-G) concentrations as follows: serum at 24, 72 and 96 hours and CSF at 72 hours. Blood and CSF samples were centrifuged at 3500 rpm. Both serum and CSF samples were stored at -80 °C until analyses.

*Materials*

Morphine-d3 (1.0 mg/ml in methanol), morphine-3-β-D-glucuronide (M-3-G) and morphine-6-β-D-glucuronide (M-6-G) were obtained from Toronto Research Chemicals (Toronto, ON Canada). These are pure compounds used to prepare the standards as below:

***LCMSMS Sample preparation***

Morphine calibration standards were prepared by first diluting a 1000 μg/L stock solution into serum (2 x dilutions). Subsequent dilutions were made with 500 μl standard + 500 μl blank serum. This produces the following calibration standards: 500,

250, 125, 62.5, 31.25, 15.63, 7.9, 3.8, 1.95 μg/L morphine + morphine metabolites in serum.

The serum or CSF samples for analyses were constructed by transferring 100 μl serum or CSF sample/standard + 100 μl 10 mM ammonium carbonate into 2 ml Eppendorf vortex wells. Of the above mixture 200 μl was transferred into an Oasis HLB Prime well (96 well plate). Each well was washed with 200 μl 95% water/5% methanol, dried under vacuum and then eluted with 250 μl 50/50 methanol/acetonitrile containing 5 μg/L morphine-d3. Eluates were collected in a 350 μl collection plate, sealed with a silicon pad and transferred to the autosampler for analysis.

***Analyses of Morphine by LC-MSMS***

A Waters Acquity ultra performance liquid chromatograph (UPLC) coupled to a Xevo TQ-MS mass spectrometer (MS/MS) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS/MS analysis. Separation was achieved on an Acquity UPLC BEH C18 (2.1 x 100 mm; 1.7 μm particle size) column at 40 ˚C and a flow rate of 0.35 ml/min. Data was acquired with multiple reaction monitoring (MRM) using electrospray positive ionization. The operating parameters used were as follows: capillary voltage, 3.5 V; cone voltage range, 10-35 V; collision energy range, 5-40 eV; source temperature, 140 ˚C; desolvation temperature, 400 ˚C; desolvation gas, 800 L/h and cone gas, 50 L/h. An injection volume of 5 μl was used and the mobile phase consisted of 10 mM ammonium carbonate in water (A) and 10 mM ammonium carbonate in 25ml water/225 ml acetonitrile (B).

The gradient consisted of a flow rate 0.35 ml/min, starting with 95% A to 100% B over 5 minutes, with a 1 minute wash step at 100% B, followed by re-equilibration to initial

conditions over 2 minutes. Analytes were quantified using the following MRMs: morphine 286 ->152 and 286->165, morphine-d3 289->153, 289->165, morphine-3-β-D-glucuronide 462->286, .0; morphine-6-β-D-glucuronide 462->286. Limit of detection was 0.05 μg/L.

*Statistics*

GraphPad software (GraphPad Software Incorporated, La Jolla, CA, USA) was used for statistical analysis. Chi-square and Fisher's exact two-tailed tests were used to compare categorical data, and the t-test or Mann Whitney U test were used to compare continuous data variables between the groups. Statistical significance was set at $p<0.05$.

**Results**

Baseline characteristics of the infants treated with TH plus morphine are shown in Table 7.1. During the admission, the morphine group had a lower proportion of infants with liver dysfunction and a significantly lower seizure burden (Table 7.2). Other clinical parameters were similar between the groups, with no significantly increased need for ventilation or hypotension requiring inotropic support in the morphine group. Time to discharge was not increased in the therapeutic-hypothermia-plus-morphine group.

*Morphine*

19/23(82.6%) infants in the TH+M group group had samples recorded for morphine and metabolite analyses; 12(63.2%) of them had the full set of analyses performed, including both serum and CSF.

Median concentrations and AUCs of morphine and its metabolites at 24, 72 and 96 hours in serum and at 72 hours in CSF are depicted in Table 7.3. Individual and mean concentrations of morphine and metabolites in serum and CSF are depicted in Figures 7.1-7.2. Relative mean concentrations of morphine and its metabolites between CSF and serum at 72 hours are shown in Table 7.4. Morphine CSF: serum concentration was 2.6 and 3.6 times higher than that of M-3-G and M-6-G, respectively.

There were no differences in the mean serum and CSF concentrations of morphine and its metabolites between the infants with liver dysfunction and those without (Table 7.5).

There was no correlation between morphine serum concentrations and the two metabolites M-3-G and M-6-G (Spearman r correlation coefficients -0.1662 and 0.3556, respectively), nor was there correlation was between M-3-G and M-6-G (Spearman r = 0.2860).

There was positive correlation between serum metabolite to morphine ratios: M-6-G/M and M-3-G/M (Spearman r = 0.9654; P < 0.0001).

**Discussion**

In this study we describe the concentrations in serum and cerebrospinal fluid of morphine and its metabolites, morphine-3-glucuronide (M-3-G) and morphine-6-glucuronide (M-6-G), in a cohort of infants with HIE treated with therapeutic hypothermia. Based on prior literature, we expected to find high concentrations of morphine and its metabolites during the hypothermic period due to reduced liver metabolic activity and renal clearance.

We found median serum morphine concentrations of 6.1 and 7.3 μg/L at 24 and 72 hours, respectively, after a constant infusion at a dose of 25 μg/kg/h for 72 hours (Table 7.3). The serum concentrations were below 20 μg/L at all time points (Figure 7.1). Serum concentrations of morphine required for adequate neonatal/infant analgesia vary widely, and have been reported to range from 4 to 125 μg/L[7,9,12]. The median AUC for serum morphine was 538 and mean 547 μg.h/L.

These results differ from data reported in other studies on neonates with HIE treated with therapeutic hypothermia, with only 50% of our infants with concentrations above 10μg /L up to 72 hours (Figure 7.1). In the majority of these studies, morphine dosage in excess of 10 μg/kg/h resulted in morphine serum concentrations above the generally accepted therapeutic range of 10-40 μg /L (up to 125 μg /L in neonates), with some even in the toxic range >300 μg/L[7,10,13] due to reduced clearance of the drug. One study had a mean serum morphine AUC 32 times greater than what we found at 18 608 (8384)ng/h/ml[7].

There were significant differences in how morphine was administered in these studies compared to ours:

Roka et al[7] used a loading dose of 50-100 μg/kg, followed by an infusion adjusted according to clinical status up to a maximum dose of 30μg/kg/h. Favie et al[10] generally allowed a loading dose of between 50-100 μg/kg, followed by a continuous infusion with doses varying between 5 and 25 μg/kg/h adjusted at physician discretion. In the study by Frymoyer[13], the morphine dosing regimen was decided by the treating clinical team, with different practices between the two centres. In one centre the recommended morphine starting dose was 20 μg/kg/h via continuous intravenous

infusion, with a planned dose reduction to 10 µg/kg/h 24 hours after the onset of hypothermia treatment. At the other centre, morphine was given either at a dose of 40 µg/kg every 6 hours or as a continuous intravenous infusion of 10-20 µg/kg/h. At both centres, the dose was adjusted based on assessment of clinical need (pain, discomfort, and shivering) and additional intermittent bolus doses of 50–100 µg/kg morphine could be given as determined by the treating clinical team. In contrast, loading doses were not administered in our study and all the infants received a standard dose of 25 µg/kg/h by continuous infusion from the onset of therapeutic hypothermia for 72 hours. The differences in dosing regimens likely played a role in the significantly higher concentrations achieved in these studies compared to our study.

Importantly, some studies[7,14] showed that infants on morphine had more complications such as increased need for ventilation or circulatory support and increased length of hospital stay without added neuroprotection. In our study we found the opposite: no significant increase in ventilatory or circulatory support or increased duration of admission.

Morphine metabolite concentrations were also much lower than has been reported in literature. Neither the M-3-G nor M-6-G concentrations exceeded 50 µg/L at any time point, whereas in the one study where some of the infants received morphine at similar dosages to ours during hypothermia[10] M-3-G and M-6-G concentrations reached 931 and 211 µg/L respectively.

Median concentrations of morphine, M-3-G and M-6-G at 72 hours in the CSF were all below 10 µg/L (Table 7.3). However, we have not found studies in newborns looking at CSF morphine and/or metabolite concentrations to determine whether these levels

were within acceptable therapeutic range or not. One study in older children with leukaemia and not subjected to hypothermia demonstrated maximum CSF concentrations of 16ng/ml(μg/L) and 34ng/ml of morphine (2 hours) and M-6-G (8 hours), respectively, after a single intravenous dose of morphine[15]. Due to the fact that our infants had HIE, received therapeutic hypothermia, and were more immature we might have expected higher concentrations than those demonstrated in older children.

We found no correlation between morphine serum concentrations and the two metabolites M-3-G and M-6-G, nor between the metabolites themselves. However, we did find a significant positive correlation between ratios of metabolites to morphine (M-3-G/M and M-6-G/M (Spearman r = 0.9654; P< 0.0001). This might suggest that the capacity for morphine glucuronidation was not saturated (taking into consideration that no loading dose or dose changes were made and generally higher morphine infusion rates were administered in our study in comparison to the other studies). Another factor that could possibly support this is the fact that there was no significant liver injury in our cohort, with very few infants with ALT >100 U/L. This would indirectly indicate that the metabolic activity of the liver, and hence glucuronidation, was not greatly affected.

A systematic review[16] of 1232 patients that included neonates from the pre-cooling era looked at factors that influence the ratios of morphine and its metabolites in both serum and CSF. They found that metabolite concentrations were increased in the presence of renal impairment, there was good correlation between the serum levels of the two metabolites regardless of clinical situation, and relative morphine CSF concentrations were 4 times higher than those of the metabolites. We also found that the relative morphine CSF penetration was higher than that of the metabolites in our

study, with CSF:serum concentration of morphine 2.6 and 3.6 times higher than that of M-3-G & M-6-G, respectively (Table 7.4). We did not find higher concentrations of metabolites in patients with renal impairment in our cohort (data not shown), nor did we find higher concentrations of morphine or metabolites in those with liver dysfunction (Table 7.5). This may be due to the relatively small numbers.

Various possible reasons were considered for the different pharmacokinetics found in our study.

While it is known that the risk of incorrect drug concentration is increased while preparing small volume infusions for neonates in the ward [17], an attempt was made to mitigate this risk by having a standard infusion prescription for all these infants and as far as possible, having a senior investigator double check all the prescriptions of enrolled infants.

Another possibility considered is that of the use of expired drug. While drug storage conditions and controls are quite strictly enforced at TBH, in the unlikely event that this was the case, it would appear from the Shelf Life Extension Program program of the FDA[18] that morphine has quite extended validity (>7 years) beyond the recorded expiry dates.

The possibility that the infants were not significantly hypothermic to affect morphine metabolism is another consideration, but this was unlikely as we have shown our ability to maintain the temperature within the target range[19].

Other possible factors that could account for the unexpected results in our study are genetic polymorphisms affecting transport, metabolism, pharmacodynamic factors, etc. Studies, mainly in adult cancer and post-operative patients, have found conflicting

results with respect to the role of polymorphisms in response to morphine and which responses are clinically significant. Some have suggested that combinations rather than single genetic variants (e.g. OMRM1 & COMT) may be responsible for variations in morphine response. However, it is thought that physical factors in the patient such as liver or kidney dysfunction and age may have more of an influence on pharmacokinetics. Some drugs are thought to up- or down-regulate expression of some pharmacologically important gene products. Thus, variability in response between individuals may be due to a combination of several genetic variants and non-genetic factors acting together[20] and it is possible that this may have a role in our results.

Much has been written about inter-individual and inter-/intra-ethnic differences in response to morphine based on variations in genes coding for receptors or metabolising enzymes such as the uridine diphosphate-glucuronosyltransferase-2B7 (UGT2B7) isoenzyme. The human UGT-glucuronosyltransferase superfamily contains more than a dozen members with important roles in the transfer of the glucuronic acid group of uridine phosphoglucuronic acid to various endogenous and exogenous compounds and facilitating their excretion in bile or urine. Morphine is conjugated by the cation of Phase II enzyme UGT into morphine-3-glucuronide and morphine-6-glucuronide. The isoenzyme UGT2B7 is the major enzyme responsible for morphine glucuronidation, while UGT1A1 plays a more minor role.

Hirota described differences in UGT2B7 alleles and μ opioid receptors (MOR1) in 2 cancer patients with differing morphine response, and also differences in UGT2B7 alleles amongst a Japanese population[21]. Similarly, Zhang found different variants of UGT1A1 among different groups within a Chinese population, which resulted in

different glucuronidation activity[22]. On the other hand, two studies found racial differences in UGT2B7 and UGT1A1 haplotypes among African-American, Caucasian and Japanese groups in both paediatric and adult populations, although there was not always direct association with morphine clearance[23,24]. A study looking at long-term effects of morphine in preterm infants, found that the effects were influenced by genetic variants in the metabolic pathway (particularly UGT1A1 and COMT) but that there was a complex interaction between these genetic and clinical factors that influenced outcome[25].

All these studies support the possibility of a significant contribution of genetic polymorphisms (in particular of the UGT2B7 isoenzyme), probably in combination with other clinical factors, in accounting for the different findings in our study. This deserves further exploration in future studies.

Finally, despite the differences in the pharmacokinetic data found in our study, it is reasonable to accept that this simple validated method, that includes solid-phase extraction, enabled us to quantify high recoveries of morphine and its metabolites in plasma and CSF samples.

**Conclusion**

We found that continuous morphine infusion at a dose of 25 μg/kg/h for 72 hours was tolerated well in this cohort of infants treated with therapeutic hypothermia, and did not result in serum concentrations that would be considered toxic. Serum concentrations were within or below the generally accepted therapeutic range in the first 72 hours. We also demonstrated that morphine penetrates the CSF in higher concentrations relative to the glucuronide metabolites.

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**Table 7.1: Baseline characteristics of patients in TH+M group**

| | **Hypothermia + Morphine (Group 1) N=23** |
|---|---|
| Maternal age, mean (SD), years | 26.1 (6.2) |
| Primiparity, n (%) | 13 (56.5) |
| Inborn, n (%) | 11 (47.8) |
| Gestational age, mean (SD), weeks | 38.86 (1.46) |
| Birthweight, mean (SD), grams | 3263 (646) |
| Male, n (%) | 15 |
| Head circumference, mean (SD), cm | 35.1 (1.9) |
| Sentinel event*, n (%) | 7 (30.4) |
| Apgar at 5 minutes <5, n (%) | 7 (30.4) |
| pH within 1st hour <7, n (%) | 11 (47.8) |
| Time to admission, h mean (SD) | 4.4 (1.3) |
| Time to target (33.5°C), h mean (SD) | 7.1 (2.8) |
| HIE 2#, n (%) | 21 (91.3) |
| HIE 3#, n (%) | 2 (8.7) |

TH+M: therapeutic hypothermia plus morphine

* Sentinel event: uterine rupture, abruptio placentae, cord prolapse, acute exsanguination

#HIE 2: Thompson score 7-14 (or moderately abnormal aEEG); HIE 3: Thompson score ≥15 (or severely abnormal aEEG)

**Table 7.2: Clinical course and complications in the two treatment groups**

| Clinical course | Hypothermia + Morphine (Group 1) N=23 | Hypothermia only (Group 2) N=22 | P-value |
|---|---|---|---|
| Ventilated, n (%) | 16 (69.6) | 11 (50) | 0.231 |
| Duration of ventilation, days Mean (SD) | 4.36 (4.03) | 2.86 (3.71) | 0.206 |
| MAS/PPHN, n (%) | 5 (21.7) | 4 (18.2) | 1.000 |
| Inotropic support, n (%) | 6 (26.1) | 4 (18.2) | 0.722 |
| Coagulation abnormality, n (%) | 11 (47.8) | 13 (59.1) | 0.554 |
| Thrombocytopaenia, n (%) | 11 (47.8) | 8 (36.4) | 0.550 |
| Culture-positive infection, n (%) | 4 (17.4) | 1 (4.5) | 0.346 |
| NEC, n (%) | 1 (4.3) | 1 (4.5) | 1.000 |
| Renal dysfunction, n (%) * | 5 (21.7) | 8 (36.4) | 0.337 |
| Creatinine, mean (SD) | 57.77 (30.57) | 62.23 (30.18) | 0.376 |
| Creatinine, median (range) | 55 (12-201) | 59.5 (14-174) | 0.295 |
| Liver dysfunction, n (%) * | 2 (8.7) | 8 (36.4) | **0.035** |
| ALT, mean (SD) | 54.69 (62.81) | 77.38 (92.67) | 0.133 |
| ALT, median (range) | 28 (11-319) | 37 (13-452) | 0.119 |
| Hypoglycaemia, n (%) # | 9 (39.1) | 8 (36.4) | 1.000 |
| Hyperglycaemia, n (%) | 4 (17.4) | 7 (31.8) | 0.314 |
| Significant seizure burden, n (%)$ | 3 (13.0%) | 13 (59.1%) | **0.002** |
| LOS, days - mean (SD) | 12.7 (5.9) | 11.9 (6.9) | 0.699 |

*Transient, not severe. ; # Both early transient & requiring treatment (only 1 required treatment, in TH only group).

## Both early transient & requiring treatment (4 in TH only and 1 in TH+M group needed treatment)

$ needing 2 or more anti- epileptic drugs

MAS: meconium aspiration syndrome; PPHN: persistent pulmonary hypertension of the newborn; NEC: necrotising enterocolitis. ALT: alanine transaminase. LOS: length of stay

**Table 7.3: Serum and CSF concentrations of morphine and metabolites in whole group**

| | Serum – median (range), μg/L | CSF – median (range), μg/L |
|---|---|---|
| Morphine 24h | 6.05 (1.20-16.26) | |
| Morphine 72h | 7.27 (1.91-14.10) | 5.09 (1.39-8.65) |
| Morphine 96h | 1.17 (0.21-7.30) | |
| AUC | 537.5 (112.8-1053.0) | 201.6 (45.9-285.5) |
| M-3-G 24h | 9.35 (2.07-34.40) | |
| M-3-G 72h | 10.88 (3.99-49.45) | 4.08 (0.79-8.71) |
| M-3-G 96h | 4.60 (0.66-18.10) | |
| AUC | 887.2 (164.1-3090.0) | 134.6 (26.1-287.4) |
| M-6-G 24h | 1.97 (0.40-8.46) | |
| M-6-G 72h | 2.63 (0.91-13.99) | 0.65 (0.13-1.48) |
| M-6-G 96h | 1.12 (0.19-4.27) | |
| AUC | 188.9 (35.0-832.7) | 21.5 (4.3-48.8) |

AUC: area under curve; M-3-G: morphine-3-glucuronide; M-6-G: morphine-6-glucuronide

**Table 7.4: Relative CSF penetration of morphine and metabolites**

| | Ratio of mean concentrations |
|---|---|
| CSF:serum morphine | 0.64 |
| CSF:serum M-3-G | 0.25 |
| CSF:serum M-6-G | 0.18 |

M-3-G: morphine-3-glucuronide; M-6-G: morphine-6-glucuronide

**Table 7.5: Drug levels in those with or without liver dysfunction**

| Sample | Liver dysfunction Mean (SD) N=2 | No liver dysfunction Mean (SD) N=8 | P |
|---|---|---|---|
| 24h serum morphine | 7.490 (6.364) | 7.004 (3.923) | 0.876 |
| 72h serum morphine | 8.370 (6.718) | 8.109 (3.662) | 0.930 |
| 96h serum morphine | 3.160 (3.338) | 2.425 (2.382) | 0.695 |
| 72h CSF morphine | 4.780 (3.734) | 5.270 (2.571) | 0.818 |
| 24h serum M-3-G | 14.905 (7.856) | 12.985 (9.830) | 0.795 |
| 72h serum M-3-G | 11.905 (8.577) | 15.063 (12.324) | 0.733 |
| 96h serum M-3-G | 5.255 (4.716) | 6.092 (5.421) | 0.838 |
| 72h CSF M-3-G | 5.145 (5.042) | 3.410 (2.287) | 0.414 |
| 24h serum M-6-G | 3.410 (2.051) | 2.977 (2.304) | 0.803 |
| 72h serum M-6-G | 2.685 (1.676) | 3.569 (3.222) | 0.713 |
| 96h serum M-6-G | 1.325 (1.195) | 1.529 (1.363) | 0.843 |
| 72h CSF M-6-G | 0.9150(0.799) | 0.557 (0.350) | 0.282 |

CSF: cerebrospinal fluid; M-3-G: morphine-3-glucuronide; M-6-G: morphine-6- glucuronide

**Figure 7.1: Individual and mean serum concentrations of morphine and metabolites**

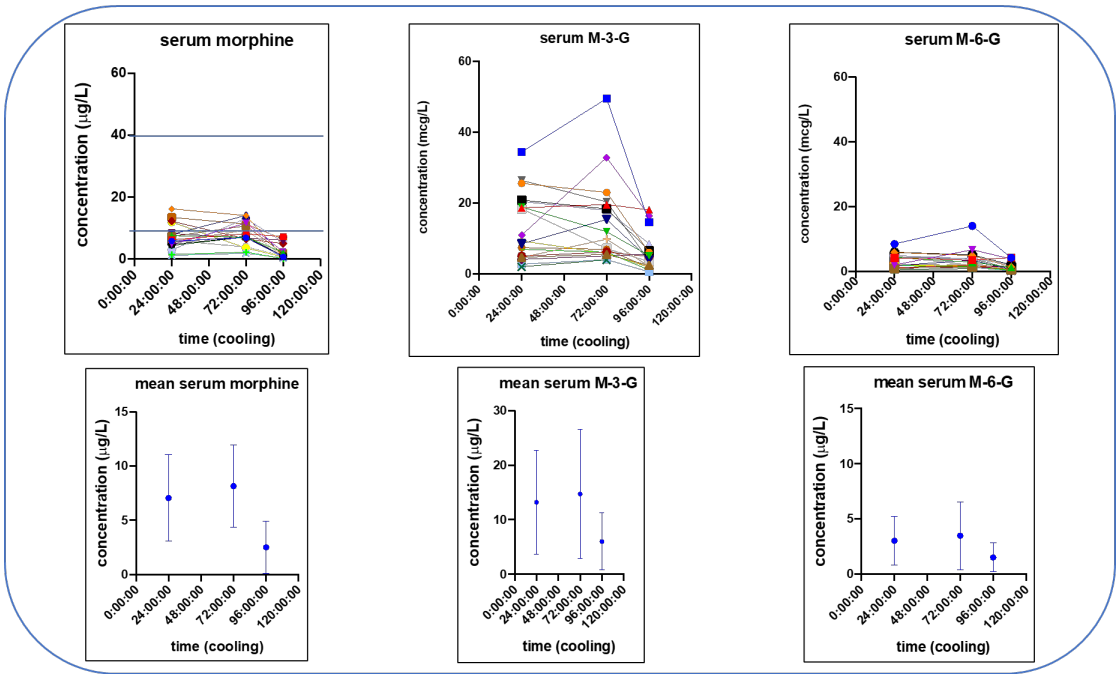
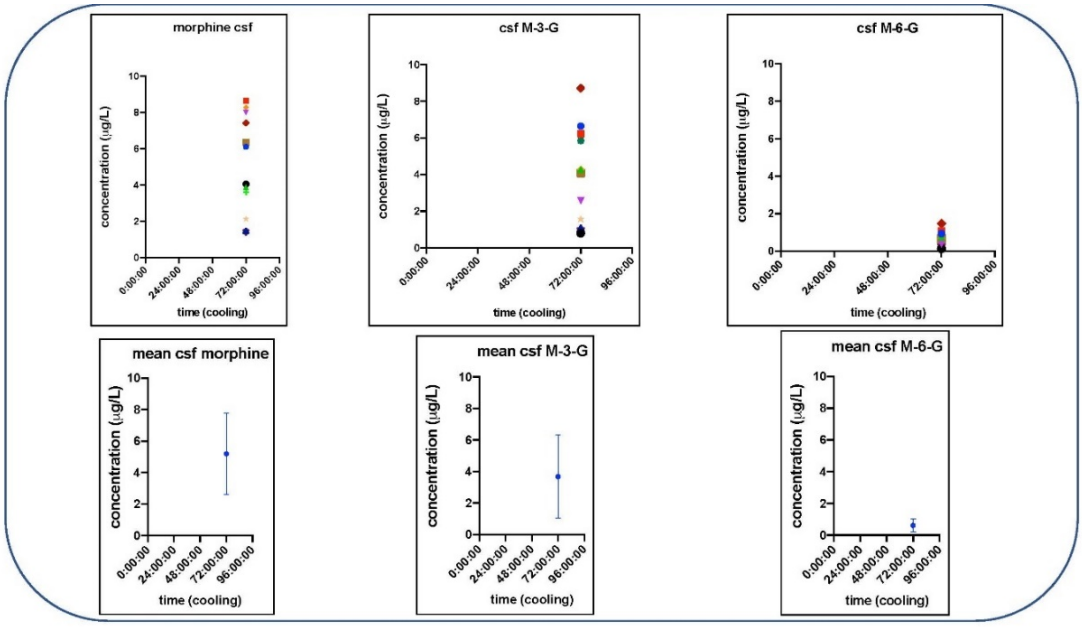



**Figure 7.2: Individual and mean CSF morphine and metabolite concentrations**



# CHAPTER 8

## Addendum

In chapters 1 and 2, we discussed the importance of identifying and selecting infants for neuroprotective therapies correctly, and having simple tools for achieving this goal. This study assessing the role of nucleated red blood cells in predicting disease severity and outcome in HIE presents the output from a masters thesis that I conceptualized and supervised.

### Nucleated RBCs in neonates with HIE treated with hypothermia

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### Introduction

Neonatal hypoxic ischaemic encephalopathy (HIE) or perinatal asphyxia is a leading cause of term neonatal death world wide with a higher incidence in middle and low income settings (1). To date therapeutic hypothermia (TH) is the only interventional strategy internationally accepted as effective in neonatal HIE(2). We have previously demonstrated its safety and efficacy in a sub Saharan setting (3). The majority of high risk neonates present with low Apgar scores at delivery and would require active resuscitation. Current clinical practice would recommend clinical assessment of the neonate, combined with evidence of fetal distress and hypoxia from both cardiotocography and umbilical cord pH to confirm a diagnosis of probable HIE . Active TH is then started as soon as possible, usually following confirmation of abnormal brain activity on cerebral function monitoring (CFM), if available(4). In low and middle income settings, CTG facilities, blood gas machines and CFM equipment

may not be universally available, particularly at district level hospitals in South Africa where a significant number of asphyxiated infants are born.

There is a need, 1therefore, for simple markers that can reliably identify at risk infants and especially those asphyxiated infants that are most likely to respond to treatment.

There are several published studies reporting an association between the number of neonatal nucleated red blood cells (NRBC) and perinatal asphyxia (5); demonstrating a significant association between absence of fetal heart rate accelerations and elevated NRBC in asphyxiated neonates(6), and a correlation with increased NRBC and degree of acidosis and low Apgar scores (7). Tungalag et al found that increased cord blood NRBC in asphyxiated infants predicted the occurrence and severity of HIE, proposing its use as an inexpensive assessment tool in settings where there are no facilities for blood pH measurements (8).

A raised NRBC count in the umbilical cord blood, combined with other markers such as lactate, have also been used to predict the severity of HIE and neurodevelopmental outcome in infants not treated with TH (9).

In the TH era, Li and co-workers concluded that NRBC counts could predict neurological outcomes at 2 years in cooled and non-cooled asphyxiated neonates, albeit not as well as a brain MRI at 2 weeks post delivery (10)

In utero subacute or chronic hypoxia stimulates fetal erythropoiesis, resulting in an increased release of immature red blood cells (NRBC) into the fetal circulation with reports suggesting that it takes 4-5h from onset of hypoxia to increase levels of erythropoietin and then approximately 24h for NRBCs to appear on a peripheral film (11). However, acute stress can lead to release of marrow stores of NRBCs in a shorter period (12). NRBC, in combination with other biomarkers, may be useful in identifying those infants where there is no clear sentinel event (13).

Although NRBC can be found in neonatal blood following low risk pregnancy and delivery, the NRBC/100 white blood cells (WBC) seldom exceeds 10/100 WBC (5). There are however several factors that appear to increase NRBC including not only asphyxia, but also prematurity, Rh-sensitization, maternal diabetes mellitus and fetal growth restriction (8,14).

Phelan et al described NRBC > 10/100WBC as a marker for fetal asphyxia (15). Li et al concluded that both the NRBC count per 100WBC and absolute NRBC taken within 6 hours in asphyxiated infants had a significant association with abnormal 2-week MRI findings (P = 0.006) and 2-year neurodevelopmental sequelae (P = 0.021) , in both hypothermic and normothermic infants. The association was stronger for cerebral injury on MRI (10).

Most hospitals in South Africa, including district hospitals, are attached to a functional NHLS laboratory service. Access to FBC is therefore a widely available special investigation. However, the ability of hospitals at different levels to identify NRBC varies with some machines only having the ability to identify the presence of NRBC and requiring referral of the peripheral smear to the main tertiary laboratory for analysis and to quantify the NRBC. While ability to quantify NRBC should pose no additional demand on resources, the turn-around time may vary from a minimum of 30 minutes to 1 hour to a few hours depending on these logistical factors.

This current study sought to determine whether NRBC could be used to predict the degree of severity in hypoxic ischemic encephalopathy in full term neonates in a tertiary referral hospital in South Africa, and whether NRBC levels could predict long-term neurodevelopmental outcome in HIE neonates treated with therapeutic hypothermia in order to identify those who might benefit from additional neuroprotection.

**Specific aims** were, therefore**:**

- To report the NRBC count in the first 6 hours in a cohort of neonates with HIE treated with therapeutic hypothermia (TH) at TBH between 2008 and 2011
- To determine the association between NRBC counts and severity of HIE as determined by the Thompson encephalopathy score
- To determine the association between NRBC counts and neurodevelopmental outcome at 12 months.

## Methodology

This was a retrospective sub-study within a larger study investigating therapeutic hypothermia in neonates suffering from hypoxic ischemic encephalopathy [N10/05/157]

The study was conducted at Tygerberg Hospital (TBH), a tertiary health facility in Cape Town which serves the eastern part of Cape Town metropole as well as the northern and eastern rural districts of the Western Cape.

All neonates with moderate to severe hypoxic ischemic encephalopathy born in Tygerberg Hospital or referred from surrounding districts that meet criteria for therapeutic hypothermia are admitted to the neonatal intensive care unit (NICU).

Infants born at TBH or referring hospitals over a three year period between November 2008 and November 2011, that met cooling criteria (as below) for the treatment of HIE and were admitted to TBH NICU, were included in the study.

Inclusion criteria based on TBH cooling criteria for neonates diagnosed with HIE were:

Infants of ≥36 completed weeks gestation with at least one of the following:

Apgar ≤7 at 10 minutes of age; continued need for resuscitation, including endotracheal intubation or mask ventilation at 10 minutes of age; acidosis within 60 minutes of birth, defined as any occurrence of umbilical cord, arterial or capillary blood pH <7 or base deficit ≥ 16 within 60 minutes of birth.

Infants that met the above criteria were further assessed for neurological abnormality using a combination of modified Sarnat criteria and a Thompson score of ≥10 (16) or presence of seizures by the attending physician.

Exclusion criteria included those neonates that did not meet the above minimal requirements and would not normally be cooled: presence of major congenital abnormalities, prematurity (<37 weeks gestation) and severe growth restriction (birthweight <1.8kg), over 6 hours after delivery at time of assessment, presence of maternal medical illness that could affect NRBC count.

All patients admitted for therapeutic cooling were treated according to a set protocol (3). The treatment included the following:

Patients were cooled using the total body hypothermia for 72 hours using the Tecotherm TSmed 200 N system, and then re-warmed slowly over a minum of 6 hours.

Blood samples, including those for the determination of NRBC count, were taken within the first hour of admission which was within 6 hours of birth, and repeated according to the cooling protocol.

Amplitude integrated electro-encephalogram (aEEG), when available, was applied to monitor brain activity and seizures, and seizures were treated using the TBH neonatal seizure protocol.

Cranial ultrasound imaging was done on all infants within 72 hours of birth.

The Thompson score (16) assessing the severity of HIE was performed on admission and repeated every 24 hours. Encephalopathy grade was assigned based on maximum score after admission to NICU.

The infants were discharged to the neonatal ward following successful therapeutic hypothermia and re-warming, and subsequent to hospital discharge followed up to 1 year of age at the neonatal high-risk clinic where neurodevelopmental assessments were performed by a single neurodevelopmental specialist.

A retrospective review of the clinical notes on Enterprise Content Management (ECM) database and cooling data booklets used routinely for all cooled neonates was performed.

A database with clinical information of these patients from a previous study (N10/05/157) was also accessed and compared with collected data for consistency.

Clinical data of participants is summarised in Table 8.1.

To determine individual NRBC data, the NHLS database system TrackCare (DISA) was accessed. Blood samples were analysed retrospectively.

An automated system was used for detecting presence of and a threshold nucleated red blood cell level of 30/100 WBC, using the Siemens Advia® machine. If the initial machine report was less than 30 NRBC/100 WBC, quantification of the exact number was not performed. If ≥30 NRBC/100 WBC were reported, a smear was done by the haematologist for further manual quantification and description of the NRBC. This is the current standard protocol in the NHLS laboratory at TBH.

The first NRBC samples taken at the time of admission for cooling were used and compared with the first Thompson score subsequent to admission to the NICU (<6 hours of age).

Long term neurological assessment:

A single developmental specialist using the Bayley Scales of Infant Development Third Edition Screening Test (Bayley-111® Screening Test) reviewed all recruited patients in the neonatal high-risk clinic at 3 months and 12 months of age. The 12-month data were used in this study.

Statistical analysis:

All the data considered from the laboratory and ECM was transferred to Excel spread sheets. Data variables were allocated numerical codes and exported to statistical software (ANOVA and NCSS 12 data [NCSS LLC, 329 North East, Kaysville, Utah, USA] analysis software) for further analysis.

Data was presented as means (±SD). The quantitative variables were compared using Student's t-test and analysis of variance (ANOVA).

The association between NRBC proportion and the following outcomes were determined: severity of HIE as determined by maximum Thompson score, neonatal death (short term) and the long-term neurological outcome.

Bivariate data were assessed using Pearson's chi square test and Fisher's test for categorical variables. P-value < 0.05 was considered statistically significant.

Ethical approval was obtained from the Human Research Ethics Committee (HREC) of Stellenbosch University. As this was a retrospective analysis of routinely collected

data for which ethical permission had previously been obtained and no new patient interventions were to be performed, a waiver of individual informed consent was granted by Stellenbosch University's HREC. [**S17/03/066**]. Permission to conduct the study was also granted by the management of TBH.

## Results

A total of 100 patient folders from 1st November 2008 and 30th November 2011 were reviewed for inclusion into the study. Of the total 100 files accessed, 25 were excluded due to significant missing data (those patients that were excluded either did not have a valid NRBC done within 6 hours of life/at commencement of cooling, no clear HIE score documented within 6 hours and no follow up information was available). The remaining 75 infants constituted the study cohort.

The study cohort was categorized according to their Thompson score into the following clinical categories: mild HIE (n=42 [56%]), moderate HIE (n=20 [26.7%]) and severe HIE (n=13 [17.3%]). (Figure. 8.1)

**Figure 8.1*:* Flow chart illustrating patient selection and outcome according to grade of HIE severity. HIE= Hypoxic Ischemic Encephalopathy.**

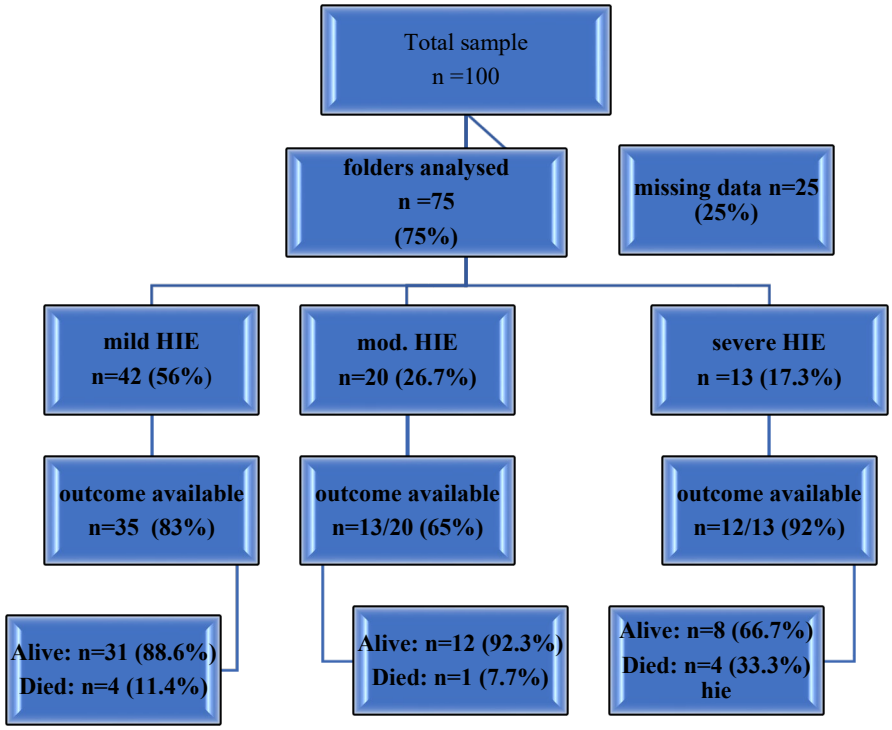


**Table 8.1. Clinical characteristics of cohort**

| Clinical Data | N-75 |
|---|---|
| **Maternal age (mean ± SD), n=73** | 25.9 (±7.3) |
| **Primiparity; n (%)** | 24 (32) |
| **HIV positive; n (%)** | 13 (17) |
| **Male; n (%)** | 46 (61) |
| **Gestational age; weeks (mean ± SD)** | 38.6 (± 4.8) |
| **Birth weight; grams (mean ± SD)** | 3117 (±610) |
| **Apgar score 5'; (mean ± SD)** | 4.7 (±1.6) |
| **Mild HIE; n (%)** | 42 (56) |
| **Moderate HIE; n (%)** | 20 (26.7) |
| **Severe HIE; n (%)** | 13 (17.3) |
| **Neonatal Deaths; n (%)** | 9 (12) |

The mean gestation and birth weight was 38.6 (±4.8) weeks and 3117(±610) grams, respectively. There was no statistically significant association between the gestational age and birth weight, and the degree of HIE (Figures 8.2 and 8.3).

Infants with a gestational age greater than 40 weeks ccounted for 12% (n=9/75) of the cohort and 3 infants had a weight greater than 4000 grams. Infants with fetal growth restriction were excluded.

24 (32%) infants were inborn, while 51 (68% ) were referred in for treatment. There was no statistical difference in the severity of encephalopathy between the inborn infants and the outborn infants.

**Figure 8.2: The relationship between the birth weight and the severity of hypoxic ischemic encephalopathy.**

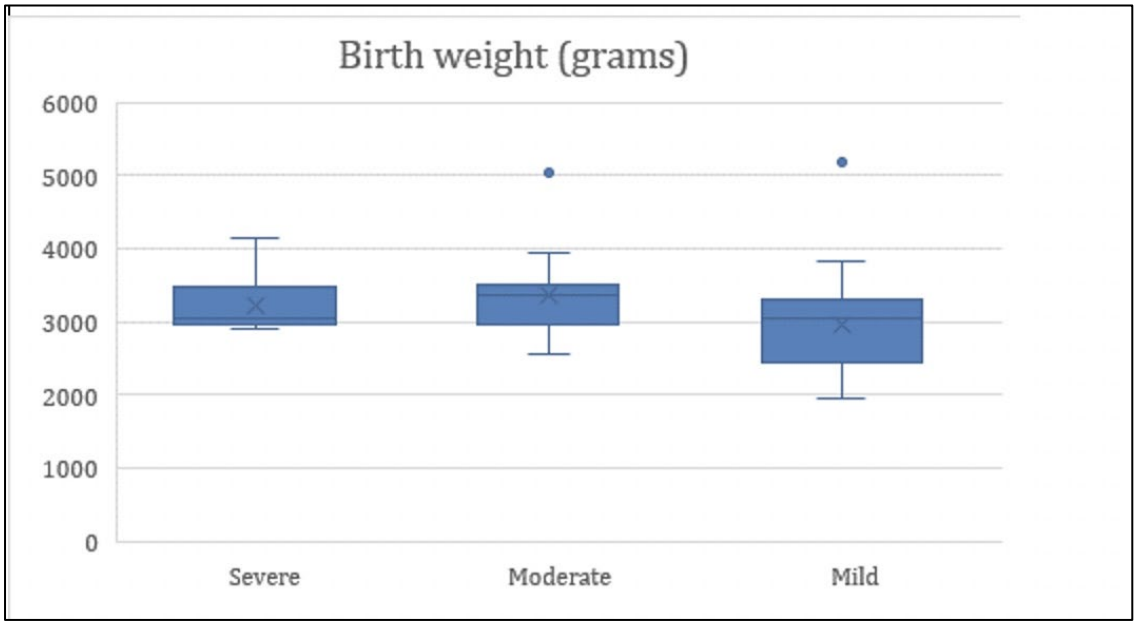
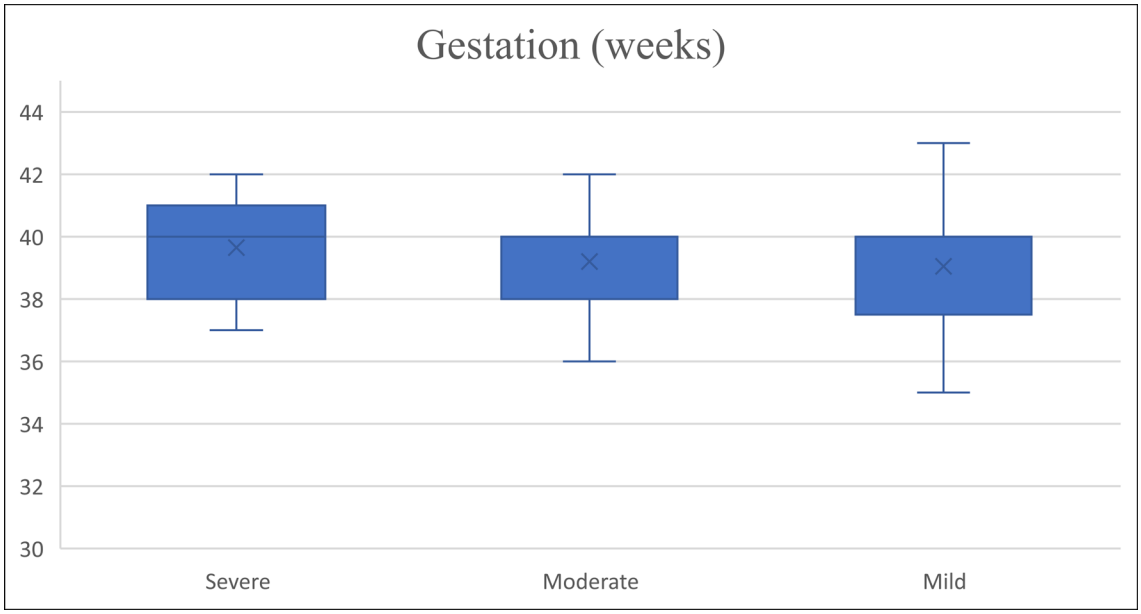


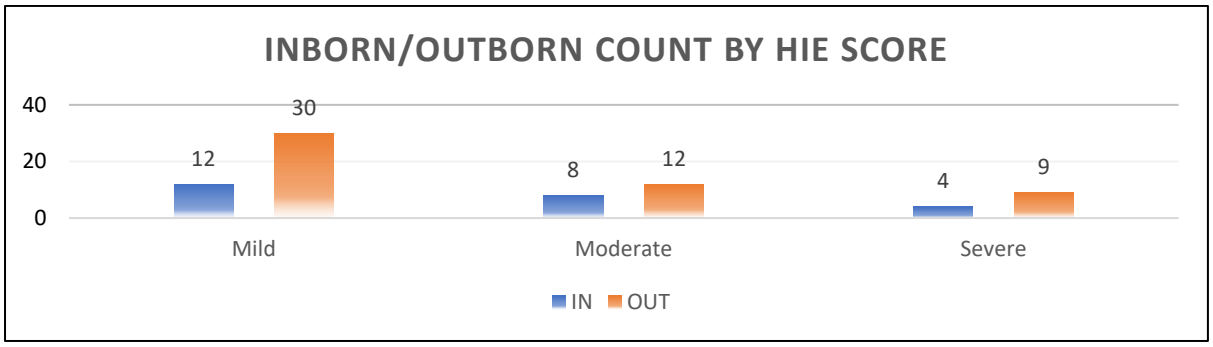

**Figure 8.3: The relationship between gestational age and severity of hypoxic ischemic encephalopathy.**




The categorization of the severity of HIE (mild ≤ 10, moderate 11-14, severe ≥15) in the inborn infants was as follows: mild HIE 50% (12/24), moderate HIE 33.3% (8/24) and severe HIE 16.7% (4/24); and in those that were referred (outborn) for cooling: mild HIE 58.8% (n=30/51); moderate HIE 23.5% (n=12/51) and severe HIE 17.7% (n=9/51). There was no statistically significant difference between the proportions of

moderate-severe HIE in the inborn and outborn infants (inborn 12/24 vs outborn 21/51; P=0.473), (Figure 8.4).

**Figure 8.4. Comparing the severity of hypoxic ischemic encephalopathy in inborn infants to out-born infants.**




All the patients (n=75) had full blood counts (FBC) done within the stipulated first 6 hours from delivery. NRBC were detected in 39 (52%) of the samples. The distribution of the NRBC in the 75 samples shown in Table 8.2. On days 2 and 3, only 23 and 11 patients had reported NRBCs, respectively.

50% of neonates with mild HIE had no documented NRBC, compared with 55 % of neonates with moderate HIE and 31% of those with severe HIE. There was no significant association between the category of NRBC and HIE severity (p=0.265).

The median NRBC value in the infants ≥ 30/100 was 83 (37 - 141).

**Table 8.2. Distribution of NRBC in the different HIE categories.**

| | **Total** | **mild HIE n=42** | **moderate HIE n=20** | **severe HIE n=13** |
|---|---|---|---|---|
| **NRBC = 0** | n = 36 | 21/42 (50%) | 11/20 (55%) | 4/13 (30.8%) |
| **NRBC = 1-29** | n = 31 | 18/42 (42.9%) | 5/20 (25%) | 8/13 (61.5%) |
| **NRBC = ≥ 30** | n = 8 | 3/42 (7.1%) | 4/20 (20%) | 1/13 (7.7%) |
| **Total with NRBC** | N = 39 | 21/42 (50%) | 9/20 (45%) | 9/13 (69.2%) |
| **NRBC ≥ 30 Median (range)** | N =8 | 52 (37 – 72) | 97.5 (63 – 108) | * 141 |

NRBC: nucleated red blood cells/100 white blood cells. HIE: hypoxic ischemic encephalopathy

*only one patient with severe HIE had NRBC≥ 30.

When comparing the long-term outcome of the children when evaluated at 12 months, increased NRBC in the first 6 hours after delivery was associated with a poorer outcome. Those children with NRBC counts of ≥30/100 soon after birth had an increased likelihood of having cerebrtal palsy (CP) or impaired neurodevelopment at long-term follow up (P=0.013; Odds ratio 20.17; 95% CI 1.017 – 399.6); on the other hand, they were likely to be normal at 1 year if the NRBC count was <30/100 [Table 8.3].

**Table 8.3. Association of NRBC with the grading of HIE, the short term and long term outcomes**

| | **NRBC<30** | **NRBC ≥30** | **Test** | **P=value** |
|---|---|---|---|---|
| Mild HIE | 39/67 (58.2%) | 3/8 (37.5%) | Chi-square | |
| Mod/severe HIE | 28/67 (41.8%) | 5/8 (62.5%) | Chi-square | **0.265** |
| NDD/CP | 14/46 (30.4%) | 4/4 (100%) | Fischer | |
| Normal | 32/46 (69.6%) | 0/4 (0%) | Fischer | **0.013** |
| Alive (incl NDD) | 47/52 (90.4%) | 4/8 (50%) | Chi-square | |
| Died | 5/52 (9.6%) | 4/8 (50%) | Chi-square | **0.008** |
| Inborn | 18/67 (26.9%) | 6/8 (75%) | Chi-square | |
| Outborn | 49/67 (73.1%) | 2/8 (25%) | Chi-square | **0.005** |

HIE: hypoxic ischaemic encephalopathy. NDD: neurodevelopmental delay. CP: cerebral palsy.

Similarly, infants that did not survive the initial admission for TH in the neonatal period were more likely to have NRBC ≥30/100 soon after birth when compared to those that survived (P=0.008; Odds ratio 9.40; 95% CI 1.7791 to 49.6649) (Table 8.3). In contrast to this there was no significant association between death and the severity of HIE (P=0.359).

The baseline characteristics and outcome of the 25 excluded patients did not differ significantly from those included in the analysis (Table 8.4). Ten of these infants had NRBC done but no outcome information; 15 had outcome information available (but no HIE grading)– 7 died, 4 had cerebral palsy/neurodevelopmental impairment and 4 were normal. 2/11 (18%) with known poor outcome had NRBC >30.

**Table 8.4: Comparison of included and excluded patients**

| Clinical Data | Included patients N=75 | Excluded patients N=25 | p-value |
|---|---|---|---|
| **Primiparity; n (%)** | 24 (32) | 13 (52%) | 0.095 |
| **HIV positive; n (%)** | 13 (17.3) | 5 (20%) | 0.769 |
| **Male; n (%)** | 46 (61.3) | 10 (40%) | 0.102 |
| **Gestational age; weeks (mean± SD)** | 38.6 (± 4.8) | 39 (±1,9) | 0.656 |
| **Birth weight; grams (mean ±SD)** | 3117 (±610) | 3089 (±495) | 0.820 |
| **Apgar score 5'; (mean ± SD)** | 4.7 (±1.6) | 4.8 (±2.0) | 0.821 |
| **Neonatal Deaths; n (%)** | 9 (12) | 7 (28) | 0.111 |
| **CP/NPP; n (%)** | 18/50 (36) | 4/8 (50) | 0.462 |

## Discussion

In this study we have demonstrated a significant relationship between an early increase of NRBC in asphyxiated infants treated with TH, and both short-term (neonatal death) and long-term outcome (cerebral palsy/neurodevelopmental delay). We also found an association between low NRBC (<30/100 WBC) and normal outcome at 1 year. However, we found no significant relationship between early increase in NRBC and either HIE severity or Apgar score. The lack of significant association with HIE severity may be related to the timing of clinical assessment, as it is known that HIE is an evolving process in the early stages.

Several studies have postulated an increase in NRBC in infants with birth asphyxia. Boskabadi et al found a significant relationship between increased NRBC and infant mortality and morbidity (short-term and long-term outcomes). Among 36 infants who had HIE in their study 16 had adverse outcomes, of whom 10 demised within a month

and 6 developed neurodevelopmental sequelae. They highlighted that the infants with adverse outcomes had significantly increased NRBC/100 WBC [18.63 (±16.63) vs 3.87 (±5.06), p<0.001] compared to those with favourable outcome (17).

The significance of NRBC in asphyxia has been defined both in terms of NRBC/100 WBC and absolute NRBC count. Perrone et al defined the decreasing physiological nature of absolute NRBC in relation to gestation and fetal weight, and the normal reference ranges of absolute NRBC in term and preterm infants (18).

Phellan et al described NRBC > 10/100WBC as a marker for fetal asphyxia (15). Jingang Li et al concluded that both the NRBC count per 100 WBC and absolute NRBC taken within 6 hours in asphyxiated infants had a significant predictive value as a marker for abnormal MRI findings (p = 0.006) and 2-year neurodevelopmental impairment (p = 0.021)(10).

We had set out to test whether in a low resource setting such as South Africa, and where a majority of infants are referred in for cooling, raised NRBC could be a useful marker for those infants that would develop moderate-severe HIE and qualify for cooling and those that would go on to have poor outcome.

In our study 9 patients demised. A larger proportion [4/8 (50%)] of infants with NRBC ≥30/100 WBC died compared to those with NRBC <30/100 WBC [5/52 (9.6%)]; P=0.008. This outcome from this study further affirms available literature, that the higher the NRBC the more severe the short-term adverse outcome, including when early onset seizures are used as a marker of neurological injury (19)

In our study we had a total of 18 infants that developed neurodevelopmental sequelae, while 15 were lost to follow up. Of the 18 with neurodevelopmental impairment,

4/4(100%) had NRBC ≥30/100 WBC and 14/46 (30%) had NRBC <30/100 WBC; P = 0.013. The infants with no follow up did not differ from those whose outcome was known (Table 8.5). Our data were consistent with previously published literature that NRBC can be used as a surrogate marker of birth asphyxia and predictor of long term neurological outcome.

**Table 8.5: Baseline characteristics of infants with known and unknown outcomes**

| Clinical Data | Outcome known N=60 | Outcome not known N=15 | p-value |
|---|---|---|---|
| **Primiparity; n (%)** | 47 (78.3) | 10 (66.7) | 0.824 |
| **HIV positive; n (%)** | 11(18.3) | 3 (20) | 1.000 |
| **Male; n (%)** | 36 (60) | 8 (53) | 0.903 |
| **Gestational age; weeks (mean± SD)** | 38.3 (±5.3) | 39.7 (±1.2) | 0.187 |
| **Birth weight; grams (mean ±SD)** | 3120 (±643) | 3092 (±458) | 0.842 |
| **Apgar score 5'; (mean ± SD)** | 4.5 (±1.6) | 5.4 (±1.7) | 0.078 |
| **Mild HIE; n (%)** | 35 (58.3) | 8 (53.3) | 0.854 |
| **Moderate HIE; n (%)** | 13 (21.7) | 6 (40) | 0.439 |
| **Severe HIE; n (%)** | 12 (20) | 1 (6.7) | 0.501 |

In 1953 Apgar et al published an article proposing a clinical method of assessing newborn infants. Subsequent research on the value of Apgar score has exposed the limitations of this clinical method in assessing asphyxiated infants. The ACOG discussion document (2015) further suggests that it is inappropriate to use the Apgar score to establish the diagnosis of asphyxia as it has a low predictive value for mortality and/or neurological outcome (20). In our study we found this to be consistent with available literature. There was a weak correlation between 5-minute Apgar score and HIE severity (r =0.0203).

In our current study we could not further stratify and describe the range and pattern of NRBC in asphyxiated infants. This is largely due to the post hoc nature of the study, where we could not pre-specify how we required the NRBC to be analysed. The current practice at Tygerberg NHLS is to only quantify NRBC ≥30 /100 WBC count. This current practice and policy is governed by the fact that there is no available local

literature that encourages further quantification of NRBC <30/100WBC count as contributing to clinical care.

Weaknesses and strengths

The strength of our study was that data on cooled infants was collected prospectively, and all infants were routinely followed up for 1 year by one experienced neurodevelopmental expert. Their outcomes were assessed using a standardized neurodevelopmental assessment tool (Bayley Scales of Infant Development Third Edition Screening Test [Bayley-111® Screening Test]).

Our study contributes to local knowledge and the growing interest of the potential association between NRBC, birth asphyxia and short- and long-term outcomes. To our knowledge, it is the first such study to be conducted in the sub-Saharan region.

A limitation of the study was that we could not answer the primary question of the study to the extent envisaged, because prospectively collected data was retrospectively analyzed and the NHLS laboratory were not analyzing NRBC <30/100 WBC at the time the study was conducted. We also could not look systematically at serial measures of NRBC in order to understand the relationship with the perceived injurious process.

The study is further limited as 25% of the selected study population did not meet the inclusion criteria, as either the NRBC count or the 12-month neurological development were not determined.

**Conclusion**

This study showed that NRBC ≥30/100 WBC had a significant correlation with early mortality and neurodevelopmental impairment at 1 year of age in this cohort. To gain a better understanding of the association between NRBC and HIE in the era of cooling in our local setting, there is a need for a larger multi-center study. This large multicentre study should focus on describing normal term infant NRBC in uncomplicated pregnancies and seek to further delineate the association of NRBC with clinical outcomes before it can be applied to select patients for neuroprotective therapies.

**Funding and Conflict of Interest:**

No external funding was provided for this study. The authors declare no conflict of interests

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# CHAPTER 9

## Discussion

### *Context*

Neonatal encephalopathy following intrapartum events accounts for 23% of infant mortality worldwide, with 96% of cases occurring in low-middle income countries (LMIC). The highest mortality is found in low income countries and highest disability in middle income countries where there may be inadequate rather than non-existent neonatal intensive care (1). Neuroprotective therapies are therefore most urgently needed in these settings if we are to improve newborn and child survival and development (2).

Therapeutic hypothermia, the only currently proven treatment for HIE, has been mainly studied in settings that are different from those of LMIC where resource capacity varies widely. Concerns about the applicability of TH in these settings are based largely on data from a very low-income setting which showed increased mortality in cooled infants (3). There were also concerns about converting mortality to increased survival with disability, resulting in even greater burden for low resource settings.

### *What this work has shown*

TH (or cooling) is feasible in a middle-income country when applied within a strict protocol similar to those used in the large published trials, and in an intensive care setting where ventilation facilities are available. Significant proportions of our patients in both cohorts (34% retrospective, 60% prospective) required ventilation.

TH can be optimized by more training in various aspects such as neurological assessment in order to select the most appropriate patients.

It may be possible to use simple blood tests, such a measurement of NRBC counts, to aid in patient selection.

There is a need for development of less costly equipment for both treatment and selection of patients for treatment. Servo-controlled cooling systems achieve better temperature stability and require less nursing input, but currently available devices are generally quite expensive. However, while the cheaper low technology methods of cooling tend to be more labour-intensive (which can be a challenge where

nurse:patient ratios are low), they have been shown in a meta-analysis including studies from low resource countries to be as effective in improving outcomes as the high technology methods when used in an intensive care environment (4). This highlights the fact that it is the close monitoring and maintaining the temperatures within target range that have a greater significance than the actual device used, and absence of high technology devices should not result in deserving infants being deprived of this beneficial therapy.

Our patients did not have more complications compared to the patients studied in high income settings. In particular, we were able to show that the patients in these cohorts did not have higher rates of infection than those in high income countries, with culture confirmed infection rates of 8-11% in the 2 cohorts. This is relevant because there has been a concern that a presumed higher prevalence of infection in LMIC may make TH less effective due to sensitization of the brain by inflammation. Our results are in keeping with literature from Uganda and India, where they showed a prevalence of infection of <10% in encephalopathic infants, similar to rates in high income countries where the cooling trials were performed (5,6).

Outcomes in our cohorts were good, with the majority (>80%) of patients surviving without major neurodevelopmental impairment. This is in contrast with a meta-analysis of TH in LMIC, which showed no beneficial effect of TH and suggested that it should not be offered in these settings until more trials are performed (7). Some of the factors that may have contributed to this negative treatment effect included lack of ventilation and monitoring facilities and no sedation in many infants. Most of the trials in this study also did not have long-term outcome data. A large meta regression analysis including infants from low resource settings showed a reduction in mortality following TH, which was not influenced by the gross domestic product of the country where it was applied (8). It would be of interest to compare the BSID-III scores of our normal survivors with those of a normal local cohort in order to fully assess the impact of the neuroprotective therapies on neurodevelopment in our setting.

Our data showed that morphine co-treatment may augment the neuroprotective effects of TH, and resulted in reduced seizures and liver dysfunction in the randomized trial. It did not cause increased need for ventilation, inotropic support or prolonged hospital stay as shown in previous studies (9,10). The lack of significant effect on the outcome at 18 months may be related to the small sample size, and would need to be

re-tested in a larger trial as there was a trend towards improved survival with normal outcome. The significance of lower liver dysfunction in relation to possible better outcome needs further exploration. One aspect that could be pursued is whether this could be related to levels of ammonia, which is thought to have an effect on cellular glutamate concentrations and neurotransmitter signaling resulting in neural cell damage (11).

We showed that morphine enters the brain (CSF) well, and does so better than its metabolites. We did not demonstrate toxic concentrations at the administered dose of 25μg/kg/h. The serum concentrations were much lower than has been found in literature at doses exceeding 10μg/kg/h with TH (9,12). This could be on the basis of a combination of genetic polymorphisms and non-genetic factors that may be unique to our cohort.

Tools

We have shown that both clinical (Thompson HIE score) and neurophysiological tools (aEEG) are useful for selecting patients for treatment and predicting outcome. This is helpful in settings where costs are a major factor, as shown in the study by Biselele (13). Their predictive ability assists in directing care and utilising limited resources optimally.

The advantages of aEEG are that it is a more objective tool for continuous assessment of brain activity, which can also be sent for a second opinion particularly by more inexperienced staff.

While MRI in the first few weeks after birth is the optimal imaging modality for assessing brain injury and predicting specific outcome, ultrasound is emerging as a promising substitute where MRI is unavailable if the correct scanning protocols and equipment are used (14,15). In our prospective cohort, severe CUS abnormalities were predictive of abnormal outcome.

Where MRI is available, it can have an important role in assessing newer neuroprotective therapies. In our cohorts, we could show that in those infants that were imaged it had a high predictive accuracy (PPV 95%) for later outcome.

### *Limitations*

The loss of aEEG recordings in the prospective cohort meant that they could not be evaluated to confirm their predictive ability, and assess the effect of morphine on the predictive ability.

Follow up is an ongoing challenge in this setting with 33% and 16% of the retrospective and prospective cohorts, respectively, lost to follow up. This is a common problem in similar settings, with a follow up rate of 29% described in another South African study (16) and creative solutions are needed to assess outcomes of therapies in these situations. These could include adaptation and validation of available developmental screening tools such as the Ages and Stages Questionnaire (17) for administration telephonically. However, although most studies recommend follow up to a minimum of 18 months to assess the effects of interventions on developmental outcomes, in our prospective cohort there were similar proportions of normal (84%) and abnormal (16%) development in those infants that were assessed at 12 and 18 month points. This suggests that in settings where follow up is difficult, aiming for a minimum follow up duration of 12 months may be reasonable.

Limited access to MRI meant that infants could not all be imaged in the first 3 weeks as planned. Many infants had to be re-admitted for the scan. This contributed to the loss to follow rate as some did not return. Having early MRI on all the infants would have assisted in inferring outcome even in those that did not attend follow up. New technologies are constantly being developed, such as low field mobile MRI that may play an important role in these settings where access to conventional MRI is a challenge.

### *Strengths*

This work has confirmed that in a middle income setting with adequate facilities for monitoring, TH can be implemented simply using the available protocols, and should be progressively rolled out to where the need is. We have also shown that we have the capability to test other neuroprotective therapies in combination with TH.

These studies are some of the few in LMIC countries that have long term outcome data following the use of TH.

## Future directions

Future research in this field should include:

- Exploration of the mechanism of possible morphine neuroprotection, including its influence on cytokines and chemokines. This work is ongoing.
- Determining the optimal dose of morphine needed for neuroprotection without adverse events.
- Exploring genetic polymorphisms that determine response to morphine. This would allow us to offer tailored treatment to patients.
- Confirming and then exploring the significance and mechanism of lower liver dysfunction with TH and morphine co-treatment
- Multipronged strategies looking at different combinations with TH, eg. EPO (can be used later), drugs that can be given to mother when there is evidence of fetal compromise eg. allopurinol
- Exploring the role of NRBC in selecting patients for neuroprotection, particularly at referring centres where costly neurophysiological tools are unavaible
- Validating tools for assessing outcome telephonically such as the Ages and Stages Questionnaire Third Edition which has already been validated in a South African and Zambian cohort (Hsiao 2016)
- Comparing BSIDIII scores of these infants with those of a normal local cohort
- Assessing use of low field mobile MRI in neonates for predicting outcome

# CHAPTER 10

## Conclusions

Neuroprotective therapies such as therapeutic hypothermia are feasible and necessary in LMIC, and do not result in survival with disability, but must be offered with adequate monitoring to be safe.

Cheaper automated devices for TH and brain monitoring should be developed to make an impact where the need is greatest.

Nucleated red blood cells may be useful in selecting patients for TH.

The routinely used early predictors of outcome have good predictive ability.

Combining TH with morphine did not improve long-term outcome significantly when compared to TH alone, but did improve some early clinical markers of disease.

Morphine pharmacokinetics in this population were different from what has been described in literature, and require further exploration in future studies.

Long-term follow up studies in these settings are needed, as well as other surrogate tools to determine outcome as follow up is usually challenging with high attrition rates.

There is an urgent need to have the ability to predict outcome at the time of discharge, and innovative tools such as mobile low field MRI may play an important role in this respect.

Future work should look at combination therapies with TH while exploring mechanisms in which they may act.