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Karoline Aker

Perinatal asphyxia in a global perspective

How can we improve outcomes?

NTNU
Norwegian University of
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Thesis for the Degree of Philosophiae Doctor

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Norwegian University of Science and Technology
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Fødselsasfyksi i et globalt perspektiv: hvordan kan vi forbedre utkommet?

Fødselsasfyksi er en tilstand der mangelfull gassutveksling og/eller blodstrøm fører til oksygenmangel til fosteret i tiden omkring fødsel. Dette kan påvirke alle organer, men skade på hjernen, hypoksisk iskemisk encefalopati (HIE), er den mest fryktede komplikasjonen på grunn av dens alvorlige konsekvenser. Fødselsasfyksi er den viktigste årsaken til varig hjerneskade hos terminfødte barn og omkring 1 million nyfødte dør og 1.2 millioner barn overlever med funksjonshemning som følge av denne tilstanden hvert år. Forekomsten av fødselsasfyksi og HIE er viktige indikatorer på mødre- og nyfødthelse, men det er vanskelig å sammenlikne forekomsten mellom studier og land fordi ulike definisjoner brukes.

Nedkjøling til en kjernetemperatur på 33-34°C i 3 døgn, igangsatt innen 6 timer etter fødsel, reduserer forekomsten av død og alvorlige utviklingsforstyrrelser blant barn med moderat til alvorlig HIE i høyinntektsland. Studier fra lav- og middelinntektsland, der >95% av dødsfall forårsaket av asfyksi og HIE forekommer, har ikke kunnet reprodusere den positive effekten av denne behandlingen. Det er derfor stor usikkerhet rundt hvorvidt kjølebehandling bør anbefales til nyfødte med HIE i lav- og middelinntektsland.

Dette doktorgradsarbeidet har som hovedformål å bedre utkommet til barn med fødselsasfyksi i et globalt perspektiv. Arbeidet består av en behandlingsstudie av kjølebehandling ved et sykehus i India og en retrospektiv, populasjonsbasert observasjonsstudie på forekomst og utkomme etter fødselsasfyksi i Sør-Trøndelag.

Den første studien var en randomisert kontrollert studie ved Christian Medical College i Vellore i Sør-India der 25 barn fikk kjølebehandling og 25 barn fikk standard støttebehandling med normal kroppstemperatur. Kjølebehandlingen ble gjennomført ved hjelp av en billig, lokalt utviklet kjølemadrass. Det primære endepunktet i studien var tegn til hjerneskade på MR tatt ved 5 dagers alder. Vi fant at de kjølte barna hadde mindre hjerneskade enn de som fikk standard behandling målt med ulike MR metoder. De kjølte barna hadde også lavere forekomst av død eller utviklingsforstyrrelse ved 18 måneders alder. Konklusjonen på studien var at kjølebehandling var trygg og effektiv på det aktuelle sykehuset, men at det trengs mer forskning på hvilket nivå av støttebehandling som er nødvendig.

Vi undersøkte også hvor godt MR og analyse av barnets spontanbevegelser ved 3 måneders alder (General Movements Assessment / GMA) kunne forutsi hvilke barn som fikk en utviklingsforstyrrelse, og vi fant at begge disse undersøkelsene var gode. GMA er en enkel undersøkelse som gjøres ved å ta en kort videofilm av barnet. Det kan derfor med enkle grep innføres i en lavressurssetting der MR ikke er tilgjengelig for å plukke ut de barna som trenger videre oppfølging.

I Norge mangler nyere studier over forekomst og effekt av innføring av kjølebehandling. I den andre studien undersøkte vi derfor forekomst av og utkomme etter fødselsasfyksi og HIE i Sør-Trøndelag i en 9-års periode før og etter innføring av kjølebehandling i juni 2007. Vi inkluderte alle barn som ble innlagt med fødselsasfyksi ved nyfødtavdelingen, St. Olavs hospital, fra 2003-2011. Med detaljert gjennomgang av medisinske journaler og data fra Norsk kvalitets- og

oppfølgingsregister for cerebral parese (NorCP) undersøkte vi om det har vært endringer i innleggelsespraksis til nyfødtavdelingen etter 2007, i hvor stor grad kjølebehandling har vært benyttet til de barna som oppfyller internasjonale behandlingskriterier og hvor stor andel av barna som døde eller utviklet CP.

Resultatene viste at forekomsten av HIE økte etter innføringen av kjølebehandling til tross for lik innleggelsesrate for fødselsasfyksi. Dette mener vi kan skyldes økt oppmerksomhet for nevrologiske symptomer og tegn. I tillegg fant vi at en tredjedel av de nyfødte med moderat til alvorlig HIE ikke ble kjølebehandlet fordi de ikke ble identifisert før etter 6 timers alder. Disse barna hadde høyere forekomst av død eller CP ved 9-10 års alder sammenliknet med barna som fikk kjølebehandling. Basert på disse funnene anbefaler vi at det gjøres mer forskning på hvordan helsepersonell tidlig kan fange opp de barna som kan ha nytte av kjølebehandling. Det er også behov for mer kunnskap om hvilke kriterier som på en objektiv og enkel måte kan brukes til å plukke ut de rette pasientene til denne effektive, men også intensivkrevende behandlingen.

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Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i medisin.

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Perinatal asphyxia in a global perspective: How can we improve outcomes?

Perinatal asphyxia is a condition characterised by reduced blood- and/or oxygen supply to the foetus around the time of birth. It can affect all organs, but an injury to the brain is the most feared complication due to its irreversible nature and devastating consequences. The disturbed brain function caused by perinatal asphyxia is the condition called hypoxic-ischaemic encephalopathy (HIE). Perinatal asphyxia and HIE continue to be the leading cause of permanent brain injury in term-born infants, and it is estimated that around 1 million infants die and 1.2 million infants survive with disability every year. The incidence of perinatal asphyxia and HIE are important indicators of maternal and newborn health, however, as definitions vary between studies, it is challenging to compare temporal and geographical trends.

Therapeutic hypothermia or cooling to a core body temperature of 33-34°C for 72 hours started within 6 hours after birth, reduces the combined outcome of death and severe disability (including cerebral palsy (CP)) in infants with moderate or severe HIE in high-income countries. Studies from low- and middle-income countries, where more than 95% of deaths caused by perinatal asphyxia and HIE occur, have failed to reproduce the same positive effect of this treatment. It is, therefore, still a knowledge gap on whether cooling should be recommended for newborns in low-and middle-income countries.

The overall aim of this PhD thesis was to improve outcomes for infants exposed to perinatal asphyxia and HIE in high- as well as in low- and middle-income countries. The PhD project consists of one intervention study of therapeutic hypothermia at a hospital in India and one retrospective, population-based observational study on incidence and outcome after perinatal asphyxia in the county of Sør-Trøndelag in Norway.

The first study was a randomised controlled trial at the Christian Medical College in Vellore in south India where 25 infants received hypothermia and 25 infants received standard care with normothermia. Hypothermia was induced by a locally developed, low-cost cooling device. The primary outcome was brain injury on MRI at five days of life. We found that cooled infants had less brain injury on MRI compared to non-cooled infants. The cooled infants also had a lower occurrence of death or disability at 18 months of age. We concluded that therapeutic hypothermia is neuroprotective and safe when provided in this hospital, but more research is needed to identify the adequate level of supportive care during cooling.

We also explored how well MRI and analyses of the infants' spontaneous movements at 3 months of age (General Movements Assessment / GMA) could predict developmental disorders at 18 months of age, and we found that both these assessments were good. GMA is a simple assessment based on a short video film of the infant. It can, therefore, be

implemented in a low-resource setting where MRI is not available to select the correct infants for further follow-up.

There are no recent studies on the incidence of perinatal asphyxia and HIE in Norway, and no studies investigating the effect of the implementation of therapeutic hypothermia in clinical practice. In the second study, we explored the incidence and outcomes after perinatal asphyxia and HIE in Sør-Trøndelag, Norway, in a 9-years period before and after the implementation of therapeutic hypothermia in June 2007. We included all infants admitted for neonatal care due to perinatal asphyxia to the neonatal intensive unit at St. Olavs Hospital between 2003 and 2011. Based on a detailed review of medical records and data from the Norwegian Quality and Surveillance Register for Cerebral Palsy (NorCP) we explored whether admission rates to neonatal care due to perinatal asphyxia and HIE changed after 2007, whether the infants receiving cooling met the international treatment criteria and the occurrence of death and CP in this group.

We found that the incidence of HIE increased after the implementation of therapeutic hypothermia, despite similar rates of admission for perinatal asphyxia. This is possibly explained by increased attention to neurological symptoms and signs. We also found that one-third of the infants with moderate to severe HIE were not offered cooling, mainly due to delayed identification after 6 hours of life. These children had a higher occurrence of death or CP at 9-10 years of age compared to the cooled infants. We hope that this knowledge will help direct research on how health personnel can improve the early diagnosis of HIE. There is also a need for research on more objective and simple criteria to select the correct infants for cooling, which is an effective, but also intensive care demanding treatment.

This thesis has been found worthy of public defence for the degree of PhD in Medicine.

The public defence will take place in LA21 on Friday the 13th of May, 2022, at 12.15 pm.

Acknowledgement

"It always seems impossible until it's done."

Nelson Mandela

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List of papers

Paper I

<u>Aker K</u>, Støen R, Eikenes L, Martinez-Biarge M, Nakken I, Håberg AK, Gibikote S, Thomas N. **Therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy in India (THIN study): a randomised controlled trial.** Arch Dis Child Fetal Neonatal Ed. 2020 Jul;105(4):405-411. doi: 10.1136/archdischild-2019-317311. Epub 2019 Oct 29.

Paper II

<u>Aker K</u>, Thomas N, Adde L, Koshy B, Martinez-Biarge M, Nakken I, Padankatti CS, Støen R. **Prediction of outcome from MRI and general movements assessment after hypoxic-ischaemic encephalopathy in low-income and middle-income countries: data from a randomised controlled trial.** Arch Dis Child Fetal Neonatal Ed. 2022 Jan;107(1):32-38. doi: 10.1136/archdischild-2020-321309. Epub 2021 Jun 10.

Paper III

<u>Aker K</u>, Syltern JM, Martinez-Biarge M, Støen R. **Contemporary trends in admissions for perinatal asphyxia and hypoxic-ischemic encephalopathy – a population-based study.** Submitted.

Other papers published during the PhD

Thomas N, Støen R, Aker K, Martinez-Biarge M, Nakken I, Håberg AK, Eikenes L. **Rise and Fall of Therapeutic Hypothermia in Low-Resource Settings: Lessons from the HELIX Trial: Correspondence.** Indian J Pediatr. 2021 Oct 5. doi: 10.1007/s12098-021-03967-3. Epub ahead of print.

Aker K, Støen R, Martinez-Biarge M, Thomas N. **Questions about the HELIX trial.** Lancet Glob Health. 2021 Dec;9(12):e1651. doi: 10.1016/S2214-109X(21)00496-4. PMID: 34798020.

Abbreviations

ADC	Apparent diffusion coefficient
aEEG	Amplitude integrated electroencephalogram
Bayley-III	Bayley Scales of Infant and Toddler Development, third edition
BGT	Basal ganglia and thalami
Cho	Choline
CI	Confidence interval
CMC	Christian Medical College
CP	Cerebral palsy
Cr	Creatine
CS	Composite score
DAMA	Discharged against medical advice
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
EEG	Electroencephalogram
FA	Fractional anisotropy
FMs	Fidgety movements
FS	Form stable
GA	Gestational age
Gln/Glu	Glutamine/glutamate
GMA	General Movements Assessment
GMDS	Griffith Mental Development Scales
GMFCS	Gross Motor Function Classification Scale

HIE	Hypoxic-ischaemic encephalopathy
ICD-10	International Classification of Diseases, 10th Revision
IQR	Interquartile range
IRR	Incidence rate ratio
LMICs	Low- and middle-income countries
MD	Mean diffusivity
mI	Myo-inositol
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NAA	N-acetyl-aspartate
NE	Neonatal encephalopathy
NICU	Neonatal intensive care unit
NorCP	Norwegian Quality and Surveillance Registry for Cerebral Palsy
NPV	Negative predictive value
PCM	Phase changing material
PLIC	Posterior limb of the internal capsule
PPV	Positive predictive value
RCT	Randomised controlled trial
ROI	Region-of-interest
SC	Standard care
SD	Standard deviation
TBSS	Tract-Based Spatial Statistics
TE	Time to echo
TH	Therapeutic hypothermia
TR	Repetition time
WM	White matter

Background

Perinatal asphyxia

Perinatal asphyxia (from Greek "stopping of the pulse") is a condition of impaired gas exchange across the placenta, leading to progressive hypoxaemia (lack of oxygen) and hypercapnia (increased carbon dioxide levels) in the blood of the fetus and newborn.[1] The impaired gas exchange can occur before, during, or after the delivery. The most common causes are maternal disease (diabetes mellitus, hypertension, preeclampsia, infection, hypotension/shock, uterine rupture, and severe anaemia), placental factors (abruption, fetal-maternal haemorrhage, umbilical cord compression, infection/inflammation, and velamentous cord insertion), and neonatal conditions (airway anomalies, neurologic disorders, severe cardiopulmonary disease, severe circulatory compromise, infection, and medication effect).[2]

History

In early printed obstetric books, perinatal asphyxia was first classified as apparent death ("mors apparens").[3] As early as in 1472, 200 years before oxygen was named, perinatal asphyxia was described by an Italian professor Paolo Bagellardo:[3 4]

"*[The midwife should examine] whether the infant is alive or not, or spotted, that is: whether black or white or of livid colour, and whether it is breathing or not. If she finds it warm, not black, she should blow into its mouth, if it has no respiration, or into its anus.*"

The term asphyxia was introduced in the late 18th century. At that time, there was increased knowledge about this condition of newborns who had difficulties initiating breathing and lacked oxygen.[3] This changed the opinion of an unavoidable "apparent death" to a condition that could be prevented and treated. Virginia Apgar, an American

anaesthesiologist, invented the well-known and still widely used clinical score (Apgar score) in 1953 to assess newborn babies' health immediately after birth and detect asphyxiated babies in need of resuscitation.[5] The score was soon found to be poorly correlated with neurodevelopmental outcomes.[6] Apgar and colleagues became increasingly aware of the respiratory and metabolic acidosis following asphyxia and that pH and base deficit in cord blood was a measure of its severity.[7] Although the term asphyxia already had been in use for 200 years, it was not until 1992 that the American College of Obstetrics and Gynaecology presented the first definition of perinatal asphyxia. This definition included four criteria: pH <7.0, Apgar score <4 at 5 minutes, neonatal neurologic sequelae (such as seizures, coma, or hypotonia) and multiorgan dysfunction.[8] However, the same institution stated in 2005 that the term perinatal asphyxia should not be used due to its poor specificity.[9]

Pathophysiology

Reduced blood- and/or oxygen supply to the fetus or newborn affects all organs and can lead to multiorgan dysfunction. Injury to the brain is of most concern due to its irreversible nature and potential long-term consequences.[10] Reduced blood- and/or oxygen supply causes a redistribution of blood flow to the most vital organs, including the brain.[11] An adaptive decrease of resistance in the cerebral vessels follows hypoxaemia.[12] There is a critical threshold where the blood pressure is too low to maintain blood flow to the brain. If there is only a modest reduction in cerebral blood flow, the brain prioritises maintaining perfusion to the brainstem, cerebellum, and basal ganglia.[13][14] Other adaptive responses, such as using alternative energy sources, occur to preserve the brain, and there is also a protective effect of the fetal haemoglobin.[15] The newborn has, therefore, a high tolerance for hypoxia/ischaemia.[2] However, when the fetal oxygen demand is higher than what is delivered, the energy must be gained through anaerobic glycolysis, causing accumulation of lactic acid and a decrease in pH. Cord blood pH is, therefore, a marker of perinatal asphyxia, although not very precise.[16-18]

The pathophysiological effects of a hypoxic-ischaemic insult are complex and evolve over time. The processes can last for weeks, months or even years after an insult.[19] In the event of asphyxia, there is insufficient delivery of oxygen and energy substrates, such as glucose.

The high-energy phosphate compounds decrease, lactic acid accumulates, and the membrane ion pumps fail.[20] This again leads to an influx of sodium and subsequently water into the cells, causing cell swelling (cytotoxic oedema).[21] Other consequences are an influx of calcium to the cells initiating release and inhibited uptake of neurotransmitters such as glutamate, formation of free radicals, production of nitric oxide, lipid peroxidation of cell membranes, and mitochondrial dysfunction. This is the process of primary energy failure resulting in oxidative metabolism failure, cytotoxic oedema, and accumulation of excitotoxins leading to neuronal cell death via necrosis and also apoptotic cascades are activated.[21 22] During necrosis, the cells swell and rupture, resulting in inflammation. There is also an innate immune response activated by hypoxia.[23]

The oxidative metabolism may partially or entirely recover depending on the duration and severity of the primary energy failure.[24] As reperfusion occurs, there is increased tissue oxygenation, and the neurotoxic cascade is assumed to be inhibited.[25 26] This brief period, where oxidative mitochondrial metabolism recovers and many neurons at least partly recover, is called the latent phase.[27] However, inflammation and the activated apoptotic cascades continue in this phase.[21]

Six to 48 hours after the hypoxic-ischaemic event, there is a phase of secondary energy failure leading to extensive cell death. Although the circulation might be adequate, this phase involves progressive failure of oxidative metabolism. The process is characterised by oxidative stress, excitotoxicity, inflammation due to overproduction of free radicals and excessive levels of extracellular neurotransmitters, and mitochondrial dysfunction, which has a central role in the pathway of delayed programmed cell death.[26 28] This secondary phase causes cell death and irreversible brain injury.[29] The tertiary phase involves different events affecting the brain, such as persistent inflammation, susceptibility to seizures, impaired maturation and connectivity, epigenetic changes, and late cell death, which can persist for months to years.[20 30]
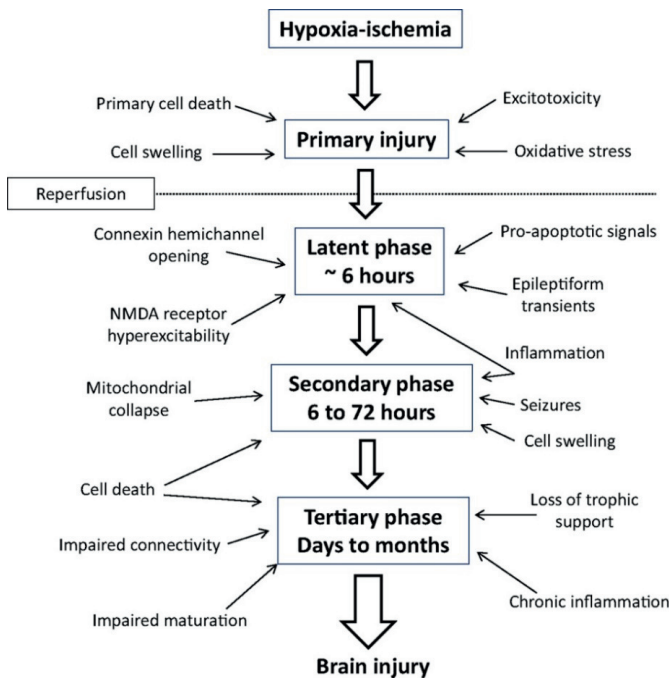
Figure 1: Mechanisms of evolving neural injury in the primary phase, latent phase, secondary phase, and tertiary phase that contribute to long-term brain damage and disability. From Davidson JO et al.[31] Licence: CC BY 4.0.

Hypoxic-ischaemic encephalopathy

Newborns who have been exposed to perinatal asphyxia and present with neurological symptoms shortly after birth are diagnosed as suffering from hypoxic-ischaemic encephalopathy (HIE) or neonatal encephalopathy (NE).[22] Neonatal encephalopathy is a clinical syndrome with disturbed neurologic function regardless of the underlying cause.[32] The clinical symptoms and signs of NE include altered level of consciousness, seizures, tone or reflex abnormalities, and feeding difficulties. This condition can have many causes, including brain malformations, vascular/perfusion-related and neuromuscular diseases, genetic disorders, inborn errors of metabolism, systemic or intracranial infections, and toxic or metabolic disturbances.[33] There are large varieties in the estimated rate of NE that is secondary to perinatal hypoxia-ischaemia.[34] Studies based on clinical data, electroencephalogram (EEG) and magnetic resonance imaging (MRI) have estimated that HIE is the most common cause of NE, accounting for 50 to 80% of NE cases.[10 35-37] On the

other hand, others claim that the contribution of asphyxia to NE is over-estimated, particularly in high-income settings.[38] However, in many cases, it might be difficult to attribute the exact cause of the encephalopathy. This thesis will focus on NE related to hypoxia-ischaemia, and the term HIE will be used for consistency, even when referring to studies where the term NE is used.

Classification of HIE

HIE is the leading cause of brain injury in term newborns. The condition is graded from mild (HIE stage 1) to severe (HIE stage 3) based on the severity of the neurological symptoms and signs. Sarnat and Sarnat made the first classification of HIE in 1976.[39] This classification was based on the clinical course of 21 infants with encephalopathy after a "well-defined episode of fetal distress or an Apgar score of 5 or less at one and five minutes after delivery." Serial neurological examinations and EEG recordings were conducted. Mild HIE was characterised by increased muscle tone and behavioural abnormalities with irritability, excessive crying, and/or sleepiness of only a few days duration. A neonate with moderate HIE was described as lethargic or had reduced arousal, with significant hypotonia and/or absent or diminished reflexes. Seizures were quite common within the first 48 hours among infants with moderate HIE. Severe HIE was characterised by stupor, coma and no reaction to stimulus, irregular breathing with the need of respiratory support and absent reflexes.

Other classifications, such as the Thompson score, have been suggested and used in research and clinical practice.[40] The Thompson score is a numeric score evaluating nine items of neurological symptoms and signs. Each item is scored from 0 to 3, and the assessment can be done without any specific training or equipment. Both Sarnat and Thompson scores were made with the primary purpose of predicting later outcomes and showed that serial neurological examinations were necessary to give the best prediction. The Sarnat score and simplified versions of it are the most widely used to identify infants with HIE in both research and clinical practice.[41] The clinical features of the three clinical stages of HIE, according to Sarnat, are shown in the table.

Table 1: Original stages of HIE published by Sarnat and Sarnat in 1976[39]

Clinical Feature	Stage 1	Stage 2	Stage 3
Level of consciousness	Hyperalert	Lethargic or obtunded	Stuporous
Neuromuscular control			
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive	Decreased or absent
Segmental myoclonus	Present	Present	Absent
Complex reflexes			
Suck	Weak	Weak or absent	Absent
Moro	Strong; low threshold	Weak; incomplete; high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or absent
Tonic neck	Slight	Strong	Absent
Autonomic function	Generalised sympathetic	Generalised parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Variable; often unequal; poor light reflex
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial and salivary secretions	Sparse	Profuse	Variable
Gastrointestinal motility	Normal or decreased	Increased; diarrhoea	Variable
Seizures	None	Common; focal or multifocal	Uncommon (excluding decerebration)
Electroencephalogram findings	Normal (awake)	Early: low-voltage continuous delta and theta. Later: periodic pattern (awake). Seizures: focal 1 to 1 ½ Hz spike and wave	Early: periodic pattern with isopotential phases. Later: totally isopotential
Duration	Less than 24 hours	2-14 days	Hours to weeks

Challenges with definitions and diagnosis

Although the pathophysiological processes of perinatal asphyxia are thoroughly described, there is a lack of consensus on the definition of perinatal asphyxia. This is a challenge since it is a premise for appropriate research on prevalence, causes and consequences. Present definitions comprise a combination of clinical signs and symptoms, biochemical indicators and specific injury patterns shown on early cerebral imaging, as well as disabilities diagnosed in childhood which are in accordance with the pattern of injury expected from a

hypoxic-ischaemic insult to the developing brain.[17 42] The World Health Organization defines perinatal asphyxia as "the failure to establish breathing at birth", which is more broad and unspecific but can be used regardless of setting.[43] The ICD-10 (International Classification of Diseases, 10th Revision) diagnoses of perinatal asphyxia rely on the 1-minute Apgar score alone, which is subjective and affected by other causes than perinatal asphyxia, such as maternal drugs, congenital malformations, and gestational age (GA).[44 45] Metabolic acidosis in cord blood has the advantage of being an objective measure; however, many infants with metabolic acidosis appear well at birth and are not at risk of adverse neurodevelopmental outcomes.[46] To get a more robust and accurate estimate of the prevalence of clinically significant perinatal asphyxia, results of register-based studies need to be supplemented by studies with access to more detailed clinical data.[47 48]

There is also a lack of consensus on the definition and severity of HIE, and studies use different inclusion criteria for defining both any HIE and moderate/severe HIE. The neurological assessment used to diagnose HIE is subjective, and the inter-rater reliability of HIE staging in clinical practice has not been thoroughly investigated.[49 50] Adding to this complexity, the neurological symptoms and signs in infants with encephalopathy are dynamic and evolve over time.[39 51]

Perinatal asphyxia and HIE in a global perspective

Even though there have been significant improvements in perinatal care over the last decades, perinatal asphyxia continues to be an important cause of neonatal mortality and morbidity worldwide. In high-income countries, the estimated incidences of perinatal asphyxia and HIE are 3-5 and 1-3 per 1000 live births, respectively, whereas the incidences are 10-20 times higher in low-resource settings.[52-54] Perinatal asphyxia and HIE is one of the three leading causes of mortality in early childhood, and more than 95% of these deaths occur in low- and middle-income countries (LMICs).[55 56] Adding to this, perinatal asphyxia is ranked among the top 20 causes of death at any age globally,[57] and it is estimated that 1.2 million infants survive with disability every year.[58]

The Millennium Development Goal number 4 was to reduce the under-five mortality rate by two thirds between 1990 and 2015.[59] Although the global rate of under-five mortality

declined by more than half, the rate of neonatal mortality has shown less progress, especially in some LMICs.[60] Five countries account for more than half of the neonatal deaths in the world, and India has the highest number.[61] Relatively simple and cost-effective interventions such as proper antenatal care, skilled births attendants and basic neonatal resuscitation skills could prevent asphyxia-related deaths in low-income countries.[55 62]

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Figure 2: Neonatal mortality rate (per 1000 live births) in 2019. The Global Health Observatory. World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.

Therapeutic hypothermia

Before the first randomised controlled trial (RCT) on therapeutic hypothermia (TH) for infants with moderate or severe HIE was published in 2005, there was no specific neuroprotective treatment for infants with HIE other than supportive care. [63] This was a significant breakthrough in the treatment of these infants who carry a high risk of adverse

outcomes. Several RCTs and systematic reviews with meta-analyses have documented that a moderate decrease in core temperature to 33.5°C for 72 hours, initiated within six hours after birth in infants with moderate or severe HIE, reduces mortality and major disability in survivors.[63-69] The latest Cochrane review included 1505 term and near-term infants from 11 RCTs and reported a relative risk reduction in the combined outcome of mortality and major disability by 18 months of 0.75 (95% confidence interval (CI) 0.68-0.83) with a number needed to treat for an additional beneficial outcome of seven (95% CI 5-10).[64] The beneficial effect of TH has also been shown to extend into childhood.[70-72] TH was recommended as standard of care for infants with moderate or severe HIE in international resuscitation guidelines published by the International Liaison Committee on Resuscitation (ILCOR) in 2010.[73] Although several other neuroprotective treatments have been studied, none have yet been implemented in clinical practice.

History

Hypothermia is both an adaptive and protective response after a hypoxic-ischaemic insult in many animals,[29 74] and an observational study of newborn babies reported significantly lower temperatures in those who were asphyxiated compared to normal controls.[75] Treatment with hypothermia was described back in the time period of ancient history when Egyptians used cool media to treat non-infectious chest blisters. More than 1000 years later, Hippocrates and his school of medicine suggested hypothermia as a treatment for haemorrhage and were the first to introduce whole-body hypothermia as a treatment for systemic disease.[76 77] The first small studies on induced hypothermia in newborn animals and infants with perinatal asphyxia were done in the 1950s and 1960s.[76 78-82] In 1959, Westin and co-workers published a study of six newborns with perinatal asphyxia that were cooled in running water of 8-14°C for 4-39 minutes and claimed that the cooling had a beneficial effect.[78] However, the studies on TH for HIE were soon paused for several years because of concerning reports on the occurrence of adverse events among hypothermic infants, including subcutaneous fat necrosis, and especially the high oxygen requirements and high mortality among preterm infants.[83-87] After carefully controlled studies in adult animals with varying degrees of neuroprotection, studies on newborn

animals showed permanent neuroprotection in various experimental models and further led to RCTs of hypothermia in newborn infants.[74]

Initiation of therapeutic hypothermia

The inclusion criteria for TH in clinical practice are the same as those used in the large RCTs. These include term and near-term infants with signs of perinatal asphyxia (10-minute Apgar score ≤5, continued need for positive pressure ventilation at 10 minutes, and/or pH <7 or base deficit ≥16 in cord blood or blood sample within one hour's age) and clinical evidence of moderate or severe encephalopathy. TH is usually conducted by whole-body cooling with a rectal temperature maintained at 33.5±0.5°C for 72 hours. This is followed by controlled rewarming at a rate of maximum 0.5°C per hour. Servo-controlled cooling devices are the most widely used as they ensure a constant temperature with minor fluctuations.

TH is usually well tolerated, and adverse events are most often benign. The most common adverse effects of TH, and the only significant adverse effects reported in the latest Cochrane review, are sinus bradycardia and thrombocytopenia.[64] There have also been concerns about persistent pulmonary hypertension and hypotension.[88 89] The large RCTs have also monitored other potential side effects, including coagulopathy, other cardiac arrhythmias, prolonged QT interval, leukopenia, anaemia, hypoglycaemia, hypokalaemia, elevated lactate, renal impairment, sepsis, hepatic dysfunction, and persisting metabolic acidosis. These effects are also commonly described after perinatal asphyxia but are not more frequently reported in cooled infants than in normothermic infants. Subcutaneous fat necrosis has also been a concern, although its association with hypothermia is not clear.[90 91]

Therapeutic hypothermia is assumed to work by decreasing the cerebral metabolism leading to a reduction in the total brain energy expenditure.[92 93] Several mechanisms may explain the neuroprotective effect. These include attenuated release of excitatory amino acids, lowered production of free radicals and nitric oxide, reduced brain alkalosis and lactate, suppressed microglia activation, and eventually decreased cytotoxic oedema and apoptotic neuronal death.[94-96] Many molecular pathways and responses of hypothermia

have been described,[27 97] but these will not be covered in more detail here. It is essential that the treatment is initiated during the latent phase, within six hours after the hypoxic-ischaemic event, before the secondary energy failure leads to irreversible cell death and established brain injury.[27] Initiation of cooling as early as possible is preferable to enhance and optimise the neuroprotective effect.[98]

Figure 3: The three metabolic phases after perinatal asphyxia. From Solevåg and Nakstad.[99] ©The Journal of the Norwegian Medical Association. Reproduced here with permission.

Therapeutic hypothermia in low-resource settings

Although the main burden of perinatal asphyxia and HIE are in LMICs, most RCTs on TH were conducted in North America, Europe, and Oceania.[63 67-69] Until recently, there were only three RCTs from LMICs, two from China and one from India, reporting neurodevelopmental outcomes beyond 12 months of age. These studies included 469 infants, and all concluded with a beneficial effect of TH.[100-102] One study from China, which contributed with the majority of infants, had 20% of participants with mild HIE and more than 80% boys.[101] The second study from China included infants for cooling up to ten hours of age.[100] The study from India was designed and powered to detect a decrease in DNA damage in blood, but showed, with cooling, a significant reduction in developmental delay at 12 months (36% vs 9% in non-cooled and cooled infants, respectively).[102]

A South African retrospective study of 99 infants admitted for TH to one neonatal intensive care unit (NICU) reported that cooling was safe, and most infants survived without major impairment at 12 months of age.[103] Additionally, several smaller trials have been reported without demonstrating clinically relevant outcomes. A pilot study of 36 infants in Uganda reported five times higher mortality in the cooled versus standard care group.[104] In the neonatal unit where the study was conducted, oxygen saturation was measured every 4-12

hours, mean arterial blood pressure was not measured, and the only intensive care available were fluids, antibiotics and phenobarbitone. This study was among the seven studies included in a systematic review of 567 infants from Uganda, Turkey, China, and India.[105] The review found no statistically significant reduction in neonatal mortality in the cooled versus standard care infants (RR 0.74, 95% CI 0.44-1.25). Another systematic review of TH in different resource settings found a significant reduction in neonatal mortality, and there was no observed association between the efficacy of hypothermia and income level of the country.[106]

Trials from LMIC have not consistently demonstrated benefits of cooling,[105 107-109] and it is debated whether results can be extrapolated from high-resource to less-resourced clinical settings. In August 2021, the hypothermia for encephalopathy in low-income and middle-income countries (HELIX) trial was published.[110] This was a multicentre RCT of TH versus standard care with normothermia, including 408 infants from India, Sri Lanka, and Bangladesh. The HELIX trial reported no difference in the combined outcome of death or disability at 18 months between the groups but found increased mortality among the cooled babies. Based on these results, Thayyil and co-authors concluded that TH should not be offered and is to be considered experimental in LMICs.[110 111]

The lack of neuroprotective effect in LMICs has been explained by "population differences" with increased incidence of congenital infections, intrauterine growth restriction and maternal morbidities. Moreover, practical considerations such as availability and level of intensive care, access to the advanced technical cooling equipment, and transport systems are limited in LMICs.[112]

Even though efficacy and safety are questioned, TH has been implemented in many hospitals in LMICs. More than 50% of clinicians in South Africa offered cooling in 2012, and the number has probably increased after that.[113] A survey of national practices in India from 2015 found that 51% of responding units offered cooling, and another 44% wanted to offer cooling but were unable due to lack of cooling device and trained staff.[114]

Low-cost cooling devices

The servo-controlled cooling devices used in the large RCTs and clinical practice in high-income settings are expensive and not affordable in many low-resource settings. Trials from LMICs have therefore used either passive cooling or different low-cost cooling devices such as ice gel packs, servo-controlled fans, and water bottles.[107][115] These devices and methods have been criticised for not having the same ability to induce and maintain a constant temperature over time as the servo-controlled devices. A low-cost mattress made of phase-changing material (PCM) has been shown to provide temperature stability comparable to servo-controlled devices.[116][117] PCMs consist of salt hydride, fatty acid and esters or paraffin, melting at a set point and with the ability to store and release heat at a nearly constant temperature.[116] This allows a more stable temperature and has shown promising results in both a feasibility trial and a multicentre trial in India, and a prospective non-randomised interventional study from Vietnam.[116-118]

Long-term neurodevelopmental outcomes

The long-term outcomes after perinatal asphyxia and HIE range from complete recovery in most infants with mild HIE to death or survival with severe disability in most infants with severe HIE.[119][120] Neurodevelopmental impairments include cerebral palsy (CP), epilepsy, developmental delay, hearing loss, reduced visual function, and cognitive and behavioural impairments such as attention problems, memory deficits, lower IQ scores and learning difficulties.[121-123] One review from the precooling era reported adverse outcomes in none of the infants with mild HIE, 32% in moderate HIE and almost 100% in severe HIE.[124] After the implementation of TH, the prevalence of adverse outcomes of infants with moderate or severe HIE have declined. However, almost half of these infants still die or survive with disabilities.[65][72] Although outcomes of infants with mild HIE have been reported to be mostly normal, there is increasing evidence that these infants are at risk of adverse outcomes.[125-127] Infants with mild HIE more frequently develop cognitive and behavioural impairments, including lower intelligence quotient.

Cerebral palsy

Cerebral palsy is the most common motor disability in children, with a prevalence of 2.1 per 1000 live births.[128] It is a heterogenic group of disorders resulting from a non-progressive injury to the developing fetal or infant brain, which affects the ability to move and maintain balance and posture.[129] [130] The Surveillance of Cerebral Palsy in Europe divides CP into three main categories based on the predominant neurological findings:[131]

- Spastic: characterised by increased tone and pathological reflexes.
- Dyskinetic: characterised by involuntary and uncontrolled stereotyped movements
- Ataxic: characterised by lack of muscle control or coordination of voluntary movements. Tremor and low tone are common.

Spastic cerebral palsy is by far the most common category. There is also a functional classification of CP, the gross motor function classification system (GMFCS), divided into five levels according to the gross motor skills.[132] Level 1 indicates the least and level 5 the most severe limitations in activity. Although a CP diagnosis is mainly based on motor function, children with CP often have comorbidities that might be more disabling than the motor impairment.[133-135]

The aetiology in most CP cases is unknown but are usually thought to be of perinatal origin. It is estimated that between 10 to 20% may be caused by a hypoxic-ischaemic event around the time of birth.[134] [136] [137] CP is the most common disability in survivors after severe perinatal asphyxia, and infants with moderate or severe HIE are at the highest risk. HIE is primarily associated with CP of the dyskinetic or spastic quadriplegic type. In the precooling era, the severity of CP among survivors of HIE was mainly severe. TH has proven to reduce the occurrence of CP among infants with moderate/severe HIE and has also been shown to reduce the severity of CP.[64] [138] Four of the large RCTs on TH reported rates of CP among survivors of 13-28% among cooled and 29-48% among non-cooled infants.[63] [67] [69] [139]

Prediction of outcome

Prediction of outcome after perinatal asphyxia is important to direct follow-up and care for the affected infants and their families. Early diagnosis of neurodevelopmental impairments is important as this enables early interventions.[133 140] The recommended assessments for early prediction of CP are brain MRI in the neonatal period and general movements assessment (GMA) at 10-15 weeks postnatal age.[133] Other predictors of long-term outcomes for infants with HIE are clinical neurological assessments, and neurophysiological (amplitude integrated EEG (aEEG) and EEG) and biochemical examinations. Clinical neurological assessments with repeated scoring of HIE severity discovering the evolution of encephalopathy over the first three days is a more accurate predictor of outcome than the initial level of HIE during the first postnatal hours.[51 141] Multiorgan dysfunction and biochemical derangements are associated with adverse outcomes but not sufficient to predict outcomes alone.[142-150] An abnormal aEEG pattern within the first six hours of life was reported to be highly predictive of outcomes in the precooling era, however, the prognostic accuracy has been lower in studies of cooled infants.[151-153]

Several predictive models for infants with HIE have been suggested. However, many include items or variables not readily available in clinical practice. One predictive model in the pre-hypothermia era suggested three variables that are readily available within four hours of birth: chest compressions for more than one minute, age at onset of respiration ≥30 minutes, and base deficit of more than 16 mmol/L.[154] Infants with all predictors present had a 93% chance of an adverse outcome. Thoresen et al. found excellent prediction of adverse outcomes by combining findings on neonatal MRI with early clinical data.[155] There is currently no easily accessible and feasible prediction model used in clinical practice. MRI continues to be the single best predictor of outcomes after HIE.[156 157]

MRI

Magnetic resonance imaging is a relatively new imaging technique, with the first whole-body MRI machine developed by Dr Raymond Damadian in the 1970s.[158] He published the first MR image of the human body in 1977.[159] This was a breakthrough in medical imaging

as the images obtained showed the potential of visualising soft tissues, such as the brain. Former imaging techniques of the brain for neonates were cerebral computer tomography scans and cerebral ultrasound. However, both techniques have poor sensitivity and specificity for soft tissue pathology compared to MRI. Furthermore, MRI scanning allows for the depiction of various types of pathology and measurements of brain metabolites with proton MR spectroscopy (MRS). In addition, computer tomography makes use of radiation. From the late 1980s, brain MRI was increasingly used for infants with HIE.[160][161] MRI provides information about the location, timing, and severity of brain injury, and it is also used to guide clinical decision-making.

Conventional T1 and T2 weighted sequences

T1 and T2 weighted images are the most common MRI sequences. These images give detailed information about brain and vascular anatomy on a macrostructural level. The type of sequence obtained depend on the chosen time to echo (TE) and the repetition time (TR).[162] TR is the time between each excitation pulse for each slice and controls the amount of T1 relaxation (longitudinal relaxation) that is allowed to occur. TE is the time from delivering the radiofrequency pulse to the peak signal is induced in the coil and controls the magnitude of T2 relaxation (transverse relaxation). T1 weighted images are produced by selecting short TE and TR times, while T2 weighted images are obtained using longer TE and TR times. Tissues have different T1 and T2 relaxation properties that determine the signal intensity and, thereby, the contrast in the image.

Basic principles of MRI, including more details on T1 and T2 relaxation, are written in Appendix I.
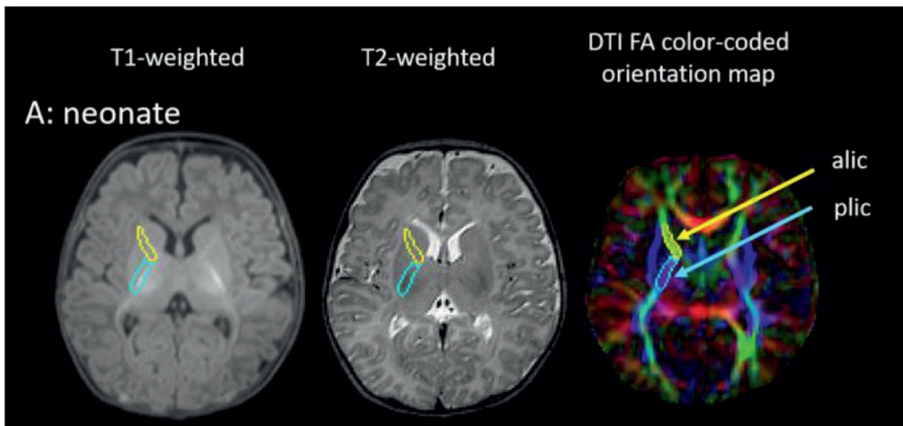
Figure 4: T1-and T2-weighted images and diffusion tensor imaging (DTI) of a neonatal brain. DTI provides greater contrasts in the white matter areas compared to T1-or T2-weighted images. This feature of DTI is particularly advantageous in identifying white matter bundles in the neonatal brain. The anterior limb of the internal capsule (ALIC, yellow contour) and the posterior limb of the internal capsule (PLIC, cyan contour) are almost invisible on conventional T1-and T2-weighted images of the neonatal brain. From Oishi et al.[163] Licence: CC BY-NC-ND 4.0.

Diffusion-weighted imaging

Diffusion-weighted imaging (DWI) is an MRI technique where the contrast is gained from the random molecular movement, which is called diffusion.[164] This method measures the natural motion of water molecules. Extracellular water molecules have relatively free diffusion, while intracellular molecules have restricted diffusion. The brain is highly heterogeneous, with many compartments and barriers. Furthermore, pathological processes such as oedema or tissue loss (tissue death) affect the diffusion. The movement of water can differ in different directions, for example restricted in one or more directions.[165] DWI produces quantitative maps of the diffusion of water molecules that can reveal microscopic details of normal and damaged tissue.[166] The DW image has limited image resolution by today's clinical MRI scanners and techniques.[166]

The DWI scan has a corresponding apparent diffusion coefficient (ADC) map. The ADC is a quantitative measure of diffusion describing movement per second and is frequently used in clinical DWI. Neonates have very high ADC values at term age, compared to adults. The higher rates of water diffusion, the higher ADC values and lower signal intensity in the DW image.[167] The diffusion and subsequently the ADC values increase with most types of

pathology in the brain, except for acute hypoxia-ischaemia.[167] Water diffusion and ADC values decrease significantly soon after hypoxic-ischaemic brain injury, and the signal on the DW image is high.[168]

Brain injury after HIE on neonatal conventional MRI

A combination of conventional MRI techniques such as T1-, T2- and diffusion-weighted images in the neonatal period is highly predictive of later neurodevelopment.[169] The prognostic accuracy is regardless of treatment with hypothermia.[170 171] In general, the brain injury following a hypoxic-ischaemic insult is bilaterally symmetrical. An acute and total/severe asphyxia is characterised by lesions in the basal ganglia and thalami (BGT), typically also involving the posterior limb of the internal capsule (PLIC), brain stem, and hippocampus.[172 173] This is the most common pattern of injury after acute sentinel events during delivery, and it is estimated to account for 25-75% of cases with moderate/severe HIE.[174 175] The BGT are susceptible to these events due to a high metabolic rate and great energy demand.[176] The PLIC is a white matter (WM) structure that passes by the BGT and connects the cerebral hemispheres with subcortical structures, including the corticospinal tract. White matter and cortical lesions can evolve secondary to the BGT injury.

The second typical brain injury in infants with HIE is predominant WM injury that may also involve the cortex, often called the watershed pattern of injury. Infants experiencing more mild, chronic, and possibly repetitive insults generally have more WM and cortical lesions.[177 178] The distributions of these lesions are typically parasagittal, involving the subcortical areas between the major arteries (often called the watershed zones) typically located to the parieto-occipital and posterior temporal lobes more than the anterior regions.[179] Perinatal WM injury can also be a result of inflammation/infection, hypoglycaemia, hemodynamic instability or other causes.[13 180]

Although there are two typical and distinct patterns of brain injury following hypoxia-ischaemia, these events are often overlapping in duration, type, and severity, resulting in a combination of abnormalities on MRI.[179] Typical findings within the first week after a hypoxic-ischaemic insult are brain swelling, abnormal signal intensity in the PLIC and BGT, loss of differentiation between the white and grey matter and cortical highlighting.[181] When

the insult is both severe and long-lasting, a more severe injury pattern involving the whole brain may evolve, often labelled total brain injury.[179]
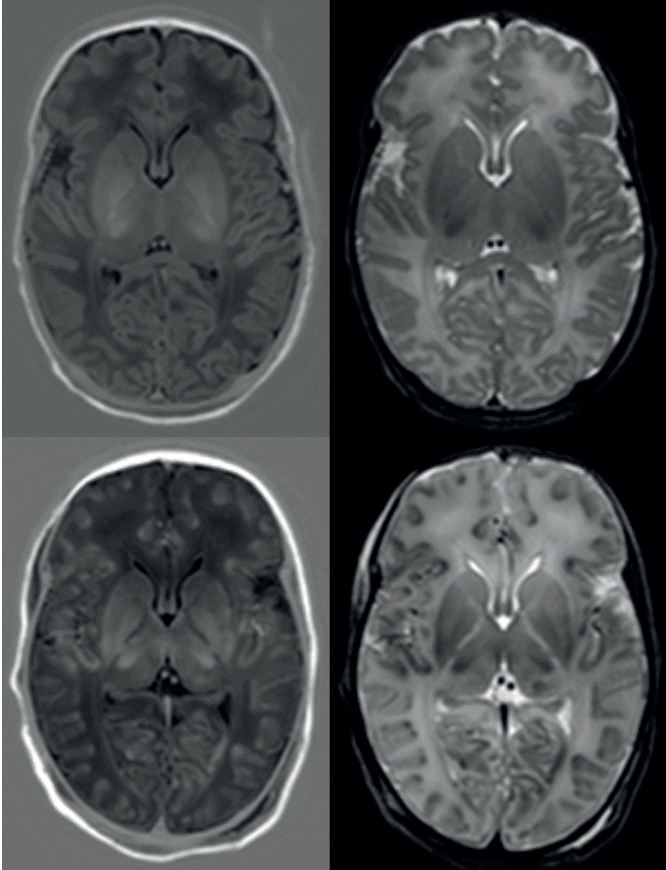
Figure 5: MRI of two neonates with HIE. The two images on top are T1 (left) and T2 (right) weighted images with normal findings. The two images below are T1 (left) and T2 (right) weighted images with total brain injury pattern involving both basal ganglia and thalami, posterior limb of the internal capsule, white matter, and cortex. Images obtained in the THIN study.

The pattern of brain injury of HIE in term infants seen on MRI correlates with the severity and type of insult.[179 182 183]Both the severity and pattern of brain injury are related to later neurodevelopmental outcomes.[184] The severity of BGT injury is directly associated with the outcome, especially later motor impairment and CP.[185] Another accurate predictor of poor

outcome, including death and CP, is an abnormal signal in the PLIC.[186] Lesions in the brainstem usually follow the most severe cases of BGT injury and are strongly associated with death.[187] The WM or watershed pattern typically results in more cognitive impairments and less severe motor problems.[177 188]

To guide the assessment of patterns of brain injury on MRI, several standardised scoring systems have been developed. The Barkovich scoring system,[189] the MRI scoring system by Weeke et al.,[190] Rutherford et al.,[170] and the NICHD brain injury pattern[191] are scoring tools used to classify the different patterns that can predict later outcomes.

DWI shows restricted diffusion immediately after the onset of hypoxia/ischaemia, and the abnormalities are most apparent one to four days after the insult. During the first week, DWI is reported to be more sensitive than T1 and T2 weighted images.[192] Lower ADC values in the thalamus within the first week of life have been reported among the best predictors of adverse outcomes in infants with HIE.[193] It is, therefore, recommended to include DWI in the standard MRI protocol for infants with HIE. However, the predictive accuracy of DWI is highest within the first week due to the pseudonormalisation of ADC values during the second week.[192 194]

The abnormal signal intensities on T1 and T2 weighted images take days to develop and gradually increase over the first week of life.[179] It is usually most evident between one to two weeks after the insult. On the other hand, DWI is very useful in early imaging of infants with HIE as the T1 and T2 weighted images might underestimate the injury in the first few days.[192] This is important to recognise when choosing the time and modality of the scanning and when interpreting the results.

Diffusion tensor imaging

Diffusion tensor imaging (DTI) is a quantitative MRI technique based on the principles of DWI where also direction and magnitude of water diffusion are quantified. In WM, where the neural tissue is arranged in parallel fibre bundles (or tracts), the diffusion of water occurs primarily in the direction along the fibres. When the diffusion occurs mainly in one direction, it is called anisotropic diffusion. The presence of spatially oriented structures explains the direction-dependence or the anisotropy.[166] This is in contrast to the diffusion

of water in most fluids, such as the cerebrospinal fluid, where the diffusion is mainly free. Free diffusion is equal in all directions, and this is called isotropic diffusion. DTI can be used to infer the organisation of WM and can detect WM abnormalities not visible on conventional MRI.[195]
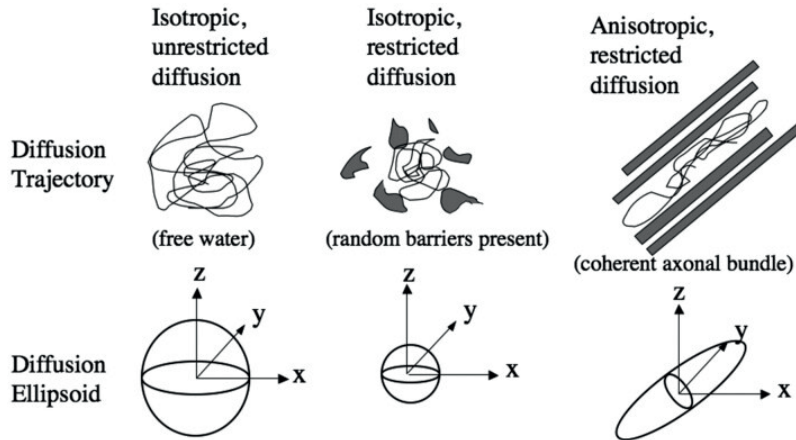
Figure 6: Isotropic and anisotropic diffusion in the brain. The diffusion ellipsoids for isotropic unrestricted diffusion, isotropic restricted diffusion, and anisotropic restricted diffusion are shown. From Mukherjee et al.[167] Reproduced here with permission.

Fractional anisotropy (FA), a DTI metric, is a measure of the overall directionality of water in a voxel. Its value is dependent on the tensor shape ranging from 0 in isotropic diffusion to 1 in anisotropic, highly directional diffusion. Decreased FA is a common feature of cerebral WM abnormalities.[196] Another metric derived from DTI is mean diffusivity (MD), a measure of the mean water diffusion, and can be compared to the ADC derived from DWI. Typically, MD increases in damaged tissue due to enhanced free diffusion but often decrease shortly after hypoxia-ischaemia.[196-198]

DTI is a sensitive technique that examines microstructural tissue properties, and it is associated with the anatomy and structure of WM, including fibre orientation.[197] A common approach for obtaining metrics from DTI data is extracting this information from a region of interest (ROI). WM tractography is another approach to obtain a three-dimensional

presentation (picture) of WM organisation.[199] DTI is time-consuming and very sensitive to motion both on a micro- and macroscopical level.[197]

Currently, DTI is mainly used for research purposes in neonates. DTI metrics have been shown to be associated with long-term outcomes in infants with HIE.[200] Significant differences in FA between cooled and non-cooled infants suffering from perinatal hypoxic-ischaemic injury have been demonstrated in several WM tracts with a sample size of 10-15 infants in each group.[201] The development of a multi-contrast human neonatal brain atlas based on multimodal MRI has provided another technique to demonstrate differences in FA and MD using small sample sizes.[202] DTI may be used as a surrogate outcome for neurodevelopment in clinical trials to reduce the large sample sizes necessary to detect differences in long-term, clinical outcomes.[201 203]

MR spectroscopy

MRS of the brain began by examining newborn infants, including some with perinatal asphyxia, in the early 1980s.[204 205] This is another quantitative MR technique that can provide information on more than 20 metabolites and compounds in the brain and their concentration.[206] In the clinic, single-voxel MRS is most commonly used, and the concentration of metabolites provided is relative to other metabolites. The different molecules can be distinguished and recognised due to their slightly different magnetic properties. As water is the dominating molecule in the brain, the water signal is suppressed. The result of an MRS acquisition is not an image per se, rather it is a complex spectrum where all the metabolites are present as partially overlapping peaks.[207] The metabolites can be expressed in absolute concentrations or as peak-heights and peak area-under-the-curve. The type and number of metabolites that can be quantified depend on the chosen pulse sequence and parameters and also the field strength.[208] The dominating peaks in the MR spectra of the brain are from creatine (Cr), choline (Cho) and N-acetyl-aspartate (NAA), but glutamine/glutamate (Gln/Glu) and myo-inositol (mI) can also be detected. Lactate is normally too low to be detected in the brain but increases after a hypoxic-ischaemic insult.

Currently, MRS is frequently implemented in clinical MRI protocols for infants with HIE and has been shown to be an accurate predictor of outcomes after HIE.[209] Brain injury after HIE usually leads to increased levels of lactate and mI and decreased levels of NAA.[210] MRS of the BGT, particularly the lactate-NAA and lactate-Cr ratio, has been shown to predict outcomes in infants after HIE with high accuracy which persists beyond the first week of life.[193 209 211]
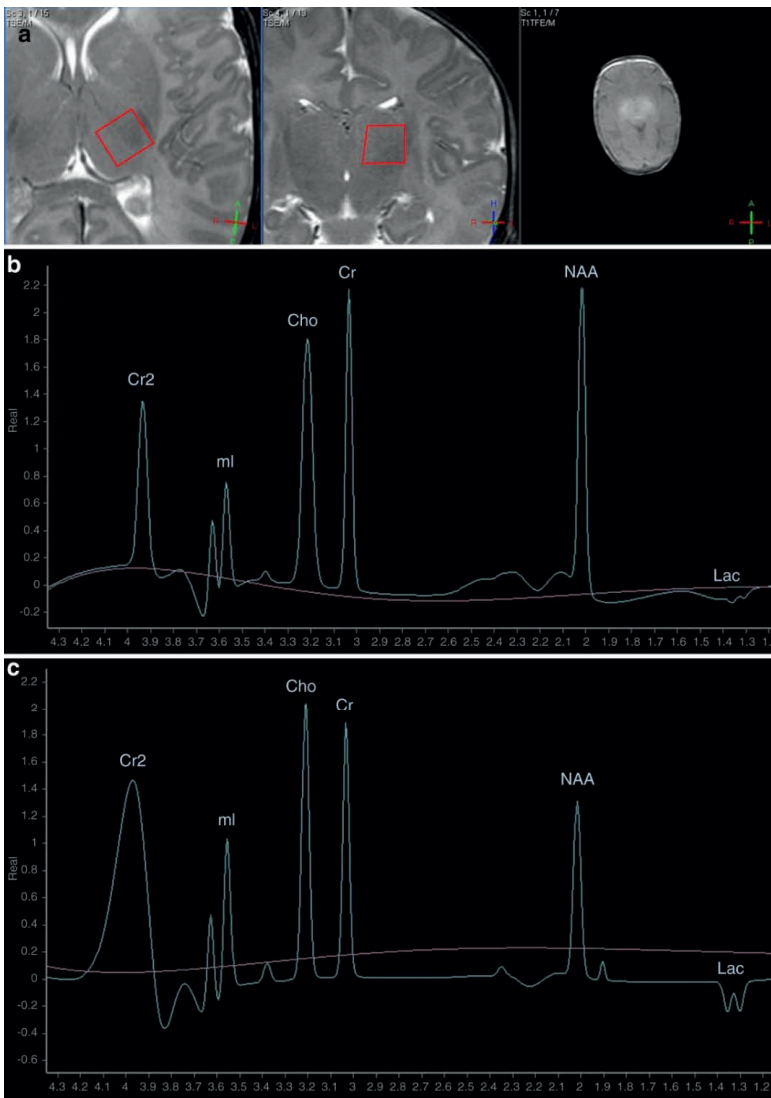
Figure 7: Representative H-MRS spectra of two newborns with moderate-to-severe HIE. **a** Gradient echo survey images acquired with echo time (TE) = 5 ms, repetition time (TR) = 75 ms, and 30° flip angle for selection of volume of interest (VOI) from the left thalamus of infants (red frame). **b** H-MRS of a newborn with good outcome at TE = 144 ms. **c** H-MRS of a newborn with poor outcome at TE = 144 ms. Cho choline, Cr creatine, Cr2 creatine, second peak, Lac lactate, doublet peak, mI myo-inositol, doublet peak, NAA *N*-acetyl-aspartate. *x* axis represents chemical shift in parts per million (ppm), *y* axis represents signal intensity in arbitrary units. From Barta et al.[212] Licence: <u>CC BY 4.0</u>

General movements assessment

The GMA is a systematic observation and classification of an infant's spontaneous movements and was developed by Professor Heinz Prechtl and co-workers in the 1990s.[213] General movements (GMs) are a set of typical spontaneous movements involving the whole body lasting from seconds to several minutes. These movements are present from 8-10 weeks' gestation until the voluntary movements appear around 15-20 weeks' post-term age.[214 215] The infants' GMs are classified according to their qualitative characteristics, which have been found to correlate with later neurodevelopment.

The GMA is a low-cost, non-invasive, and highly predictive assessment that is based on infants' spontaneous movements in a video. It is important that the infant is in a good state, being awake, calm, and alert. The parents or health personnel should not interact while recording, and the infant must not be fuzzing or crying. The infant is filmed for three to six minutes, and the assessment is thereafter scored by trained personnel, often physiotherapists or physicians, by observing the video.

The GMA before five months' post-term age is increasingly being used to predict outcomes, especially motor outcomes and CP, in high-risk infants.[133 216] A typical movement pattern which is part of the GMA, called fidgety movements (FMs), usually appear from 8-9 weeks up to 16-20 weeks post-term age.[217] FMs are small movements in the whole body, especially the neck, trunk, and limbs, that occur in all directions with moderate speed and variable acceleration.[218] The movement pattern often occurs continuously in an awake infant.[219] The FMs can be classified according to their presence and the length of interspersed pauses as either continual (score: F++), intermittent (score: F+), sporadic (score: F+/-), exaggerated (score: Fa) or absent (score: F-). Exaggerated FMs are FMs with exaggerated amplitude and speed, and this pattern is considered abnormal. Traditionally, FMs are considered normal and associated with a normal motor outcome if present continually or intermittently, and abnormal if sporadic, exaggerated, or absent.[220]

The absence of FMs between nine and 20 weeks' post-term age is proven to be a strong marker for later disability in general and CP in particular.[213 217 221-223] Although most studies focus on motor outcome, there are also studies reporting an association between GMA and cognitive outcomes.[224-226] Two studies have reported high sensitivity and specificity of absent FMs for predicting CP and neurodevelopmental outcomes in infants with HIE.[227 228]

However, most studies on GMA are conducted in preterm or mixed high-risk populations, and the literature on the association between GMA and outcomes in asphyxiated infants is sparse.

Aims

The overall aim of this thesis was to improve outcomes for infants exposed to perinatal asphyxia and HIE in high- as well as in low- and middle-income countries. To fulfil the overall aim, this PhD thesis consists of two studies: one interventional trial and one retrospective observational study. The efficacy and safety of TH in a low-resource setting were assessed in an RCT in India. Incidence and outcomes after perinatal asphyxia and HIE in a Norwegian county in a period before and after the implementation of TH was explored in a population-based observational study.

The aims and hypotheses are addressed in papers I-III as follows:

Paper I

The Therapeutic Hypothermia in India (THIN) study was an RCT aiming to evaluate the neuroprotective effect of TH, achieved by a low-cost, locally produced cooling device, in infants with moderate to severe HIE admitted to a tertiary NICU in India. Outcome measures were neonatal MRI biomarkers, indicating severity of brain injury. We hypothesised that TH in this setting was neuroprotective and safe.

Paper II

The aim of the second paper was to evaluate the predictive accuracy of neonatal MRI and GMA on neurodevelopmental outcomes in infants with HIE enrolled in the THIN study. We hypothesised that both MRI biomarkers and absence of FMs had high accuracy in predicting adverse outcomes at 18 months of age.

Paper III

The third paper aimed to describe temporal trends in admissions and outcomes after perinatal asphyxia and HIE before and after the implementation of TH in a tertiary care NICU in Mid-Norway between 2003-2011. Therapeutic hypothermia was implemented in this unit in June 2007, and we hypothesised that the implementation of this new treatment influenced the referral rates to the NICU.

Materials and methods

Study design

Paper I and II

The THIN study was a single-centre open-label RCT of TH achieved by a PCM-based cooling device (MiraCradle® Neonate Cooler, Pluss Advanced Technologies, India) versus standard care with normothermia in a tertiary neonatal unit in South India. Infants were included between September 2013 and October 2015 at the Christian Medical College (CMC) Vellore. The CMC is a tertiary care teaching hospital in south India with around 15 000 deliveries annually and 75 neonatal beds. The neonatal department serves an area of about 200 square kilometres with a population of approximately 10 million. Included infants underwent cerebral MRI at 5±1 days of age and a video for GMA at 10-15 weeks post-term age. Neurodevelopmental outcomes were assessed at 18 months.

Paper I reports the primary outcome of this RCT, including the initial neonatal admission and neonatal MRI biomarkers indicating the severity of brain injury. Secondary analyses of the THIN study are reported in paper II, assessing associations between patterns of brain injury on neonatal MRI, presence or absence of FMs, and outcomes at 18 months of age.

Paper III

This was a population-based retrospective observational study of term/near-term infants born in Sør-Trøndelag county between 2003-2011 and admitted to the NICU at St. Olavs Hospital, Trondheim University Hospital, due to perinatal asphyxia. St. Olavs Hospital is the only tertiary care referral centre in the county of Sør-Trøndelag, with 21 neonatal beds and 3500-4000 annual deliveries. Sør-Trøndelag is a former county comprising the southern

portion of the present Trøndelag county in mid Norway and had a population of 294 000 in 2011.[229]

Study population

Paper I and II

The THIN study included infants admitted to the NICU with moderate or severe HIE. The inclusion and exclusion criteria are presented in the figure. For outborn infants, no cry at birth was a sufficient physiological criterion.

Inclusion criteria: Should fulfil all criteria 1, 2, 3 and 4.
1. Age of newborn: <6 hours of age
2. GA >35 weeks and birth weight > 1800 g
3. PHYSIOLOGICAL CRITERIA: Any one of the following
   a. Arterial blood gas (umbilical cord/1st postnatal hour) pH <7.0 or base deficit ≥12
   b. Apgar score ≤ 5 at 5 minutes
   c. Ventilation for at least 10 minutes as part of resuscitation at birth
4. NEUROLOGICAL CRITERIA: Seizures (OR) evidence of moderate or severe encephalopathy (3 of 6 criteria in modified Sarnat)

Exclusion criteria:
1. Lack of consent
2. Major congenital malformations
3. Imminent death anticipated at the time of assessment for eligibility

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Figure 8: Inclusion and exclusion criteria in the THIN study.

Table 2: Modified Sarnat classification of encephalopathy

Category	Moderate encephalopathy	Severe encephalopathy
Level of consciousness	Lethargic	Stupor or coma
Spontaneous activity	Decreased activity	No activity
Posture	Distal flexion/ complete extension	Decerebrate
Tone	Hypotonia (focal or generalised)	Flaccid
Primitive reflexes		
Suck	Weak	Absent
Moro	Incomplete/ weak/ high threshold	Absent
Autonomic system		
Pupils	Constricted	Deviated, dilated or non-reactive to light
Heart rate	Bradycardia	Variable
Respiration	Periodic breathing	Apnoea

A total of 85 infants were assessed for eligibility, and 50 infants were included in the study according to the calculated sample size. The intervention was discontinued in three infants (two in the TH group and one in the SC group) due to death and withdrawal of life support. Another infant in the SC group was discharged against medical advice before an MRI was obtained.
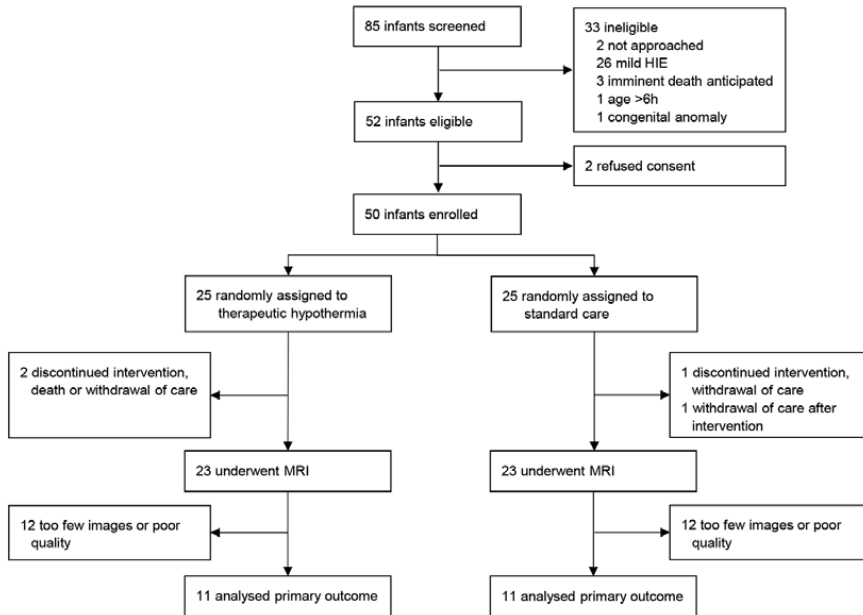
Figure 9: Flow chart of included infants in the THIN study. HIE, hypoxic-ischaemic encephalopathy.

Outcomes

The primary outcome was the value of FA by DTI in the PLIC. The prespecified secondary outcome measures included FA and MD values in other brain regions (thalami, lentiform nuclei, genu and splenium of the corpus callosum, and midbrain), peak area of major metabolites on BGT MRS, and abnormalities on conventional MRI. Other secondary outcomes were absence or presence of FMs on GMA at 10-15 weeks and neurodevelopmental outcomes at 18 months. All assessors of MRI, GMA and outcomes were blinded to the intervention and the results of former examinations.

Randomisation and blinding

Included infants were randomised using the stratified block randomisation method created by the biostatistics department at CMC. Stratification was used to balance the groups on severity of encephalopathy (moderate and severe HIE). Sequentially numbered opaque

sealed envelopes were used for allocation concealment with block sizes of four and six. Blinding was not possible due to the nature of the treatment.

Sample size

The sample size was calculated based on a 10% difference in mean in the FA of the PLIC and a power of 90%, which gave a sample size of 20 in each arm. With an estimated 20% mortality before MRI could be achieved, a sample size of 50 infants was considered sufficient to detect potentially significant differences in the primary outcome.

Paper III

Infants were included in this study if they fulfilled a set of criteria for clinically significant perinatal asphyxia, as given in the figure below. For this study, term/near-term infants were defined as infants born with GA ≥36 weeks or, if unknown GA, a birth weight >2kg.

Inclusion criteria:
- Born in Sør-Trøndelag between 1 January 2003 – 31 December 2011
- Gestational age ≥36 weeks or if unknown gestational age a birth weight ≥2kg
- Admission to neonatal care for ≥24 hours with any of the ICD-10 diagnoses:
  - P20 Intrauterine hypoxia
  - P21 Birth asphyxia
  - P90 Convulsions of the newborn
  - P91.0 Neonatal cerebral ischemia
  - P91.3 Neonatal cerebral irritability
  - P91.4 Neonatal cerebral depression
  - P91.5 Neonatal coma
  - P91.6 Hypoxic ischaemic encephalopathy of the newborn
  - P91.8 Other specified disturbances of cerebral status of newborn
  - P91.9 Disturbances of cerebral status of newborn, unspecified
- Fulfilling at least one of the following clinical criteria:
  1. Cord blood pH <7.06
  2. 5-minute Apgar score ≤5
  3. Resuscitation after birth with assisted ventilation ≥5 minutes, intubation and/or chest compressions
  4. Impaired renal function with urine production <1 ml/kg/t within the first 24 hours of life
  5. Elevated liver transaminases (ALAT >70 U/L)

Figure 10: Inclusion criteria in the population-based observational study

All data were collected retrospectively. Background data on live births were collected from the Medical Birth Registry of Norway. A local database of deliveries at St. Olavs Hospital provided additional data on birth weight, GA, 5-minute Apgar score, and cord blood pH. Clinical data from the admissions were collected from medical records.

A total of 804 infants were admitted to the NICU with any of the given diagnoses. Infants born outside Sør-Trøndelag and those born preterm (GA <36 weeks) were not eligible. The remaining infants had their medical records screened for inclusion based on their clinical course during admission. Eighty-four infants fulfilled the clinical criteria for perinatal asphyxia but were excluded due to major congenital malformations (e.g. diaphragmatic hernia, severe central nervous system malformations, severe heart diseases or malformations requiring immediate surgical interventions), syndromes expected to seriously affect the child's life span, such as trisomy 13 and 18, or other diagnoses explaining the criteria (e.g. metabolic, infections, severe lung disease, congenital anomalies, pain and drug effects, cerebral infarctions, and suspected or confirmed seizures without any signs of perinatal hypoxia-ischaemia).
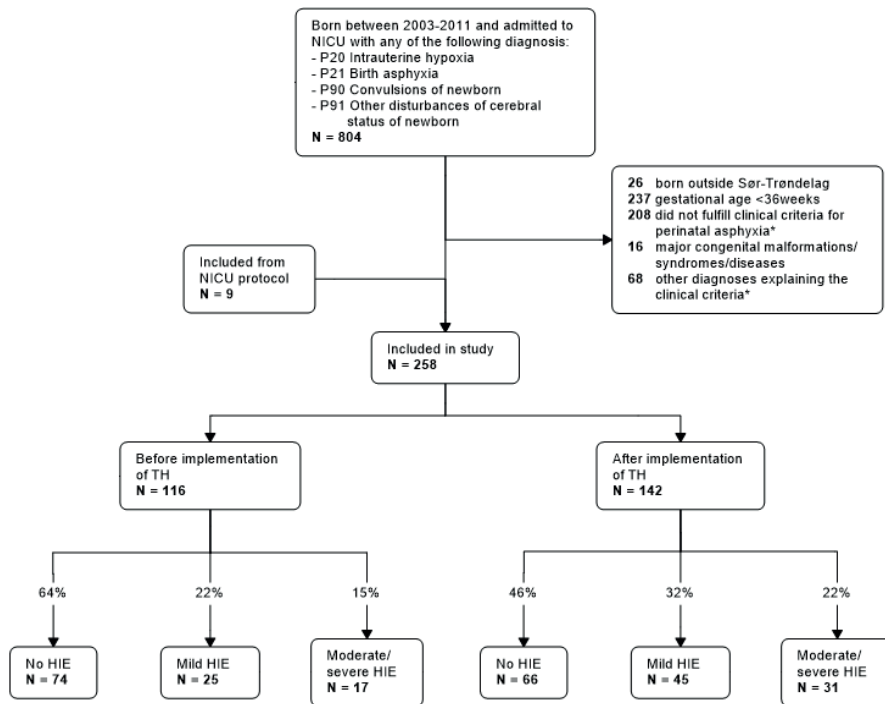
Figure 11: Flow chart of included infants in paper III before (January 2003 – May 2007) and after (June 2007 – December 2011) the implementation of therapeutic hypothermia (TH), including the severity of hypoxic-ischaemic encephalopathy (HIE).
*Clinical criteria are given in Figure 10.

Interventions in THIN study

Included infants were randomised to either TH or standard care with normothermia.

Before randomisation, all inborn infants were resuscitated and stabilised under a radiant

warmer. The infants were screened for inclusion and randomised after admission to the

NICU. Those assigned to hypothermia (TH group) were placed on the cooling device

(MiraCradle®) to induce hypothermia. The targeted core temperature was 33.5±0.5°C for

72 hours followed by controlled rewarming at 0.2-0.5°C per hour until the temperature

reached 36.5°C. Infants assigned to standard care with normothermia (SC group) were

placed under a radiant warmer, and the targeted core temperature was 37.0±0.5°C.

Rectal and skin temperature was monitored continuously. All infants had a central venous line and arterial access and were monitored continuously for oxygen saturation, heart rate, blood pressure, and respiratory rate. Urine output was monitored every six hours. Neurological examination was done at recruitment and repeated daily for four days. Both the Thompson score and modified Sarnat were performed.[40] Biochemical and haematological parameters were monitored. All treatments were as per the neonatal unit's treatment protocols. The first line anticonvulsant was phenobarbitone, the second was phenytoin, and the third was levetiracetam. Sedatives and/or analgesics were given to babies who had excessive shivering or a pain score of more than four on the Neonatal Infant Pain Scale. Mechanical ventilation was not routinely used but was provided for infants with respiratory failure.

Cooling device

The MiraCradle is a low-cost passive cooling device using PCM technology to induce hypothermia. It was developed by Pluss Technologies in collaboration with CMC Vellore. The neonate is nursed on a conduction mattress, under which the PCM blocks are placed. Two types of PCM blocks (FS-21 (form stable) and FS-29) are used with different melting points (21 and 29°C, respectively). Both types are used in the induction phase, but only FS-29 is used during maintenance. The PCM blocks are stored in the refrigerator, and the FS-29 usually does not need to be changed during TH.
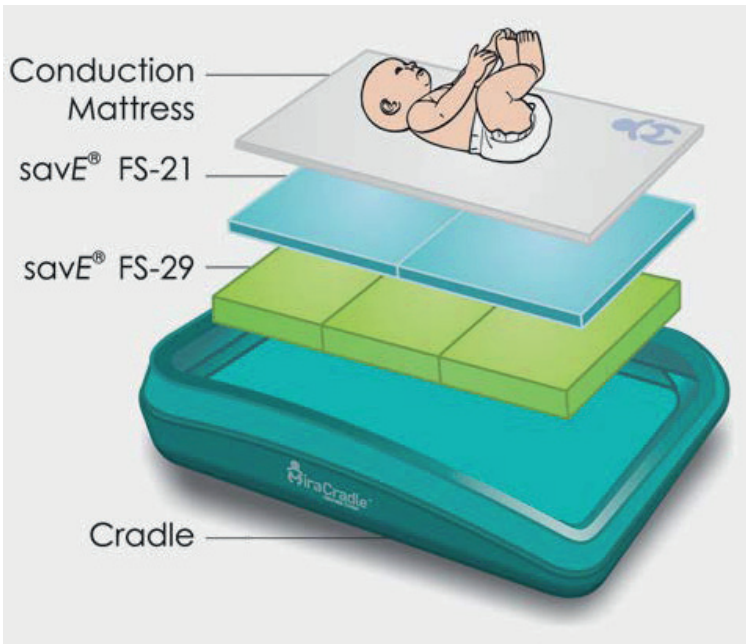
Figure 12: MiraCradle ® - Neonate Cooler. Picture from
https://miracradle.com/product.html reproduced here with permission.

Figure 13: Infant during cooling with MiraCradle ®. Picture from
https://miracradle.com/product.html reproduced here with permission.

Assessments performed in the THIN study

MRI

Cerebral MRI was done at 5±1 days of life with a 3.0T Philips Achieva scanner (Philips Healthcare, Best, Netherlands; software V.3.2.3.1) using an 8-channel head coil. Sedation was used if needed with chloral hydrate or midazolam, and pulse oximetry surveillance was performed. Extra ear protection was applied during all scans. The infants were wrapped, and foam pads were placed around the head to reduce movements. Clinical brain MRI included axial T1-weighted inversion-recovery images (TR = 5000ms, TE = 15ms, TI = 900ms), axial T2-weighted spin-echo images (TR = 7700ms, TE 140ms), sagittal three-dimensional T1-weighted images (TR = 9.9ms (shortest), TE 4.6ms), and DWI (axial plane) single-shot echo-planar imaging (TR = 2400ms, TE = 105ms (shortest), b-values 0 and 700s/mm^2). DTI was acquired using a single-shot echo-planar imaging sequence applied in 32 directions (TR = 3670ms, TE = 52ms, b-value 0 and 600s/mm^2). Multi voxel MRS was performed with 25 voxels covering the BGT (TE = 144ms, TR 2000ms and voxel size 1x1x1.5cm). The total duration of the MRI acquisition was 25 minutes.

All included infants, except four that died early, had cerebral MRI obtained at a median age of 5 days (range 4–7). Of these, only 22 had DTIs that could be analysed. One did not have a DTI sequence, and 23 could not be analysed because of too few DTI images or poor image quality due to artefacts. MRS was done in all 46 infants with MRI, but 18 failed processing (7 in the TH group and 11 in the SC group) and two were excluded due to poor quality and voxels chosen mainly within WM (TH group).

Image analysis and reading

The DTI analyses were performed by Live Eikenes, professor at the Department of Circulation and Medical Imaging at NTNU, with the tools of the FMRIB Software Library (FSL; Oxford Centre for Functional MRI of the Brain, UK; www.fmrib.ox.ac.uk/fsl). Image artefacts due to motion and eddy current distortions were minimised by registration of all DTI acquisitions to the mean b=0 image using affine registration. The brain was extracted using the Brain Extraction Tool (BET, part of FSL). FMRIB's Diffusion Toolbox (FDT) was used to fit a diffusion tensor model to the raw diffusion data in each voxel. Voxel-wise maps of

axial (AD; λ1) and radial diffusivity (RD; (λ2+ λ3) / 2), FA and MD were calculated for the TH and SC groups.

Voxel-wise statistical analysis of the DTI data was performed using Tract-Based Spatial Statistics (TBSS, part of FSL), which is described in detail elsewhere.[230][231] Voxel-wise statistics of the skeletonised FA and MD were carried out on the WM skeleton using Randomise (part of FSL) to test for group differences between the TH and SC groups. Randomise performs non-parametric permutation-based testing of inference using Threshold-Free Cluster Enhancement[232] with a correction for multiple comparisons (p<0.05, corrected for sex, birth weight and GA).

A region-of-interest (ROI) approach was also applied to extract FA and MD from PLIC, thalami, lentiform nuclei, midbrain, and genu and splenium of the corpus callosum, as defined by the JHU Neonate Brain Atlas.[202] Manual adjustments of the ROIs were performed to ensure that the ROIs were placed anatomically correct in each participant. Mean FA and MD values were calculated for all participants and ROIs.
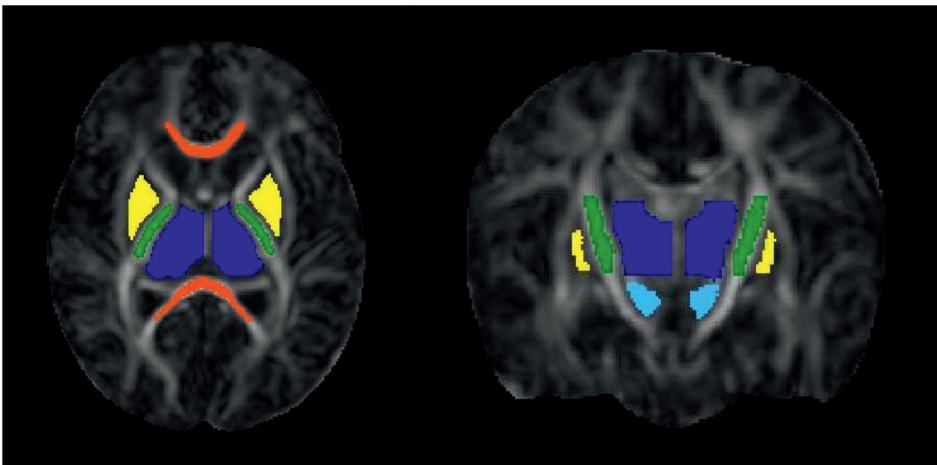
Figure 14: Region of interest location. Posterior limb of the internal capsule (green), thalami (dark blue), lentiform nuclei (yellow), midbrain (light blue), and genu and splenium of the corpus callosum (red) overlaid on JHU Neonate Brain Atlas fractional anisotropy template.

The clinical images were reviewed by Miriam Martinez-Biarge, neonatologist and researcher with experience in neonatal neuroimaging at the Imperial College London, and scored according to Rutherford et al.[170] Images were classified as either:

- Normal/mildly abnormal (any one of the following)
    - BGT: normal or mildly abnormal
    - WM: normal or mildly/moderately abnormal
- Moderately/severely abnormal (any one of the following)
    - BGT: moderately or severely abnormal
    - PLIC: absent signal
    - WM: severely abnormal

Images were also categorised in seven patterns of brain injury:

- Normal: no abnormalities
- Only mildly/moderately abnormal WM
- Only severely abnormal WM
- Mildly abnormal BGT with normal or mildly/moderately abnormal WM
- Mildly abnormal BGT with severely abnormal WM
- Moderately abnormal BGT with no or any WM injury
- Global brain injury: Severely abnormal BGT, WM and cortex with absent PLIC and brainstem abnormalities

The MR spectra were automatically processed on the Philips workstation, and one voxel of interest in the BGT from each infant was chosen. Peak area of major metabolites was calculated, including NAA, Cho, Cr, mI, and Gln/Glu. The peak area values were expressed relative to Cho and Cr.

General movements assessment

A video recording for GMA was taken at 10-15 weeks' post-term age using a standardised setup. A digital video camera was located above the infant, who was placed on a mattress in supine position. The infants were filmed once in an optimal state of active wakefulness

without crying or sucking on dummies. All the videos were later analysed for the temporal organisation of FMs according to Prechtl and co-workers as either:[213]

- - Continuous (++)
- - Intermittent (+)
- - Sporadic (+/-)
- - Exaggerated
- - Absent (-)

Fidgety movements were classified as "absent" if they were absent and "present" if present in a continuous, intermittent, sporadic, or exaggerated pattern.[233]

Figure 15: Video recording for GMA using the standardised video setup.

A video recording for GMA was conducted of 39 infants at a median age of 12 weeks (range 8-16). Two infants came for GMA before ten weeks' post-term age, but both had intermittent FMs and were included in the analysis. Two infants were lost to follow-up before GMA, and another two did not come for GMA.

Long-term outcome assessments

Long-term neurodevelopmental outcomes were evaluated at 18 months by an experienced developmental paediatrician and a clinical psychologist. A complete neurological examination, including the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III),[234] and clinical assessment of vision and hearing were performed. CP and the CP subtype were diagnosed in accordance with Surveillance of Cerebral Palsy in Europe,[235] and the gross motor function was classified using the GMFCS.[132] Adverse outcome was defined as a Bayley-III cognitive and/or motor CS <85 (−1 SD),[236][237] a diagnosis of CP GMFCS level 3–5, impaired sensory/communication outcomes (blindness or deafness), ongoing seizure disorder or death related to associated causes.

The Bayley Scales of Infant and Toddler Development

The Bayley-III is an extensive formal developmental assessment tool that evaluates cognitive, language, and motor development.[234] The assessment can be performed in infants from one and up to 42 months of age. Composite scores (CS) are derived for each of the three domains scaled to a mean of 100 with a standard deviation of 15.

The Bayley-III language CS was not used in the analysis of this study due to the infants' young age and the subjectivity of their non-English application. Assessment of outcome using Bayley-III was available in 37 infants at a median age of 18 months (range 16–21). One infant did not come for follow-up until 48 months of age and was therefore evaluated by Griffith Mental Development Scales (GMDS) assessment. This child had a normal GMDS and was assigned a Bayley motor and cognitive CS of 105 and 107, respectively, due to higher scores found in testing of typically developing children.[238][239] Another child's outcome was based on phone assessment and classified as normal in the binary analysis of outcome. Three infants were lost to follow-up.

Assessments included in the population-based study

Reclassification of HIE

All information about neurological symptoms during the first 72 postnatal hours were collected from the medical records to make a retrospective HIE classification according to a modified Sarnat score.[39 240] The severity of HIE was classified as either mild or moderate/severe because of the difficulties of differentiating the stages retrospectively. This was reviewed by a senior neonatologist unaware of the outcomes.

MRI

Of the 258 included infants, 61 underwent cerebral MRI on clinical indication. The images were scored later for the purpose of this study according to Rutherford et al.[170] Images were classified as either normal/mildly abnormal or moderately/severely abnormal, and also categorised in seven patterns of brain injury, similar to what was described above in the THIN study.

Long-term outcomes

Long-term outcomes included death or a diagnosis of CP at 9-10 years of age. There was no uniform outcome assessment for this study. Diagnoses of CP were collected from the Norwegian Quality and Surveillance Registry for Cerebral Palsy (NorCP) and the local patient administrative system.

The NorCP is an informed consent-based national medical quality and surveillance registry approved by the Norwegian Directorate of Health in 2006.[241] The primary aim is to secure equal health services for people with CP, regardless of the place of residence, and data from the registry should be used in research to gain new knowledge about CP. The registry is owned by the Hospital in Vestfold.

Statistical analysis

Demographic factors and clinical characteristics were summarised with counts (percentages) for categorical variables and means (standard deviation (SD)) or medians (interquartile range (IQR)) for continuous variables in all three papers. Group differences in demographic, neonatal and outcome data were analysed using SPSS version 24 (paper I) and version 25 (paper I, II and III) (IBM, Chicago, Illinois, USA). Two-sided P values <0.05 were considered significant in all papers.

In paper I, the primary analysis was a comparison of infants assigned to the TH group with infants assigned to the SC group (intention-to-treat analysis population). The primary outcome was analysed using TBSS as described above. In addition, the FA and MD values obtained from the ROI approach were analysed using an adjusted two-group comparison (Linear Mixed Model (LMM) and General Linear Model (GLM)), adjusted for sex, birth weight, and GA. LMM was used for PLIC, thalami, lentiform nuclei and midbrain due to dependent measurements from the left and right sides. The within-subject correlation was accounted for by subject-specific random intercept. The multivariable linear regression analyses were done with support from statistician Turid Follestad at the Unit of Applied Clinical Research at NTNU. Group differences in adverse events, complications and conventional MRI and metabolite levels from MRS were analysed by chi-square or Mann-Whitney U tests, as appropriate.

In paper II, the chi-square test, Fisher's exact test, linear-by-linear association, Student's t-test, or Mann-Whitney U test were used as appropriate to analyse group differences in outcomes. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the predictive accuracy for adverse outcome were calculated for moderately/severely abnormal MRI, moderate/severe BGT injury, absent PLIC, combined absent and equivocal PLIC, absent FMs, and combined absent and sporadic FMs. The CIs for the predictive values were calculated using a free online statistical calculator from MedCalc (MedCalc Software, Ostend, Belgium).[242] No corrections were made for multiple comparisons.

In paper III, the study cohort was divided into two groups with infants born from January 2003 until May 2007 (period 1), and from June 2007 until December 2011 (period 2), due to

the implementation of TH in the unit in June 2007. Data from the two periods were analysed separately for comparisons. The Chi-square test, Fisher's exact test or linear-by-linear association were used to assess the differences in proportions between the groups for categorical variables, and the Mann-Whitney U test or Student's T-test were used to assess the differences between groups for continuous variables. In addition, an incidence rate ratio (IRR) calculator was used to compare the incidences of perinatal asphyxia and HIE between the two periods. The IRR was calculated in STATA Statistical software version 17 (StataCorp, Texas, USA).

Ethics

In the THIN study, written parental consent was obtained after giving the parents an information leaflet and oral explanation. Information about the study was provided in different languages (English, Tamil, and Telugu) to ensure that the parents were informed in their native language. The study was approved by the Institutional Review Board at the CMC (number 2013/8223) and the Regional Committee for Medical and Health Research Ethics in Central Norway (number 2013/2167).

The population-based study was approved with waiver of consent for neonatal data, including MRI and CP diagnosis and CP subtype by the Regional Committees for Medical and Health Research Ethics in Central Norway (number 2014/478).

Results

Paper I

Fifty infants (mean birth weight of 2395 grams and GA 39+1weeks) were randomised at a mean age of 3.2 hours. Primary outcome data were available for 22 infants (44%), 11 in each group.

The TBSS analysis of whole-brain WM showed higher FA values in the TH group compared with the SC group in the left PLIC. Mean FA value in left PLIC was 0.407 (SD 0.032) and 0.390 (SD 0.029) in the TH and SC groups, respectively; in right PLIC 0.409 (SD 0.038) and 0.390 (SD 0.036), respectively. Higher FA and lower MD values were also found in several WM tracts.
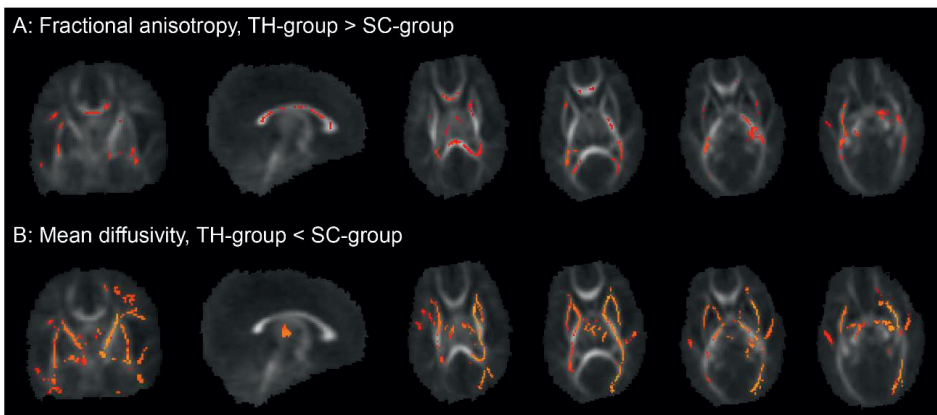
Figure 16: Tract-based spatial statistical analysis of whole-brain white matter. (A) There was significantly higher fractional anisotropy in the therapeutic hypothermia group (TH group) compared with the standard care group (SC group) (p<0.05, non-parametric permutation test, corrected for multiple comparisons, sex, birth weight and gestational age). Areas included the posterior and anterior limb of the internal capsule in the left hemisphere, bilaterally in the external capsule, the genu, splenium and body of corpus callosum, forceps major, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, uncinate fasciculus; superior corona radiata on the left side, and cerebral peduncle and superior longitudinal fasciculus in the right hemisphere. (B) There was significantly lower mean diffusivity in the TH group compared with the SC group. Areas included the posterior and anterior limb of the internal capsule, external capsule, superior and inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, cingulum, uncinate fasciculus, splenium of the corpus callosum, corticospinal tract, cerebral peduncle and thalami bilaterally; and centrum semiovale, anterior, superior and posterior corona radiata in the left hemisphere. From paper I

The DTI analysis by the ROI approach gave an unadjusted mean FA value in PLIC of 0.414 in the TH group compared with 0.397 in the SC group, and after adjusting for sex, birth weight and GA, there was a mean difference between groups of 0.026 (95% CI 0.004 to 0.048, p=0.023). There was also significantly higher FA in the thalami, lentiform nuclei, and midbrain (see Table 2 in the paper).

Conventional MRI was moderately/severely abnormal in 2 (9%) and 10 (43%) infants in the TH and SC groups, respectively (p=0.007). Four infants had a global injury pattern, all in the SC group. There were no significant differences between the groups in major adverse events. Two infants in the TH group died or had withdrawal of life support due to poor prognosis before discharge compared to five infants in the SC group.

Paper II

Forty-six of the 50 infants included in the THIN study had conventional MRI, 39 came for GMA and 47 had available follow-up data. Twelve (26%) infants had moderately/severely abnormal MRI. The most frequent pattern of brain injury on MRI was mildly abnormal BGT with normal or mildly/moderately abnormal WM (57% and 26% in the TH and SC group, respectively). Nine (23%) had absent FMs and 30 (77%) had present FMs: ten (26%) sporadic, 18 (46%) intermittent, one (3%) continual and one (3%) exaggerated.

Seventeen (36%) infants had adverse outcomes, five cooled and 12 non-cooled (21% vs 52%, p=0.025). Eight infants died, seven in the neonatal period and one at 12 months of age. The nine survivors with adverse outcomes included two with CP GMFCS level 5 and seven who had a Bayley-III motor and/or cognitive CS <85.

The occurrence of adverse outcomes was higher among infants with moderately/severely abnormal compared to those with normal/mildly abnormal MRI (8 of 11 and 5 of 32, respectively; p<0.001). The eight infants with moderately/severely abnormal MRI and adverse outcomes had a global brain injury pattern or moderate BGT injury with absent or equivocal PLIC. All five infants with normal/mildly abnormal MRI and adverse outcomes had mild BGT injury with normal or equivocal PLIC and/or moderate WM injury.

Six of the nine (67%) infants with absent FMs had adverse outcomes: one died, two developed severe CP, and three had Bayley-III motor and/or cognitive CS <85. Most infants with present FMs (25 of 29) had a normal outcome. The four infants with present FMs and an adverse outcome all had Bayley-III motor and/or cognitive CS 70–85, and none of them developed CP. The sensitivity, specificity, PPV, NPV, and accuracy of MRI and GMA for adverse outcomes are shown in the table below.

Table 3: Predictive ability of MRI abnormalities and general movements assessment for adverse outcome[*]

	Sensitivity	Specificity	PPV	NPV	Accuracy
Moderately/severely abnormal MRI[†]	61.5 (31.6 to 86.1)	90.0 (73.5 to 97.9)	72.7 (45.6 to 89.5)	84.4 (72.9 to 91.6)	81.4 (66.6 to 91.6)
Moderate/severe BGT injury	61.5 (31.6 to 86.1)	96.7 (82.8 to 99.9)	88.9 (52.6 to 98.3)	85.3 (74.4 to 92.1)	86.1 (72.1 to 94.7)
Absent PLIC	46.2 (19.2 to 74.9)	100 (88.4 to 100)	100	81.1 (72.2 to 87.6)	83.7 (69.3 to 93.2)
Equivocal/absent PLIC	76.9 (46.2 to 95.0)	93.3 (77.9 to 99.2)	83.3 (55.9 to 95.2)	90.3 (77.5 to 96.2)	88.4 (74.9 to 96.1)
Absent FMs	60.0 (26.2 to 87.8)	89.3 (71.8 to 97.7)	66.7 (38.0 to 86.7)	86.2 (74.3 to 93.1)	81.6 (65.7 to 92.3)
Sporadic or absent FMs	70.0 (34.8 to 93.3)	57.1 (37.2 to 75.5)	36.8 (24.4 to 51.3)	84.2 (66.3 to 93.6)	60.5 (43.4 to 76.0)

Data are % (95% CI). PPV, positive predictive value; NPV, negative predictive value; BGT, basal ganglia and thalami; PLIC, posterior limb of the internal capsule; FMs, fidgety movements.
[*]Adverse outcome defined as death, cerebral palsy with gross motor function classification system (GMFCS) level 3-5 or Bayley-III cognitive and/or motor composite score <85.
[†]Moderately/severely abnormal MRI defined as moderate/severe BGT score and/or absent PLIC and/or severe WM score.

Paper III

During the 9-years study period, there were 33 403 term/near-term live births in Sør-Trøndelag county. The incidence of any HIE was significantly higher after the implementation of TH (2.69 vs 4.27 per 1000 in periods 1 and 2, respectively). Although not significant, a higher incidence of moderate/severe HIE was also observed in period 2. After the implementation of TH, 1.45 per 1000 term/near-term live births were cooled.

Table 4: Incidence of perinatal asphyxia and hypoxic-ischaemic encephalopathy (HIE) by study period[*]

	Period 1	Period 2	Total	IRR (95% CI)	*P* value
Incidence per 1000 live births					
Perinatal asphyxia	7.07	7.59	7.35	1.074 (0.834-1.384)	0.571
Any HIE	2.56	4.06	3.36	1.587 (1.075-2.372)	0.015
Moderate/severe HIE	1.04	1.66	1.37	1.599 (0.858-3.081)	0.118
Therapeutic hypothermia	-	1.39			
Incidence per 1000 live births ≥36weeks gestation[†]					
Perinatal asphyxia	7.44	7.97	7.72	1.071 (0.832-1.381)	0.584
Any HIE	2.69	4.27	3.53	1.584 (1.072-2.367)	0.016
Moderate/severe HIE	1.09	1.74	1.44	1.596 (0.856-3.074)	0.120
Therapeutic hypothermia	-	1.45	-	-	-

[*]Period 1 was before implementation of therapeutic hypothermia (January 2003 – May 2007). Period 2 was after implementation of therapeutic hypothermia (June 2007 – December 2011).
[†]If unknown gestational age; birth weight >2kg.
IRR: incidence rate ratio, CI: confidence interval

258 infants were included in the study, of whom 140 (54%) had no HIE, 70 (27%) had mild HIE, and 48 (19%) had moderate/severe HIE. More infants were identified as having HIE in period 2 (36% vs 54% in periods 1 and 2, respectively, p=0.005). In period 2, 26 infants were cooled, of whom 20 had moderate/severe HIE, five had mild HIE, and one had no reported HIE. Eleven (35%) of 31 infants with moderate/severe HIE in this period were not treated with TH. Of these, one died early, one was assumed to have a prenatal insult, one had postnatal collapse at 1.5 hours of life and was not considered for TH, and seven had a delayed diagnosis of HIE due to other concurrent disease or presentation of seizures after six hours of life.

Eight infants, all with moderate/severe HIE, died before discharge, and one died after the neonatal period of complications related to brain injury. Sixteen (80%) of 20 cooled infants with moderate/severe HIE survived without CP. Of the 70 infants with mild HIE, two developed CP and none died.

Fourteen (33%) of the 43 infants with moderate/severe HIE had moderately/severely abnormal MRI, of which five died and four developed CP (one had GMFCS level 1, three had

GMFCS level 4-5). Three infants with moderate/severe HIE and normal or mildly abnormal MRI developed CP, all with GMFCS level 1.

Discussion

Summary of main findings

With the main purpose of improving outcomes for infants exposed to perinatal asphyxia and HIE in a global perspective, we conducted two studies. One was an RCT on TH using a low-cost cooling device in a tertiary NICU in a low-resource setting (THIN study). The other was a population-based observational study on the incidence and outcomes after perinatal asphyxia and HIE in a high-income setting.

The THIN study reported a neuroprotective effect of TH on MRI biomarkers. The beneficial effect was found even though the use of sedatives/analgesics and mechanical ventilation were limited. The patterns of brain injury of the included infants were similar to those reported from high-income countries. Brain injury on neonatal MRI, particularly findings of moderate/severe BGT injury and abnormal signal in PLIC, and absence of FMs on GMA predicted neurodevelopmental outcomes at 18 months with high accuracy.

In the population-based study, there was an increase in the reported incidence of HIE after the implementation of TH, despite similar admission rates due to perinatal asphyxia. After the implementation of TH, 1.45 per 1000 live term/near births were cooled. Only 65% of infants with moderate/severe HIE were cooled, mainly due to late presentation of seizures.

Methodological considerations

Both intervention and observational studies can fail to report the true answer to the research question due to errors that can be either random or systematic.[243] Random errors will always occur due to the fact that no measurement or no data set are perfect, and these errors cannot be eliminated entirely. These can be reduced by increasing the sample size

or repeating measures, however, both our studies had a relatively small sample size. Systematic errors, or bias, are not determined by chance but due to inaccuracy in the system, causing consistent errors. Systematic errors are usually more problematic in research as they can lead to biased data and possible false conclusions, regardless of the size of the study. The two studies in this thesis had different designs, and the methodological considerations will, therefore, be discussed separately.

THIN study

The THIN study was a single-centre open-label RCT. RCTs are considered the gold standard of study design when performing interventional studies. The main strengths of an RCT are that the random assignment to the treatment groups avoids confounding and minimises selection bias. When performed correctly, randomisation ensures a balance between the groups for both known and unknown factors. The treatment and control groups can be considered equal, and the effect is isolated to the intervention.

According to the planned sample size, this study included and randomised 50 infants. Stratification was used to balance the groups on severity of encephalopathy (moderate or severe) as the neuroprotective effect has been suggested to be less in infants with severe HIE.[244][245] Based on previous data, we expected 20% of included infants to have severe HIE. However, only two infants with severe HIE were included. A possible explanation for the low proportion of infants with severe HIE is that the most severely affected were not offered intensive care and were, therefore, not considered eligible. This was supported by the finding of a very low proportion of severe HIE also among screened infants. Thus, the results of the present trial cannot be extrapolated to infants with severe HIE.

Single- or double-blinding of the treatment groups was not possible due to the nature of the intervention. However, the persons performing and interpreting the outcomes were blinded to the intervention. Selection bias is important when conducting unblinded studies with sequential enrollment. To minimise this selection bias, the study used allocation concealment by sequentially numbered opaque sealed envelopes.

The small sample size is the main limitation of this study. The primary outcome in this study was an MRI biomarker, which was considered a surrogate for later

neurodevelopmental outcomes. DTI measures, and the value of FA in PLIC in particular, are shown to accurately predict later outcomes.[200] DTI biomarkers have, therefore, been suggested as an endpoint in clinical trials.[246][247] Others have demonstrated significantly lower FA in PLIC with similar sample sizes when comparing groups of infants with HIE based on treatment with TH or adverse outcomes.[201][246] An advantage of the small sample size was the ethical challenge with including more infants to normothermia given the beneficial effect of TH already proven in other settings, but the study was not powered to detect differences in neurodevelopmental outcome between treatment groups.

Despite proper randomisation procedures, there is a risk of imbalance between groups in small trials. Such imbalance could affect the measured effect of the intervention. As this was an RCT, the neonatal baseline characteristics were not compared by statistical testing. There was an observed trend towards lower 5-minute Apgar scores among the non-cooled infants. The cooled infants had slightly lower cord pH compared to the non-cooled infants, but there was a high rate of missing data. There was also a higher number of mechanically ventilated infants in the non-cooled group (8 % of cooled vs 24% of non-cooled infants), although not statistically significant.

Another limitation is that only 11 infants in each group (44%) had DTI data for analysing the primary outcome. This was primarily due to periods of technical issues with the MR scanner giving incomplete data sets and due to artefacts in obtained images. DTI analyses are susceptible to movement and vibration artefacts, and neonates are therefore a difficult population to examine without proper sedation.[248] Despite this, we found significant differences between the treatment groups. The statistical analysis of the primary outcome was done by intention-to-treat using sex, birth weight and GA as covariates. We did not adjust for other possible prognostic factors due to the small sample size. The large amount of missing data on the primary outcome could have biased the estimated treatment effect. The four non-cooled infants with a global injury pattern on conventional MRI did not have DTI data and did not contribute to the group differences found on DTI. This suggests that the beneficial effect of cooling was underestimated.

Another limitation of this study is the short duration of follow-up. The outcome was assessed by Bayley-III at 18 months of age which is the most widely used assessment of early development. Both motor, cognitive and language development were evaluated using

Bayley-III, but language scores were not included in the outcome due to the children's low age and the subjectivity of their non-English application. The Bayley-III has been shown to under-identify later cognitive and motor impairments, and the threshold for adverse outcome was, therefore, set at -1SD.[236-238 249] Cognitive and behavioural impairments are a challenge for children with a history of HIE, also for those without CP.[250] These challenges may not be apparent until later in childhood and requires long-term follow-up.[251]

A strength of the study was that the secondary MRI outcomes (conventional MRI) were available for all, except the four that died early. These images had good quality, and all could be scored. In addition, there was a very low attrition rate in both treatment groups, with only three infants lost to follow-up.

Population-based observational study

The population-based study aimed to describe temporal trends in the incidence of admissions to a neonatal unit in Norway related to perinatal asphyxia and HIE and their outcomes over a 9-years period. The study was observational, and data were collected retrospectively. Observational studies may be prone to selection bias. Selection bias can occur when the group included fails to represent the population of interest, which could interfere with the results and affect the internal validity. As this study was population-based, we were able to include all cases from the whole source population. The selection of infants in this study was based on the ICD-10 diagnoses reported at discharge, and all diagnoses (both primary and secondary) were considered. The local admission protocol was also screened, and this resulted in the inclusion of an additional nine infants. In addition, we used broad inclusion criteria and detailed exclusion criteria to define clinically significant perinatal asphyxia. The collection of detailed clinical data from the neonatal admission ensured that all eligible infants were carefully screened. However, incorrect reporting of diagnosis and missing data in the medical records cannot be excluded.

A major limitation of the retrospective design is that all the collected data were exclusively relying on data from the medical records, which were not originally obtained for research purposes. Clinically relevant information may not be documented in the medical record and, therefore, not available. However, access to individual medical records with a large

amount of data (clinical, labs, imaging results and observation charts from doctors and nurses) ensures a much more accurate collection of data than any register-based study.

The retrospective reclassification of the severity of HIE and HIE diagnosis was also limited by the availability of information. However, this reclassification was necessary as the documentation of ICD-10 diagnosis of HIE was inadequate in many patients. Another bias is that the introduction of TH may have led to more accurate documentation of neurological status in the medical records compared to the earlier period. A relatively low number of included infants limited the power of subgroups analysis, especially when comparing cooled and non-cooled infants with moderate/severe HIE in the second period.

This study had a low attrition rate with only a 3.5% loss to follow-up due to emigration. We consider, therefore, the risk of bias due to loss to follow-up to be negligible. CP diagnoses were based on ICD-10 diagnoses in the local patient administrative system and the NorCP, which had a national completeness of CP diagnoses of 91% in 2006 to 2007.[252]

Ethical considerations in the THIN study

The THIN study was planned at a time when several large RCTs had demonstrated a beneficial effect of cooling. Many units, even in LMICs, had started to cool, and it was a dilemma whether it was ethical to conduct large RCTs of hypothermia against normothermia in LMICs.[108 253] Choosing an MRI biomarker as primary outcome had the great advantage of reducing the sample size to include fewer infants to standard care with normothermia.

Performing research in resource-limited settings and doing research that involves vulnerable individuals adds to the complexity of issues like informed consent.[254] Institutional ethical review boards and frameworks of research governance are both essential to ensure the ethical standards of clinical trials. The THIN study was initiated locally and had a local principal investigator. Local ethical committees in India and Norway approved the study, and all parents of included infants gave written informed consent. A recent systematic review evaluating informed consent rates for neonatal RCTs found that trials in LMICs reported consistently higher consents rates than trials from high-income countries.[255] Two-thirds of trials from LMICs had consent rates of more than 90%

compared to only one quarter in high-income countries, suggesting the possibility of flaws in the process of informed consent. The consent rate in our study was similar to the RCTs conducted in high-income settings (96% in THIN study, as compared to 94% in CoolCap, 95% in NICHD, 92% in TOBY, and 87% in ICE trials).[63 68 69 240] This may indicate that the availability of a potentially life-saving treatment is an extremely strong motivation for participation. It is important to recognise that these parents are in shock after such unexpectedly dramatic deliveries. Adding the short therapeutic window of cooling, there is little time for thorough consideration.[256] Regardless of the setting, it is of indisputable importance to ensure that the information given about a clinical trial is adapted to and understood by the recipient.

Discussion of main findings

Neuroprotection by TH on MRI biomarkers

The THIN study reported a neuroprotective effect of TH in a low-resource setting based on neonatal MRI biomarkers. The MRI biomarkers have been suggested as reliable end-points in clinical trials and shown by others to give an accurate prediction of long-term neurodevelopmental outcomes.[203 246] The higher FA values in several WM tracts in cooled compared to non-cooled infants is in accordance with the study by Porter et al.[201] The brain regions affected are typically regions where myelination occurs around term age and/or areas with a high metabolic rate.[257 258]

This favourable effect of TH was found by both the voxel-based analysis (TBSS) and the ROI analysis. Others have also demonstrated a good correlation between TBSS and the manual ROI approach when evaluating infants with HIE.[201 259] TBSS has the advantage of being more effective and reproducible, however, it can presently only be used for group analysis. The ROI approach may be implemented in a clinical setting if age-appropriate normal values are determined. Our findings are in contrast with the HELIX trial, which did not find any difference between the cooled and non-cooled infants on whole-brain WM FA by TBSS.[110] They also failed to show any difference between the groups on MRS.

The ADC values derived from DWI and the MD values from DTI are comparable, and both are markers of hypoxic-ischaemic brain injury. They are, therefore, often discussed

together. We consistently found lower MD values in the cooled compared to the non-cooled group, which has also been reported in a study by Artzi et al.[260] These findings are in contrast to most studies reporting lower ADC and MD values in infants with brain injury.[261-264] It is important to notice that ADC and MD values may pseudonormalise in the second week after an acute hypoxic-ischaemic insult, and this process is affected by TH.[261] The finding of lower MD values is therefore of unknown significance.

The favourable effect of cooling was also confirmed by conventional MRI. The proportion of infants with moderate to severe abnormalities on conventional MRI is lower in our study compared to both the NICHD and TOBY trials.[170][191] This is in accordance with the very low proportion of infants with severe HIE in our study.

Patterns of brain injury

The predominant pattern of brain injury in the THIN study was BGT with or without WM and/or cortical injury. BGT injury is considered the most typical injury pattern in newborns after an acute hypoxic event.[182] Only two infants had severe WM injury with normal or mildly abnormal BGT, and the distribution of WM injury in our study was similar to the TOBY trial.[170] This differs from another Indian study of infants with HIE, where more than 90% of infants had WM injury and only 27% had any BGT injury.[265] More than 50% of the included infants in that study had mild HIE, which may explain some of the differences in injury patterns.[178] A similar predominance of WM injury was also reported in the HELIX trial, although this study included only infants with moderate or severe HIE.[110] This WM injury predominance is suggested as a possible explanation for the lack of neuroprotective effect of cooling. WM injury may be a marker of more prolonged hypoxia-ischaemia or other causes of NE.[177][178][188] The high occurrence of WM injury has been used as an argument for a "population difference" between high- and low-resource settings. However, the patterns of brain injury in the THIN study, with the majority of infants having BGT injury with only minor or no WM injury, are similar to studies in high-income settings.[170][172][191] The patterns of brain injury in HIE infants from different settings should be further explored.

Prediction of outcomes by MRI and GMA

Both MRI and GMA predicted neurodevelopmental outcomes with high accuracy in our study. The highest predictive accuracy for an adverse outcome using MRI was by moderate/severe BGT abnormalities and equivocal/absent PLIC. Substudies of both the TOBY and ICE trials found similar high accuracy of neonatal MRI.[170 171] This differs from the Indian study by Lally et al. with a high occurrence of WM injury, reporting a low sensitivity of MRI abnormalities in the detection of adverse outcomes.[265]

The absence of FMs on GMA had similar predictive accuracy as neonatal MRI in our study. A recent study also reports that neonatal MRI and GMA can be used for outcome prediction with high accuracy in cooled infants with HIE, either separately or alone.[227] We also demonstrated a close association between the temporal organisation of FMs on GMA and Bayley-III motor and cognitive CS. Normal FMs at 10-15 weeks post-term age is a well-known predictor of a normal motor outcome.[266 267] The association between GMA and cognitive outcomes have also been shown by others.[224 225]

The rate of adverse outcomes was lower among cooled compared to non-cooled infants. However, the THIN study was not powered to evaluate adverse outcomes at 18 months of age, and this result must, therefore, be interpreted with caution.

Temporal trends and definitions of perinatal asphyxia and HIE

A uniform definition of perinatal asphyxia is lacking, which makes it difficult to compare trends both over time and between different countries and settings. We report an incidence of admissions for neonatal care due to perinatal asphyxia of 7.7 per 1000 term/near-term live births. Previous large, register-based studies often use proxy variables to define perinatal asphyxia, such as low Apgar scores or metabolic acidosis in cord blood.[268-270] Two European studies reported incidences of perinatal asphyxia of 5.4 and 8.5 per 1000 live births when defining asphyxia by a 5-minute Apgar score <7 alone,[271 272] but a much lower incidence was reported by a Canadian study using a much stricter definition, more similar to the entry criteria of TH.[273]

The reported incidence of HIE in this study is higher than what others have found in similar settings, whereas the incidence of moderate/severe HIE is more consistent with other studies.[137 274-278] There is, however, inconsistent use of the term HIE in the literature as some refer to HIE including all three stages, whereas others refer to only moderate/severe HIE.[137 277] A very low incidence of moderate/severe HIE was reported by a Swedish study using TH as a proxy for the diagnosis.[278]

The neurological symptoms and signs in infants with encephalopathy are dynamic and evolve over time.[39 51] In addition, the evaluation of HIE severity is subjective and based on individual judgment with uncertain inter-rater reliability.[49 50] One study that used certified examiners reported difficulties in assigning the severity of HIE within the first postnatal hours, which has become the decisive time for the neurological assessment after the implementation of TH.[279] There is also a lack of consensus between studies on defining the severity of HIE with different thresholds or number of symptoms and signs required to fulfil one level of HIE. The large RCTs on cooling versus normothermia had criteria for defining moderate/severe HIE with either strict neurological criteria alone assessed by certified examiners (NICHD trial) or less strict neurological criteria and an abnormal aEEG recording (TOBY and CoolCap trials).[63 68 240]

After the implementation of TH, there was a significant increase in the incidence of HIE in our study. This unexpected finding could indicate that infants in this period were more severely affected by the asphyxia/hypoxic-ischaemic insult. There were, however, similar admission rates related to perinatal asphyxia before and after TH and no apparent difference in illness severity. Increased awareness to and documentation of neurological signs and symptoms after implementation of TH have most likely contributed to this increased incidence.

We report that 1.45 per 1000 live-born infants were cooled after the implementation of TH. However, 35% of infants with moderate/severe HIE in the same period were not cooled, which is similar to what others have found.[274 280-282] These non-cooled infants in our study differed from the cooled infants in that they were smaller, less acidotic and were to a lesser extent resuscitated after birth. One could speculate that some of these could have prenatal insults which would not benefit from TH. However, many had a delayed diagnosis, especially due to the late presentation of seizures.

We report the outcomes death and CP at 9-10 years of age. As expected, there was a low rate of CP and no deaths among infants with mild HIE. The majority (80%) of cooled infants with moderate/severe HIE survived without CP compared to only 40% of the non-cooled infants with moderate/severe HIE admitted after June 2007 (implementation of TH). The rate of adverse outcomes among cooled infants is much lower than what was reported in the first large RCTs on TH. Although using similar entry criteria, more recent studies on cooled infants also report improved outcomes.[283][284] Possible explanations are earlier recognition and treatment with TH and general improvements in obstetric and neonatal care. However, a drift towards cooling of infants with mild HIE has also been reported, which could influence the rate of adverse outcomes and alter the balance between benefit and harm.[285-287]

Clinical implications and generalisability

Perinatal asphyxia is an extremely serious condition for those who are severely affected and continues to be the leading cause of brain injury in term infants. In a global perspective, nearly 2000 neonates die every day, and the same number survives with permanent brain damage and life-long disabilities due to this devastating condition.[52][288] If the use of TH is targeted even better towards those infants who will benefit from the treatment, and cooling is proven effective also in less resourced settings, it will make a significant impact for the lives of these babies and their families. LMICs are home to 84% of the world's population and carry the highest burden of this condition.[289]

We found that TH induced by a low-cost cooling device reduced brain injury on MRI biomarkers in infants with moderate HIE in a neonatal unit in India. The treatment was both feasible and safe, and we claim that our findings support the implementation of TH in tertiary NICUs in low-resource settings with the appropriate level of intensive care. This is contrary to the conclusion of the HELIX trial, which was the headliner on the main website of Lancet Global Health in August 2021:[110]

"*Therapeutic hypothermia should not be offered as treatment for neonatal encephalopathy in LMICs even when tertiary neonatal intensive care facilities are available.*"

Thayyil et al. claim that the lack of neuroprotective effect must be explained by population differences alone since both the cooling equipment used and the intensive care provided in this study were optimal. However, included newborns were cared for in tertiary neonatal units in public sector government hospitals, with one nurse for every two to four patients, and blood pressure was monitored non-invasively every four hours. Only 15% were recruited outside India, and two-thirds of all included infants were outborn. The high occurrence of early seizures and low incidence of sentinel events in the HELIX trial could be explained by more frequent prelabour injury but can also be a marker of less rigorous monitoring during labour and/or poor initial stabilisation. Persistent metabolic acidosis and hypotension, and prolonged blood coagulation and gastric bleeds were more common among cooled infants, questioning whether the level of intensive care offered in the hospitals participating in the HELIX trial was optimal. Biochemical markers for infants included in the THIN study will be published in a separate paper to explore any such differences between cooled and non-cooled infants in this trial.

The argument that "population differences" is the reason why cooling does not work, or even inflicts harm, in infants cared for in LMICs, has been repeated for over a decade.[54][112] This implies that the millions of babies born within the context of a LMIC have some biological similarity that make them different from babies born in high-income countries. Some NICUs in LMICs provide the same level of care as NICUs in high-income countries, whereas others are without any intensive care facilities. The group of countries classified as LMICs comprises countries and areas with huge socio-economic and cultural diversity as well as different health-related challenges.[289] Instead of using the term "population differences", a non-biased description of the setting is important. Antenatal care, intrapartum monitoring, resources for timely caesarean section, resuscitation facilities, and neonatal transport services should be considered not only when determining whether cooling should be provided but also where and how the resources should be invested to improve outcomes. Resources vary between areas and countries, and a blanket statement based on one RCT that it is malpractice to cool babies born in LMICs, is irresponsible and unethical.[290]

Early prediction is important for both parents and health care workers to inform the affected families and to direct interventions and follow-up after HIE. We have shown that

GMA has similar predictive accuracy as MRI. MR scanners are expensive both to buy, maintain and use, and are often unavailable in a low-resource setting. On the other hand, smartphones are readily available regardless of setting and can be used to obtain video recordings of the infants' spontaneous movement for GMA. The video could be evaluated by remote assessment. This is both a time- and cost-effective predictive assessment. We, therefore, claim that GMA is a good supplement, or even an alternative, to MRI, especially in low-resource settings.

Perinatal asphyxia and HIE are rare but potentially life-changing incidents for those affected also in high-income settings such as Norway. Its incidence is important as a marker of the quality of prenatal, obstetrical, and neonatal care. We found a higher incidence of any HIE compared to many others, but this may be due to wide inclusion criteria and a relatively large proportion of included infants with no or mild HIE. With an increased focus on adverse outcomes also in infants with mild HIE, there is a need for more accurate tools to identify infants who may benefit from TH.

The significant rate of infants with moderate/severe HIE that were not cooled in period 2 suggests that appropriate training and vigilance towards early neurological signs and symptoms to identify infants with HIE are necessary. Those who had an early NICU admission might have been recognised earlier if aEEG had been used more frequently. The advances and increased availability of electrophysiologic and neuroimaging techniques are promising, and more objective early biomarkers of evolving brain injury could possibly improve the selection of infants for cooling.

Future research

The beneficial effect of TH in the THIN study was found even though few infants received continuous infusion of sedatives and analgesics, and few were mechanically ventilated. This is in contrast to clinical practices in many HICs where infants treated with TH are electively intubated. Mechanical ventilation will be limited in many low-resource settings, and it is of critical importance to determine if TH can be provided in a safe and efficient way without mechanical ventilation. Stress might increase injury to a vulnerable brain, and an animal study found a lack of neuroprotective effect of TH without sedation/analgesia.[291]

Sedatives and analgesics may have neuroprotective or toxic effects on the developing brain, and a better understanding of such effects, as well as the optimal level of sedation during cooling, needs to be determined. [292] [293]

Although it is widely recognised that TH is a highly intensive treatment necessitating careful monitoring of temperature, respiration, and circulation, the required level of supportive care is not known. Close monitoring of vital functions, and the ability to provide immediate interventions and treatments for cardiorespiratory instability must be available. However, the required level of clinical and biochemical monitoring, and the use of antibiotics and nutrition during TH are examples of unresolved questions which warrant further research. Such research is necessary to optimise supportive care during TH to benefit infants with HIE in all settings.

In the population-based study from Norway, we found that a significant proportion of infants with moderate HIE was identified after six hours of life and, therefore, not eligible for TH. More widespread use of aEEG on admission may improve the early identification of infants who may benefit from TH, but additional early biomarkers are also needed. A uniform agreement on the definition and classification of HIE severity at well-defined time intervals after birth could also improve the selection of infants for neuroprotective therapies both in research and clinical practice. Extension of the criteria for TH to include late or moderately preterm infants is currently being investigated (NICHD Neonatal Research Network; Preemie Hypothermia for Neonatal Encephalopathy, ClinicalTrials.gov Identifier: NCT01793129), and RCTs on TH for infants with mild HIE is being called for and ongoing (Optimizing the Duration of Cooling in Mild Encephalopathy (COMET Study), NCT03409770).[287] [294] [295] Other neuroprotective treatments, as supplements or alternatives to TH, are also being studied but are not yet available in clinical practice.[296-304]

Increased knowledge on the patterns of brain injury in infants with HIE from different settings, the optimal level of supportive care during TH and improved selection of infants for cooling will hopefully help save even more children from dying or surviving with disabilities. The medical community also needs to stop dichotomising the world based on the country's income level. The majority of the worlds' population lives in LMICs, and both within and between countries there are huge differences in health, education, income, and wealth.[289] A phased approach, as suggested by Wilkinson et al., must be considered, where

improving antenatal, perinatal and neonatal care should be the main focus in the lowest resourced settings, while local clinicians, clinicians with experience with cooling, and policymakers should discuss if cooling is safe and effective in tertiary NICUs with proper intensive care.[112] Our vision should be in accordance with WHOs "Making Every Baby Count", which states:[305]

"*It is time to make every baby count and prevent future tragedies, by learning from and effectively responding to preventable deaths.*"

Conclusion

This thesis sought to improve outcomes of infants with perinatal asphyxia and HIE in a global perspective. We have shown that TH using a low-cost cooling device is both neuroprotective and safe in a tertiary neonatal unit in India. The neuroprotective effect was demonstrated on several MRI biomarkers, and the patterns of brain injury on conventional MRI were closely associated with outcomes at 18 months. The safety and efficacy of TH in low-resource settings are still controversial, and some claim that the treatment should still be considered experimental in all LMICs. We hope our results will contribute to a common effort to identify the required and adequate level of supportive care during TH, which is of relevance to everyone providing TH to infants with HIE. In addition, we report similar predictive accuracy of neonatal MRI and GMA and suggest that GMA is a feasible and low-cost method to prognosticate and direct follow-up in settings where MRI is not available.

This thesis also reported an increased incidence of HIE after implementing TH in clinical practice in a Norwegian county, despite similar admission rates related to perinatal asphyxia. This might be related to an increased attention to neurological signs and symptoms. A significant proportion of infants with moderate or severe HIE did not receive TH, and many of these infants were diagnosed after the therapeutic window due to late presentation of seizures. Hopefully, this knowledge will help direct research on better methods for early diagnosis and selection of infants for cooling.

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Appendix 1

Basic principles of MRI

An MRI scanner contains one main magnet coil, which generates a strong, constant magnetic field. The field strength is measured in the unit Tesla (T), and one Tesla corresponds to 20 000 times the magnetic field of the earth.[1] Most MR scanners in clinical use have a field strength of 1.5 or 3 T. Secondly, an MR scanner contains three gradient coils placed in each of the three orthogonal directions, which can create local variations in the main magnetic field. The third part of the scanner is the radiofrequency (RF) coils which transmit energy to the tissue of interest and subsequently receive the signal back from this tissue. The primary origin of the MR signal comes from the hydrogen nuclei, which contains one single proton. The proton is constantly spinning and thereby creating its own magnetic field, known as a magnetic moment.[2] In normal circumstances, the magnetic moments are randomly oriented, but when placed in an external magnetic field, they align their spin direction with this field, either parallel or antiparallel. More protons align parallel than antiparallel, and this creates a sum magnetic field aligned along the main field.

The RF energy is transmitted for a short period, called an RF pulse.[3] Pulses with the same frequency as the proton's precessional frequency can transfer energy to the protons and alter the direction of the proton spins and flip the alignment of the protons away from the main magnetic field. The result is a reduction in the overall longitudinal magnetisation and generation of transverse magnetisation.[1] This transverse magnetisation can be detected as an induced current in the receiver coil and is the basis for the MR signal. When the RF pulse is switched off, the protons return to their original position and lose energy. This process causes recovery of the longitudinal magnetisation, called longitudinal relaxation (T1-relaxation).
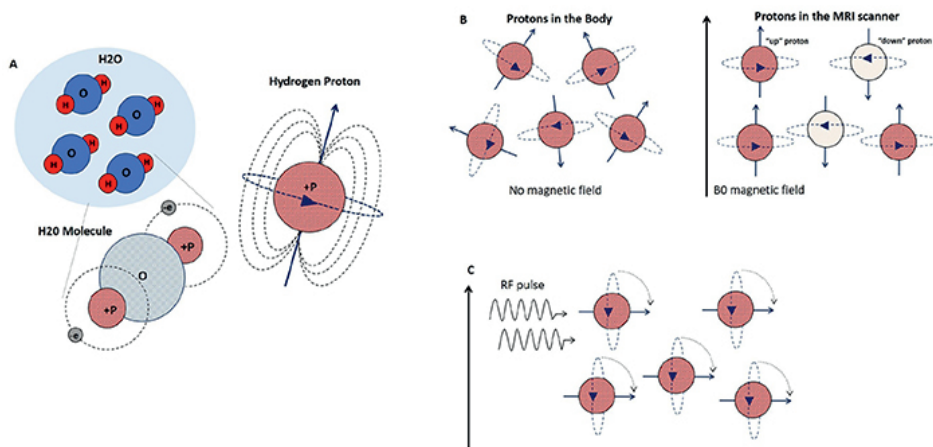
Figure 1: Hydrogen protons and how they behave in a magnetic field. **(A)** Water consists of two hydrogen atoms and one oxygen atom. The hydrogen nucleus (shown in red as P+) contains one positive charge—a proton spinning around on its axis, which acts like a tiny magnet, i.e. has a magnetic moment. **(B)** In the MRI scanner, the protons align with the B0 magnetic field, some "up" (red) and slightly less "down" (white). The total magnet field generated from all the hydrogen protons almost cancel each other out to leave only the magnetic field from the small proportion of extra "up" protons. It is this small magnetic field that we manipulate and measure using MRI. **(C)** When an RF pulse with the same frequency as the proton's precessional frequency is turned on, the "up" protons flip away from the B0 field as they absorb the RF energy. When the RF pulse is turned off, the protons return to aligning along the main magnetic field. From Broadhouse K.[4] Licence: CC BY 4.0.

Simultaneously, there is a process called transverse relaxation with a reduction in the transverse magnetisation due to interaction between neighbouring spins and loss of coherence (T2-relaxation). The relaxation process can be detected as a decreasing signal in the receiver coil. The relaxation process rate depends on the different molecules' properties of a tissue, and these differences provide the contrast in the MR image. The molecules' molecular motion, or tumbling rate, determines the T1 relaxation. Free water molecules are small and have high mobility, causing slow or less efficient T1 relaxation. However, as the water molecules are spaced apart, the interaction between the spins are less likely to occur, and the T2 relaxation is slow. Fat has low molecular tumbling but efficient energy exchange and, therefore, quick T1 relaxation. T2 relaxation is also short in fat as the molecules are close, and interaction between spins are more common.[5] Conclusively, fat has a short T1 and T2 time, and water has a long T1 and T2 time.

The intrinsic contrast parameters (T1 and T2 relaxation/ time) of the different molecules determine contrast between tissues. In a T1 weighted image, the contrast and signal intensity predominantly depend on the difference in T1 relaxation time. The T1 weighted image is characterised by low water (dark) and high fat (bright) signals. T2 weighted images are dependent on the difference in T2 times and are characterised by a high water signal (bright) and a low fat signal (dark). The weighting of the MR signal is influenced by the time between two RF pulses (repetition time, TR) and the time from applying the RF pulse to the maximal signal amplitude in the coil (echo time, TE).[3] The TR and TE are chosen and set by the operator to gain a specific tissue weighting in the image. Generally, a T1 weighted image has short TR and TE, and a T2 weighted image has long TR and TE.[6] T1 and T2 weighted images are conventional imaging techniques that demonstrate anatomical features including pathology on a macrostructure level.

To create an image, the MR signal must be localised. The signal is localised in three dimensions using three separate field gradients: the slice-selection gradient, the phase-encoding gradient, and the frequency-encoding gradient.[6] More details on the image construction and acquisition will not be covered here.
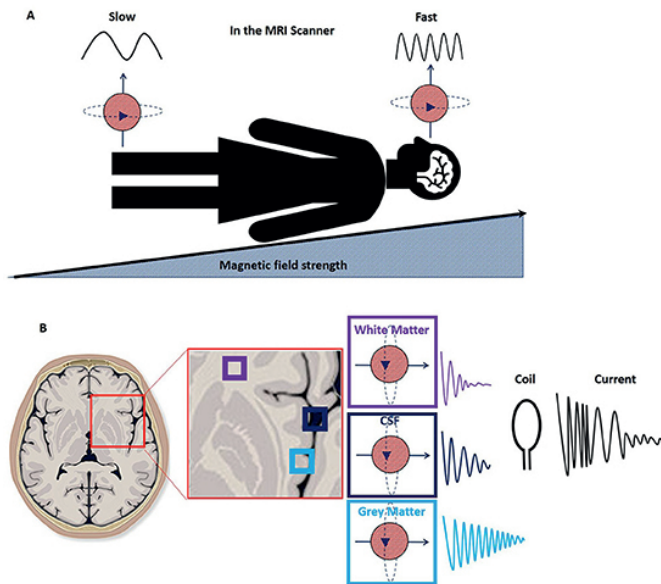
Figure 2: Acquiring an MR image. **(A)** The person is positioned inside the main magnetic field and the protons in his/her body will align along the magnetic field. A second magnetic field is applied, which increases across the body, from foot to head. Hydrogen protons in the head will then be spinning faster than those in the feet. **(B)** Different tissues, such as white matter, grey matter and cerebral spinal fluid in the brain will relax differently and therefore give off different amounts of energy to the coil around the head. This signal is decoded, and an MR image constructed. Modified from Broadhouse K.[4] Licence: CC BY 4.0.

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Appendix II

CORRESPONDENCE

Rise and Fall of Therapeutic Hypothermia in Low-Resource Settings: Lessons from the HELIX Trial: Correspondence

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To the Editor: Being responsible for the THIN study (Therapeutic Hypothermia in India), we want to comment on the review by Krishnan et al. published in *Indian Journal of Pediatrics* [1]. Erroneous reproduction of data from other studies and nondocumented insinuations and accusations, require a response.

The THIN study was a single-center, randomized trial on cooling for HIE in South India [2]. The primary outcome was signs of hypoxic–ischemic injury on MRI, and 18 mo' outcomes have recently been reported [3]. We were worried to see that Krishnan and co-workers have misinterpreted our data. The number of infants enrolled in each arm was 25 and not 11, as quoted in Table 1 of their published review [1]. Similarly, neonatal mortality was 2/25 (cooled group) and 5/25 (standard care group), and not twice in the cooled

infants compared to usual care. Similar errors could have been made with other included studies, which casts doubt on the validity of their review.

The authors also claim that we "... made a bold statement on safety and efficacy of cooling... reflecting a lack of understanding of the complexity of using MRI biomarkers in neuroprotection trials." MRI is the best predictor of outcome after HIE [4, 5]. Diffusion tensor imaging (DTI) is an extremely sensitive and accurate biomarker of neurological injury, even in group sizes as small as 10 [6, 7]. Choosing an MRI biomarker as primary outcome had the great advantage of reducing the sample size at a time when many units, even in LMICs, had started to cool based on the clear beneficial effect shown in the large RCTs [8]. We found significant differences between groups both in the primary and secondary MRI outcomes, indicating a positive effect of cooling and reiterating the sensitivity of MRI [2]. Differences in patterns of brain injury, as suggested by Krishnan et al., is not supported by findings in the THIN study where only 35% of infants had normal basal ganglia and thalami, and the distribution of white matter injury was similar to the TOBY trial [4].

The argument that "population differences" is the reason why cooling does not work, or even inflicts harm in infants cared for in low- and middle-income countries (LMICs), has been repeated for over a decade by the same research group [9]. The countries classified as LMICs have huge socioeconomic and cultural diversity. Antenatal care, intrapartum monitoring, resources for timely C-section, resuscitation facilities, and transport services should be considered not only when determining whether cooling should be provided, but also how the resources should be invested to improve outcomes. Babies in the HELIX trial were cared for in tertiary neonatal units in public sector government hospitals with one nurse for every 2–4 patients, and 2/3rd of babies were outborn [10]. A blanket statement based on one RCT that it is malpractice to cool babies in LMICs, is irresponsible and unethical.

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The authors accuse Thomas and colleagues from Christian Medical College, Vellore, of commercializing a low-cost cooling device (Miracradle). However, it was not commercialized or marketed by them, but handed over to the industry without any benefits for the individuals or the institution.

This mix of nonevidenced and vague insinuations and accusations is repeated throughout the review. Generalizing statements by the researchers about poor research design, fraud and noncredible data, naivety, "white-savior" attitudes and commercial interests appear to be targeted on everyone involved in cooling research in LMICs. We are very conscious about our responsibilities when doing research in resource-limited settings, and such issues deserve open-minded, respectful, and constructive discussions. The way this is done by Krishnan et al. does not contribute to this necessary debate, and we are afraid that it will not contribute to improving the health of infants born in LMICs.

Declarations

Conflict of Interest None.

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Appendix III

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Questions about the HELIX trial

We congratulate Sudhin Thayyil and colleagues[1] on publishing the HELIX trial in *The Lancet Global Health*. Based on their results, which showed increased mortality in the hypothermia group compared with the standard care group, they recommended that cooling for neonatal encephalopathy should not be offered in low-income and middle-income countries (LMICs). However, we feel that their results cannot be generalised.

Thayyil and colleagues argue that all infants in the HELIX trial received optimal intensive care.[1] Babies were cared for in public sector government hospitals (which were mostly in India) with one nurse per two to four babies. Blood pressure was measured non-invasively every 4 h. We feel that these measures do not constitute optimal intensive care, regardless of country or setting. The high occurrence of early seizures and the low incidence of sentinel events could be explained by more frequent prelabour injury, but could also be a marker of less rigorous monitoring during labour or poor initial resuscitation and stabilisation, especially given the fact that 70% of babies in their study were outborn. No data are presented on outcomes in relation to severity of encephalopathy or start of cooling (median age 4·30 h [IQR 1·09–6·00]). Increased mortality before hospital discharge is the greatest concern after this trial. Yet, no details are provided about the largest category of deaths—asphyxial brain injury-related complications—which could also be related to suboptimal intensive care.

Differences in patterns of brain injury, with more white-matter and less basal ganglia and thalami (BGT) injury, is suggested as an explanation for the absence of a neuroprotective effect of cooling in the HELIX trial.[1]

However, there is a notable selection bias in the reported MRI data that is not discussed in the study; 28% of deaths occurred before MRI could be done, and 74% of babies with available MRI data survived with no or mild disability. By contrast, the therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy in India (THIN) study, with data for conventional MRI available in 92% of infants, found that only 35% of infants had normal BGT and the distribution of white-matter injury was similar to the TOBY trial.[2,3]

We argue that a non-biased description of the setting is pivotal. Antenatal care, intrapartum monitoring, resources for timely caesarean section, resuscitation facilities and neonatal transport services, and detailed evaluation of supportive care should be considered when determining whether cooling should be provided and how the resources should be invested to improve outcomes. LMICs are home to 84% of the world's population and comprise countries and areas with huge socioeconomic and cultural diversity, as well as differences in infrastructure and delivery of health services.[4] To extrapolate the results from the HELIX trial to all babies born in LMICs discounts this diversity.

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Paper I

Original research

Therapeutic hypothermia for neonatal hypoxic-ischaemic encephalopathy in India (THIN study): a randomised controlled trial

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ABSTRACT

Objective To evaluate the neuroprotective effect of therapeutic hypothermia (TH) induced by phase changing material (PCM) on MRI biomarkers in infants with hypoxic-ischaemic encephalopathy (HIE) in a low-resource setting.

Design Open-label randomised controlled trial.

Setting One neonatal intensive care unit in a tertiary care centre in India.

Patients 50 term/near-term infants admitted within 5 hours after birth with predefined physiological criteria and signs of moderate/severe HIE.

Interventions Standard care (n=25) or standard care plus 72 hours of hypothermia (33.5°C±0.5°C, n=25) induced by PCM.

Main outcome measures Primary outcome was fractional anisotropy (FA) in the posterior limb of the internal capsule (PLIC) on neonatal diffusion tensor imaging analysed according to intention to treat.

Results Primary outcome was available for 22 infants (44%, 11 in each group). Diffusion tensor imaging showed significantly higher FA in the cooled than the non-cooled infants in left PLIC and several white matter tracts. After adjusting for sex, birth weight and gestational age, the mean difference in PLIC FA between groups was 0.026 (95% CI 0.004 to 0.048, p=0.023). Conventional MRI was available for 46 infants and demonstrated significantly less moderate/severe abnormalities in the cooled (n=2, 9%) than the non-cooled (n=10, 43%) infants. There was no difference in adverse events between groups.

Conclusions This study confirmed that TH induced by PCM reduced brain injury detected on MRI in infants with moderate HIE in a neonatal intensive care unit in India. Future research should focus on optimal supportive treatment during hypothermia rather than looking at efficacy of TH in low-resource settings.

Trial registration number CTRI/2013/05/003693.

What is already known on this topic?

► Therapeutic hypothermia is the only treatment shown to reduce death or disability for infants with moderate to severe hypoxic-ischaemic encephalopathy (HIE).

► Treatment effect is based on studies from high-income settings, although the majority of these infants are born in low-resource settings.

► Small trials in low and middle-income countries using different cooling devices have not demonstrated the benefit of cooling.

What this study adds?

► This study confirms a neuroprotective effect of therapeutic hypothermia induced by phase changing material in newborns with moderate HIE in a neonatal unit in India.

► The beneficial effect on MRI biomarkers was found even though the use of sedatives/analgesics and mechanical ventilation was limited.

► This supports the implementation of therapeutic hypothermia in tertiary care neonatal units in resource-limited settings.

INTRODUCTION

More than 95% of deaths from perinatal asphyxia and hypoxic-ischaemic encephalopathy (HIE) occur in low and middle-income countries (LMICs)[1]; globally, 1.2 million infants survive with disability annually.[2] Therapeutic hypothermia (TH) is the only treatment found to reduce mortality and major disability based on randomised controlled trials (RCT) from high-income countries (HICs).[3] Trials from LMICs, however, have not consistently demonstrated benefits of cooling,[4–6] and two recent reviews concluded that evidence for the safety or efficacy of TH as a neuroprotectant for HIE in LMICs is lacking and that TH in this setting is experimental.[4 7] There are only three RCTs from LMICs (China and India) reporting neurodevelopmental outcomes beyond 12 months of age, and all concluded with a beneficial effect of TH.[8–10] Population characteristics with higher incidence of fetal growth restriction and different comorbidities (eg, higher incidences of sepsis and meconium aspiration), facility characteristics with limited staffing with limited training and unavailability of expensive servo-controlled cooling equipment have been suggested as reasons for why TH can still not be recommended as standard of care for moderate to severe HIE in low-resource settings.

India carries the largest burden of neonatal deaths worldwide, and perinatal asphyxia and HIE account for a quarter of these deaths and an unknown number of survivors with lifelong disability.[11] A survey from 2015 showed that many

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of the neonatal units in India which do not offer TH are unable to do so due to lack of cooling device and trained staff.[12] Phase changing material (PCM) has been shown to provide very stable temperatures,[13 14] but its efficacy in inducing a rapid fall in temperature or in maintaining stable temperatures during the cooling phase irrespective of the ambient temperature has been questioned.[15] PCMs consist of salt hydride, fatty acid and esters or paraffin, melting at a set point, and with the ability to store and release heat at a nearly constant temperature.[14] The very low cost of PCM, even when compared with so-called low-cost, servo-controlled devices,[15] makes it an excellent alternative to servo-controlled devices if efficacy is comparable.

MRI of the brain is considered the best single predictor of outcome in infants with HIE, and conventional MRI (cMRI) in the neonatal period is highly predictive of later neurodevelopment.[16 17] Diffusion tensor imaging (DTI) is another MR method which has the ability to assess the microstructural organisation of white matter (WM),[18] and may be used as a surrogate outcome for neurodevelopment.[19 20] Fractional anisotropy (FA), a DTI metric, is a measure of the overall directionality of water, and decreased FA is a common feature of cerebral WM abnormalities.[21] Mean diffusivity (MD), another DTI metric, can be compared with the apparent diffusion coefficient (ADC) derived from diffusion-weighted imaging (DWI) and both are commonly used markers of hypoxic-ischaemic brain injury.[22]

The aim of this study was to evaluate the neuroprotective effect of TH achieved by a PCM-based cooling device in infants with moderate to severe HIE in a neonatal intensive care unit (NICU) in India. Outcome measures were neonatal MRI biomarkers, indicating severity of brain injury.

METHODS
Patients
This open-label RCT was conducted between September 2013 and October 2015 in the NICU at the Christian Medical College (CMC) Vellore, a large tertiary care teaching hospital in south India. The hospital has around 15 000 deliveries annually and 75 neonatal beds with a nurse-patient ratio of 1:3–4 for the sickest infants. This was a collaborative study between CMC and the Norwegian University of Science and Technology (NTNU) and St Olavs Hospital, Trondheim University Hospital, both located in Trondheim, Norway, and involved specialists from all centres.

Infants born at gestational age (GA) >35 weeks and birth weight >1800 g admitted within 5 hours after birth with a history of perinatal asphyxia were considered for inclusion. Physiological inclusion criteria were arterial blood gas (umbilical cord or first postnatal hour) pH <7.0 or base deficit ≥12, 5 min Apgar score ≤5, or need of positive pressure ventilation for at least 10 min as part of resuscitation at birth. For outborn infants, no cry at birth was a sufficient criterion. Eligible infants were further screened for neurological criteria, including seizures or evidence of moderate or severe HIE identical to the NICHD trial.[23] Infants with major congenital anomalies or with imminent death anticipated at the time of assessment were excluded. Written parental consent was obtained after giving the parents an information leaflet and oral explanation.

Randomisation
Infants were stratified according to the severity of encephalopathy and randomly assigned to treatment group in a 1:1 ratio. The allocation sequences were concealed in sequentially numbered, sealed and opaque envelopes with block sizes of 4 and 6, which were created by the Department of Biostatistics

at CMC. A research officer ensured adequate recruitment and collection of clinical data.

Procedures
Infants assigned to hypothermia (TH group) were placed on a PCM-based cooling device (MiraCradle Neonate Cooler, Pluss Advanced Technologies, India).[14] The target core temperature was 33.5°C±0.5°C for 72 hours followed by controlled rewarming at 0.2°C–0.5°C per hour until the temperature was above 36.5°C. Infants assigned to standard care (SC group) were placed under radiant warmer, and target core temperature was 37.0°C±0.5°C. The environmental temperature in the NICU is typically 27°C–30°C year-round.

Rectal temperature was monitored continuously in all infants and recorded every 15 min for the first 4 hours, thereafter hourly during the intervention and another 24 hours. Oxygen saturation, heart rate, blood pressure and respiratory rate were monitored continuously while urine output was monitored every 6 hours. Neurological examination using the Thompson score and modified Sarnat was done at recruitment and repeated daily until 4 days of life. Blood gas, electrolytes, glucose, kidney function, liver enzymes, coagulation parameters and full blood counts were monitored, and investigation for infection was done as clinically indicated. All treatments including medications were as per existing treatment protocols. Sedatives/analgesics were given to babies who had excessive shivering or a pain score of >4 on Neonatal Infant Pain Scale as per treating physician's discretion. Mechanical ventilation was not routinely used but was provided for infants with respiratory failure. Seizures were managed with a loading dose of phenobarbitone. Second-line anticonvulsant was phenytoin and third was levetiracetam.

MRI acquisition and analysis
All infants underwent cerebral MRI at 5±1 days of life with the same 3.0T Philips Achieva scanner (Philips Healthcare, Best, Netherlands; software V.3.2.3.1) using an 8-channel head coil. MRI scans were performed under pulse oximetry surveillance, and chloral hydrate (50 mg/kg) or midazolam (0.10–0.15 mg/kg) sedation was used if needed. Infants were wrapped, foam pads placed around the head and ear protection was applied. Conventional T1 and T2-weighted images, DWI, MR spectroscopy (MRS) and DTI were obtained (online supplementary eTable1). The duration of the MRI acquisition was 25 min. All MR images were stored deidentified on a password-protected hard drive which was brought from CMC to NTNU in person by one of the investigators.

The DTI data were analysed using Tract-Based Spatial Statistics (TBSS) and a region-of-interest (ROI) approach. The DTI analysis plan is given in the online supplementary material with ROI locations (online supplementary eFigure1). The clinical images were reviewed by one neonatologist (MMB) with experience in neonatal neuroimaging and scored in accordance with Rutherford et al.[16] The MR spectra were automatically processed on the Philips workstation, and one voxel of interest in the basal ganglia from each infant was chosen. Peak area of major metabolites was calculated, including N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), myoinositol, and glutamate and glutamine. The peak area values were expressed relative to Cho and Cr.

Outcomes
The primary outcome was FA in the posterior limb of the internal capsule (PLIC) analysed by the TBSS and ROI approach. A priori defined secondary outcomes were FA and MD in thalami,

lentiform nuclei, genu and splenium of the corpus callosum, and midbrain, peak area of major metabolites on MRS and abnormalities on cMRI. All assessors of MRI were blinded to the intervention and clinical characteristics except sex, birth weight and GA.

Severe adverse events were major cardiac arrhythmia (except sinus bradycardia), persistent hypotension (defined as mean blood pressure <GA+postnatal age in days, despite two inotropes), prolonged coagulation time (prothrombin time >20 s, activated partial thromboplastin time >60 s), thrombocytopenia (platelet count <$100x10^9$/L), severe haemorrhage, persistent pulmonary hypertension, culture-positive sepsis and death or withdrawal of care. The Data Safety Monitoring Board at the CMC oversaw the study and adverse events were reported.

Statistical analysis

A sample size of 20 in each arm was calculated to detect a 10% difference in mean PLIC FA values, with a power of 90% for a two-sided t-test at a significance level of 5%.[19] To account for the assumption that up to 20% of included infants may die before MRI, 25 infants were included in each arm. The primary outcome was analysed using TBSS based on intention to treat. In addition, the FA and MD values obtained from the ROI approach were analysed using multivariable linear regression including sex, birth weight and GA as covariates in addition to treatment group. A linear mixed model was used for PLIC, thalami, lentiform nuclei and midbrain due to dependent measurements from the left and right sides, accounting for within-subject correlation by subject-specific random intercept. Group differences in adverse events, postnatal complications, cMRI and metabolite levels from MRS were analysed by χ^2 tests or Mann-Whitney U tests, as appropriate. P values <0.05 were considered significant.

Demographic factors and clinical characteristics were summarised with counts (percentages) for categorical variables and means (SD) or medians (IQR) for continuous variables. All analyses were performed using SPSS V.24 and V.25 (IBM).

RESULTS

A total of 85 infants were assessed for eligibility, of whom 50 infants were randomly allocated hypothermia (n=25) or SC (n=25) (figure 1). TH was discontinued in two infants due to death or discharge against medical advice (DAMA). In the SC group, two infants were DAMA before MRI was acquired. All infants who were DAMA were noted as having poor prognosis, and withdrawal of care had been recommended. There were no baseline differences in delivery and neonatal demographics between groups (table 1). Mean rectal temperature during the intervention was 33.5°C (SD 0.27) and 36.9°C (SD 0.35) in the TH and SC groups, respectively (online supplementary eFigure 2).

Primary outcome data with DTI were available for 22 infants (44%): 4 died or were DAMA, 1 did not have a DTI sequence, 9 had too few DTI images to be analysed and 14 had poor quality images due to artefacts (figure 1). The TBSS analysis showed higher FA values in the TH group compared with the SC group in the left PLIC (figure 2). Mean FA value in left PLIC was 0.407 (SD 0.032) and 0.390 (SD 0.029) in the TH and SC groups, respectively; in right PLIC 0.409 (SD 0.038) and 0.390 (SD 0.036), respectively. Higher FA and lower MD values were also found in several WM tracts (figure 2). Using the ROI approach, the unadjusted mean FA value in PLIC was 0.414 in the TH group compared with 0.397 in the SC group, and after adjusting for sex, birth weight and GA there was a mean difference between groups of 0.026 (95% CI 0.004 to 0.048, p=0.023). DTI results from the ROI analysis are shown in table 2.

cMRI (n=46) demonstrated moderate/severe abnormalities in 2 (9%) and 10 (43%) infants in the TH and SC groups, respectively (p=0.007). Four (all in the SC group) had a global injury pattern (severe basal ganglia and thalami (BGT), WM and cortical injury). Six infants had moderate BGT injury, four of them with moderate/severe WM injury (all in SC group) and two with equivocal PLIC (one in each group). One infant in each group had normal/mild BGT and severe WM injury; the infant in SC group also had severe cortical injury.
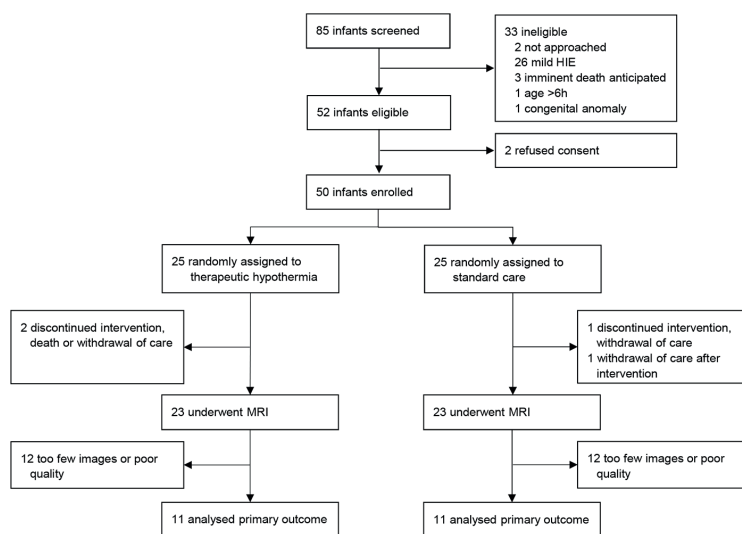

Figure 1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram. HIE, hypoxic-ischaemic encephalopathy.

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Table 1 Neonatal baseline characteristics*

Characteristic	Therapeutic hypothermia (n=25)	Standard care (n=25)
Inborn, n/N (%)	13/25 (52)	17/25 (68)
Mode of delivery, n/N (%)		
Vaginal	9/25 (36)	10/25 (40)
Vacuum/forceps	5/25 (20)	5/25 (20)
Emergency caesarean section	11/25 (44)	10/25 (40)
Male sex, n/N (%)	17/25 (68)	16/25 (64)
Gestational age (weeks), mean (SD)†	39.1 (1.3)	39.2 (1.4)
Birth weight (g), mean (SD)	2911 (483)	2960 (553)
Length (cm), mean (SD)‡	48.5 (2.5)	48.6 (3.1)
Head circumference (cm), mean (SD)§	34.0 (1.1)	34.4 (1.9)
Small for gestational age, n/N (%)¶	7/25 (28)	7/23 (30)
1 min Apgar score, n/N (%)		
0–3	17/21 (81)	13/21 (62)
4–6	3/21 (14)	7/21 (33)
7–10	1/21 (5)	1/21 (5)
5 min Apgar score, n/N (%)		
0–3	5/22 (23)	1/21 (5)
4–6	13/22 (59)	12/21 (57)
7–10	4/22 (18)	8/21 (38)
Cord/blood <60 min pH, mean (SD)**	6.81 (0.12)	6.93 (0.18)
Cord/blood <60 min base excess, mean (SD)††	−19.6 (3.2)	−16.5 (4.6)
Resuscitation at birth, n/N (%)		
Bag mask ventilation	19/19 (100)	22/23 (96)
Intubation	12/19 (63)	16/23 (70)
Chest compressions	0/19	3/23 (13)
Epinephrine	0/19	0/23
Temperature on admission (°C), mean (SD)‡‡	35.8 (0.8)	36.2 (0.6)
Age at randomisation (hour), mean (SD)	3.14 (1.48)	3.27 (1.50)
Seizures before randomisation, n/N (%)	13/25 (52)	13/25 (52)
Thompson score at randomisation, median (IQR)	9 (6–13)	9 (6–16)

*Percentages are based on the number of infants for whom data were available.
†Data were unavailable for two infants in standard care group.
‡Data were unavailable for one infant in therapeutic hypothermia group.
§Data were unavailable for one infant in each group.
¶Small for gestational age defined as birth weight less than the 10th percentile according to the Intergrowth 21st chart.
**Data were unavailable for 13 infants in therapeutic hypothermia group and seven in standard care group. Cord pH was not available in outborn infants.
††Data were unavailable for 14 infants in therapeutic hypothermia group and eight in standard care group. Cord base excess was not available in outborn infants.
‡‡Data were unavailable for three infants in therapeutic hypothermia group and five in standard care group.

MRS was done in all 46 infants with MRI, but 18 failed processing (7 in the TH group and 11 in the SC group) and 2 were excluded due to poor quality of images and voxels chosen mainly within WM (TH group). Median NAA/Cho ratio was 0.628 (IQR 0.556–0.684) and 0.559 (IQR 0.472–0.625) in TH and SC groups, respectively (p=0.013). The other MRS measures were non-significant.

Adverse events and postnatal complications are shown in table 3. Fentanyl was given as infusion (maximum 2 µg/kg/hour) to one infant in each group, and as a single bolus to one infant (SC group). Bolus doses of midazolam (0.10–0.15 mg/kg) and/or chloral hydrate (50 mg/kg) were given to 10 and 3 infants in TH and SC groups, respectively. Data on sedatives/analgesics are

missing for nine infants. Forty-one infants (82%) with clinical seizures after admission received phenobarbitone, and 21 (42%) received two or more anticonvulsants.

DISCUSSION

This RCT of TH using a PCM-based cooling device for neonatal HIE confirms a neuroprotective effect in a low-resource setting. There was higher FA in the left PLIC and several WM tracts in cooled compared with non-cooled infants. Infants in the TH group had less abnormalities on cMRI and higher NAA/Cho ratio in the basal ganglia compared with SC group. The beneficial effect of cooling demonstrated on several MRI biomarkers occurred even though the use of sedatives/analgesics was very limited, and the majority of infants were not mechanically ventilated.

The favourable effect of TH demonstrated by higher FA in the left PLIC, corpus callosum and other WM tracts is in accordance with the findings of Porter et al.[19] Treatment effect on WM integrity found by the voxel-based analysis (TBSS) was confirmed by the ROI analysis. TBSS has the advantage of being faster, more time effective and reproducible, and can investigate the entire WM. Decreased FA values reflect tissue damage and are strongly associated with neurodevelopmental outcomes,[20 24] and our findings support the use of FA as a sensitive marker for brain injury after HIE.

The finding of lower MD values in the TH group than the SC group is of more uncertain significance. Although lower MD in cooled versus non-cooled infants has been shown also by Artzi et al,[25] most studies demonstrate lower ADC values in injured brain tissue on early MRI.[26–29] ADC and MD may, in contrast to FA, pseudonormalise after an acute hypoxic-ischaemic insult, and this process is affected by TH.[26] The interpretation of MD will, therefore, depend strongly on time of imaging and injury severity.

The difference between treatment groups shown on DTI was supported by findings on cMRI and MRS. The injury pattern on cMRI was predominantly BGT with or without WM and/or cortical injury, which is the injury pattern most commonly seen in infants with HIE in HICs and comparable to the TH trials.[16 30] In a study of infants with HIE admitted to a NICU in Kerala, India, more than 90% had WM injury and only 27% had any BGT injury.[31] The predominance of WM injury could be partly explained by a large proportion of infants with mild HIE (56%) in that study, but question remains if there are differences in injury pattern between populations. It has been speculated that a high incidence of fetal growth restriction in LMICs may be related to prenatally established brain injury and, thus, reduced efficacy of TH.[5 15 32] Our study, where almost a third of infants were small for gestational age, did not support this.

There were no significant differences in major adverse events or postnatal complications between the groups and no life-threatening adverse events in the TH group. In accordance with our results, the PCM-based cooling device has recently been shown feasible and safe when used in 11 tertiary NICUs in India.[13] Our study confirms very stable temperatures in cooled infants with fluctuation of temperature (SD) of 0.27°C during intervention. The PCM mattress is produced locally and costs one-tenth of a servo-controlled, automated cooling device commonly used in HICs. Compared with other low-cost, non-servo-controlled cooling devices PCM provides excellent temperature stability and requires less nursing input.[13 14 33]

Very few infants received continuous infusion of sedatives/analgesics and respiratory support in the present study. This
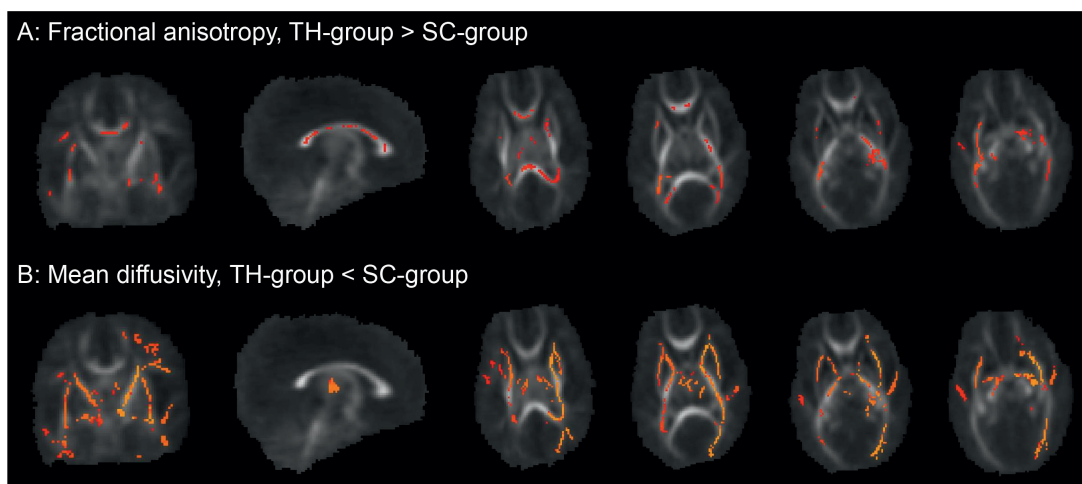
Figure 2 Tract-based spatial statistical analysis of whole-brain white matter. (A) There was significantly higher fractional anisotropy in the therapeutic hypothermia group (TH group) compared with the standard care group (SC group) (p<0.05, non-parametric permutation test, corrected for multiple comparisons, sex, birth weight and gestational age). Areas include the posterior and anterior limb of internal capsule in the left hemisphere, bilaterally in the external capsule, the genu, splenium and body of corpus callosum, forceps major, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, uncinate fasciculus; superior corona radiata on the left side, and cerebral peduncle and superior longitudinal fasciculus in the right hemisphere. (B) There was significantly lower mean diffusivity in the TH group compared with the SC group. Areas include the posterior and anterior limb of the internal capsule, external capsule, superior and inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, cingulum, uncinate fasciculus, splenium of corpus callosum, corticospinal tract, cerebral peduncle and thalami bilaterally; and centrum semiovale, anterior, superior and posterior corona radiata in the left hemisphere.

suggests that routine sedation requiring mechanical ventilation is not necessary to achieve a neuroprotective effect of TH. However, our study was not designed to detect the effect of sedatives/analgesics, and results should be interpreted with caution. A sedative effect of the anticonvulsants used must also be anticipated. There is still little knowledge about the optimal level of sedation during TH.[34] Many centres in HICs electively intubate and sedate infants during cooling, while others use clinical indications to assess the need for sedatives/analgesics and ventilatory support. Although the issue of deep sedation necessitating mechanical ventilation is of particular importance in resource-limited settings, a better understanding of the neuroprotective and/or toxic effects of sedatives/analgesics is important in all settings where TH is provided.

Limitations
The very low proportion of infants with severe HIE (2 of 50) is a limitation of the present study and is most likely due to selection bias. Infants with imminent death anticipated at the time of enrolment, and infants without established respiration at 30 min of age are not offered intensive care in the study hospital due to the poor prognosis and were not included. The sickest outborn infants may not have been referred or arrived beyond 5 hours of age to the tertiary care hospital. This is supported by a very low proportion of severe HIE also among screened infants. Thus, the results of the present trial cannot be extrapolated to infants with severe HIE.

DTI data were available for only 44% of included infants due to artefacts and incomplete data sets. The sample size was lower than expected, and the results are therefore vulnerable to the variability in the data. Despite this, we found significant differences between the treatment groups. The fact that four infants (all in the SC group) with global injury on cMRI did not contribute to the group differences found on DTI, suggests that the beneficial effect of cooling is underestimated in the present study.

Despite proper randomisation procedures, there is a risk of imbalance between groups in small trials. The randomisation

Table 2	Between-group comparisons of selected regions of interest*				
Region	Mean difference FA (95% CI)	P value	Mean difference MD (95% CI)†	P value	
PLIC	0.026 (0.004 to 0.048)	0.023	−0.051 (−0.087 to −0.014)	0.007	
Thalami	0.008 (−0.009 to 0.024)	0.354	−0.061 (−0.104 to −0.017)	0.007	
Lentiform nuclei	0.006 (−0.012 to 0.023)	0.484	−0.059 (−0.093 to −0.024)	0.001	
Midbrain	0.012 (−0.005 to 0.029)	0.147	−0.037 (−0.070 to −0.005)	0.026	
Genu of corpus callosum	0.033 (−0.002 to 0.068)	0.062	−0.020 (−0.092 to 0.053)	0.575	
Splenium of corpus callosum	0.054 (0.021 to 0.087)	0.003	−0.048 (−0.104 to 0.008)	0.087	

*Adjusted for sex, birth weight and gestational age.
†Values reported as 10^{-3}.
FA, fractional anisotropy; MD, mean diffusivity; PLIC, posterior limb of the internal capsule.

Original research

Table 3 Major adverse events and postnatal complications

	Therapeutic hypothermia (n=25)	Standard care (n=25)	P value
Major adverse events			
Death or withdrawal of care during intervention	2 (8)	1 (4)	0.55
Major cardiac arrhythmia	0	0	–
Persistent hypotension	0	0	–
Prolonged coagulation time	23 (92)	19 (76)	0.12
Thrombocytopenia	4 (16)	2 (8)	0.38
Severe haemorrhage	0	2 (8)*	0.15
PPHN	0	0	–
Culture-positive sepsis	0	0	–
Postnatal complications			
Death or withdrawal of care after intervention	0	4 (17)†	0.04
Clinical seizures	20 (80)	22 (88)	0.44
≥2 anticonvulsants	9 (36)	12 (48)	0.39
Mechanical ventilation >24 hours	2 (8)	6 (24)	0.12
Inotropic support	14 (56)	17 (68)	0.38
Treated coagulopathy	10 (40)	8 (32)	0.56
Hypoglycaemia	2 (8)	1 (4)	0.55
Hyperglycaemia requiring insulin	1 (4)	1 (4)	–

Data are n (%).
*Both were subgaleal bleed requiring packed cell transfusion.
†Denominator 24 due to one withdrawal of care during the intervention.
PPHN, persistent pulmonary hypertension of the newborn.

procedure in the present study included allocation concealment and stratification by HIE stage. Unfortunately, umbilical blood gas analysis was not available in any of the referral hospitals and could, therefore, not be used to assess severity of the hypoxic-ischaemic insult.[35 36]

CONCLUSION

TH reduced brain injury detected on all MRI biomarkers in infants with moderate HIE admitted to a NICU in India and supports that TH is feasible and neuroprotective in this setting. Future research should focus on finding the optimal supportive treatment during TH. This is of particular importance in LMICs where the burden of disease is highest.

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Supplementary online content

Contents

1

Paper I

eTable 1. MRI Scan Protocol THIN-study

Parameter	DTI	DWI	3D T1	T1 IR	T2	SWI	MRS[a]
b-value, s/mm²	b = 0 and b = 600	b = 0 and b = 700	n/a	n/a	n/a	n/a	n/a
Orientation	Axial	Axial	Sagittal	Axial	Axial	Axial	Axial
Fold-over Direction	AP	AP	AP	RL	RL	RL	RL
TR, ms	3670 (shortest)	2400	9.9 (shortest)	5000	7700	16 (shortest)	2000 (long)
TE, ms	52 (shortest)	105 (shortest)	4.6	15	140	23 (shortest)	144
TI, ms	n/a	n/a	n/a	900	n/a	n/a	n/a
No. of Slices	45	35	120	35	35	110	25 voxels
Slice Thickness, mm	2.5	3.0	1.0	3.0	3.0	1.0	1.5
Slice Gap, mm	0	0	0	0	0	0	
FOV, mm	200x200	180x180	192x192	170x170	170x170	180x180	
Matrix (resolution)	80x80	192x192	192x192	256x256	256x256	448x448	
Voxel Size, mm	2.5x2.5x2.5	1.0x1.0x3.0	1.0x1.0x1.0	0.7x0.7x3.0	0.7x0.7x3.0	0.4x0.4x1.0	1.0x1.0x1.5
k-space Coverage	Halfscan, factor 0.68	Halfscan, factor 0.72	Full	Full	Full	Elliptical	
Parallel Imaging	SENSE (p = 2)	SENSE (p = 1.5)	SENSE (p = 1)	SENSE (p = 1.4)	no	SENSE (p = 2.4)	No
Flip Angle	90°	90°	8°	90°	90° (120° refocusing)	15°	90°
Scan Technique	DTI. SS-EPI SPIR fat saturation	DWI. SS-EPI SPIR fat saturation	3D T1 TFE	TSE IR	TSE	T1 FFE	
Turbo Factor (ETL)	n/a	n/a	n/a	5	11	n/a	n/a
Diffusion Tensor Directions	High (32)	3	n/a	n/a	n/a	n/a	n/a

Abbreviations: DTI, diffusion tensor imaging; DWI, diffusion-weighted imaging; IR, inversion recovery; SWI, susceptibility weighted imaging; MRS, MR spectroscopy; AP, anterior-to-posterior; RL, right-to-left; TR, repetition time; TE, echo time; TI, inversion time; FOV, field of view; SENSE, sensitivity encoding; SS-EPI, single-shot echo planar imaging; SPIR, spectral presaturation inversion recovery; TFE, turbo field echo; TSE, turbo spin echo; FFE, fast field echo; ETL, echo train length.
[a] Multi voxel spectroscopy (25 voxels cover basal ganglia)

2

DTI analysis and reading

The diffusion tensor imaging (DTI) analyses were performed with the tools of the FMRIB

Software Library (FSL; Oxford Centre for Functional MRI of the Brain, UK;

www.fmrib.ox.ac.uk/fsl). Image artefacts due to motion and eddy current distortions were

minimised by registration of all DTI acquisitions to the mean b=0 image using affine

registration. The brain was extracted using Brain Extraction Tool (part of FSL). FMRIB's

Diffusion Toolbox was used to fit a diffusion tensor model to the raw diffusion data in each

voxel. Voxel-wise maps of fractional anisotropy (FA) and mean diffusivity (MD) were

calculated for the TH- and SC-groups.

Voxel-wise statistical analysis of the DTI data was performed using Tract-Based Spatial

Statistics (TBSS, FSL).[1][2] Voxel-wise statistics of the skeletonised FA and MD were carried

out on the WM skeleton using Randomise (FSL) to test for group differences between the TH-

and SC-groups. Randomise performs non-parametric permutation-based testing of inference

using Threshold-Free Cluster Enhancement[3] with a correction for multiple comparisons

(p<0.05, corrected for sex, birth weight and gestational age).

A region-of-interest (ROI) approach was also applied to extract FA and MD from posterior

limb of the internal capsule (PLIC), thalami, lentiform nuclei, midbrain, and genu and

splenium of the corpus callosum, as defined by the JHU Neonate Brain Atlas (eFigure 1).[4]

Each participant's individual FA image was registered to the JHU Neonate Brain Atlas using

linear and non-linear registration (FSL). Manual adjustments of the ROIs were performed to

ensure that the ROIs were placed anatomically correct in each participant, and mean FA and

MD were calculated for all participants and ROIs.

3

eFigure 1. Region of interest location
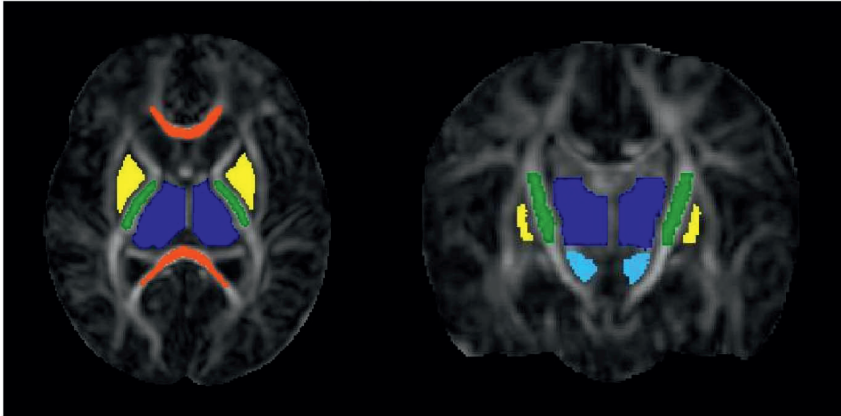
Posterior limb of the internal capsule (green), thalami (dark blue), lentiform nuclei (yellow), midbrain (light blue), and genu and splenium of the corpus callosum (red) overlaid on JHU Neonate Brain Atlas fractional anisotropy template.

4

eFigure 2. Rectal temperature profile during intervention
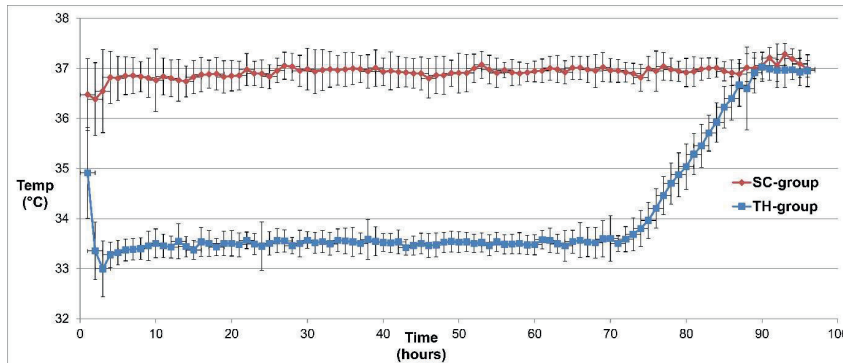
SC, standard care; TH, therapeutic hypothermia.
The vertical bars indicate mean±2 SD. Time 0 is the first temperature taken after randomization.

Mean age of starting therapeutic hypothermia (TH) was 3.75 hours (SD 1.44) and mean rectal temperature on admission to NICU was 35.8°C (SD 0.80) in TH-group and 36.2°C (SD 0.63) in standard care (SC) group. Rectal temperature dropped to target range within the first hour after initiation of cooling in 22 infants (88%). Sixty-four temperature measurements (3.6%) during TH were outside the target range. There were no temperatures ≥38°C during rewarming or 12 hours thereafter. In the SC-group, 175 temperature measurements (10%) were outside the target range. No measurements were above 38°C, but 32 measurements (2%) were between 37.5°C and 38.0°C during the first 72 hours.

5

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6

Paper II

Original research

OPEN ACCESS

Prediction of outcome from MRI and general movements assessment after hypoxic-ischaemic encephalopathy in low-income and middle-income countries: data from a randomised controlled trial

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ABSTRACT

Objective To evaluate the accuracy of neonatal MRI and general movements assessment (GMA) in predicting neurodevelopmental outcomes in infants with hypoxic-ischaemic encephalopathy (HIE).

Design Secondary analyses of a randomised controlled trial (RCT).

Setting Tertiary neonatal intensive care unit in India.

Methods Fifty infants with HIE were included in an RCT of therapeutic hypothermia (25 cooled and 25 non-cooled). All infants underwent brain MRI at day 5, GMA at 10–15 weeks and outcome assessments including Bayley Scales of Infant and Toddler Development, third edition, at 18 months. Associations between patterns of brain injury, presence/absence of fidgety movements (FMs) and outcomes were assessed.

Results Seventeen of 47 (36%) had adverse outcome (5 (21%) cooled vs 12 (52%) non-cooled, p=0.025). Eight infants died (four before an MRI, another three before GMA). Two developed severe cerebral palsy and seven had Bayley-III motor/cognitive composite score <85. Twelve (26%) had moderately/severely abnormal MRI and nine (23%) had absent FMs. The positive predictive value (95% CI) of an adverse outcome was 89% (53% to 98%) for moderate/severe basal ganglia and thalami (BGT) injury, 83% (56% to 95%) for absent/equivocal signal in the posterior limb of the internal capsule (PLIC) and 67% (38% to 87%) for absent FMs. Negative predictive values (95% CI) were 85% (74% to 92%) for normal/mild BGT injury, 90% (78% to 96%) for normal PLIC and 86% (74% to 93%) for present FMs.

Conclusion(s) Neonatal MRI and GMA predicted outcomes with high accuracy in infants with HIE. The GMA is a feasible low-cost method which can be used alone or complementary to MRI in low-resource settings to prognosticate and direct follow-up.

Trial registration number CTRI/2013/05/003693.

What is already known on this topic?

► The main burden of hypoxic-ischaemic encephalopathy is in low-income and middle-income countries, and many infants die or survive with disability.

► Neonatal MRI and general movements assessment are recommended assessments to predict cerebral palsy in infants at risk of perinatal brain injury.

► The literature on outcome and outcome prediction after hypoxic-ischaemic encephalopathy in low-income and middle-income countries is sparse.

What this study adds?

► This study from South India showed similar patterns of brain injury in infants with hypoxic-ischaemic encephalopathy as trials from high-income countries.

► Both neonatal MRI and general movements assessment predicted neurodevelopmental outcome with high accuracy.

► Assessment of fidgety movements is a feasible low-cost method to prognosticate and direct follow-up in low-resource settings.

INTRODUCTION

Perinatal asphyxia is ranked among the top 20 causes of death at any age globally.[1] The main burden of disease is in low-income and middle-income countries (LMICs), where the incidence of hypoxic-ischaemic encephalopathy (HIE) is 10–20 times higher than in high-income countries (HICs).[2] [3] Therapeutic hypothermia (TH) has been found to reduce mortality and major disability in survivors by 25% (relative risk) with a number needed to treat of 7.[4] The therapeutic hypothermia in India (THIN) study showed a neuroprotective effect of TH on early MRI biomarkers,[5] but data on neurodevelopmental outcomes in LMICs are scarce.

Outcomes after HIE range from full recovery to death or survival with different degrees of disability.[6] [7] The prevalence and distribution of outcomes may differ between settings, and factors like maternal morbidities, nutritional status and infections may influence both the pattern of brain injury and the effect of cooling in infants with HIE.[8–12] A study from South India reported more white matter (WM) injury in HIE infants as compared with studies from HICs.[13]

Prediction of outcomes in infants with HIE is essential for affected families, and important for
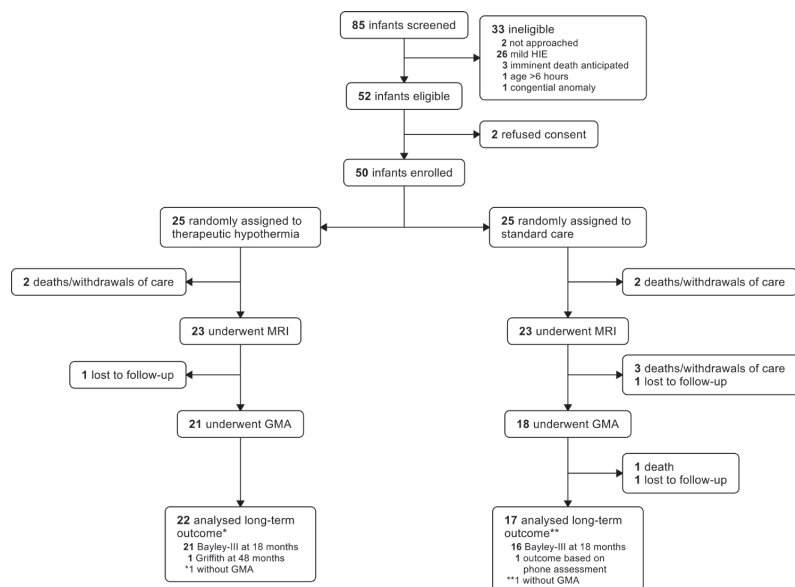
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Figure 1 Trial profile. Bayley-III, Bayley Scales of Infant and Toddler Development (third edition); GMA, general movements assessment; Griffith, Griffith Mental Development Scales; HIE, hypoxic-ischaemic encephalopathy.

Table 1 Outcomes according to neonatal MRI*

	Normal/Mildly abnormal MRI (n=34)	Moderately/Severely abnormal MRI (n=12)	Total (n=46)	P value
Fidgety movements				0.001†
Absent (−)	3/31 (9.7)	6/8 (75.0)	9/39 (23.1)	
Sporadic (+/−)	10/31 (32.3)	0	10/39 (25.6)	
Intermittent (+)	16/31 (51.6)	2/8 (25.0)	18/39 (46.2)	
Continual (++)	1/31 (3.2)	0	1/39 (2.6)	
Exaggerated	1/31 (3.2)	0	1/39 (2.6)	
Adverse outcome‡	5/32 (15.6)	8/11 (72.7)	13/43 (30.2)	<0.001
Death	0	4/12 (33.3)	4/46 (8.7)	<0.001
Bayley-III cognitive composite score				0.001
<70	0	3/7 (42.9)	3/38 (7.9)	
70–84	4/31 (12.9)	1/7 (14.3)	5/38 (13.2)	
≥85	27/31 (87.1)	3/7 (42.9)	30/38 (78.9)	
Bayley-III motor composite score				0.002
<70	0	3/7 (42.9)	3/38 (7.9)	
70–84	3/31 (9.7)	0	3/38 (7.9)	
≥85	28/31 (90.3)	4/7 (57.1)	32/38 (84.2)	
Cerebral palsy	1/31 (3.2)	3/7 (42.9)	4/38 (10.5)	0.002
GMFCS				
Level 1	1/31 (3.2)	1/7 (14.3)	2/38 (5.3)	
Level 5	0	2/7 (28.6)	2/38 (5.3)	

Data are n/N (%). Denominators vary according to available data.
*Normal/Mildly abnormal MRI defined as normal/mild basal ganglia and thalami score and/ or normal/mild/moderate white matter score. Moderately/Severely abnormal MRI defined as moderate/severe basal ganglia and thalami score and/or absent posterior limb of the internal capsule and/or severe white matter score.
†P value based on Fisher's exact test on absent versus present FMs.
‡Adverse outcome defined as death, cerebral palsy with GMFCS level 3–5 or Bayley-III cognitive and/or motor composite score <85.
Bayley-III, Bayley Scales of Infant and Toddler Development (third edition); GMFCS, Gross Motor Function Classification System.

clinicians to direct resources for follow-up. Neonatal MRI and the observation of spontaneous movements using the general movements assessment (GMA) are recommended tools to predict cerebral palsy (CP) in high-risk infants.[14] Abnormal signal intensity in the basal ganglia and thalami (BGT) on MRI, which is the typical injury pattern after an acute hypoxic-ischaemic insult, is strongly associated with later motor impairment.[15] On the other hand, predominant WM injury, carries a lower risk of motor impairment, but is more related to cognitive and behavioural impairments.[16] Absence of fidgety movements (FMs) on GMA between 9 and 20 weeks post-term age is a strong marker for later CP.[17 18] In LMICs where resources are limited and MRI not easily available, GMA could be an ideal cost-effective predictive tool. However, most studies on GMA have been done in HICs and mainly in preterm populations.

The aim of this substudy is to evaluate the ability of early MR biomarkers and presence or absence of FMs to predict neurodevelopmental outcomes in infants with HIE included in a randomised controlled trial (RCT) on TH in India.

METHODS
Study population
This is a substudy of the THIN-study, a single-centre RCT of infants admitted with HIE to the neonatal intensive care unit (NICU) at the Christian Medical College Vellore, a tertiary care teaching hospital in rural south India. Approximately 15 000 babies are born at the hospital annually. The NICU has 75 beds and is a referral unit for a population of approximately 6 million. The unit offers level 3 neonatal intensive care including high-frequency oscillatory ventilation, inhaled nitric oxide and surgery. Fifty infants at or near term admitted before 5 hours after birth with signs of moderate/severe HIE were recruited between September 2013 and October 2015. The infants were randomly assigned to hypothermia with target core temperature 33.5°C±0.5°C for 72 hours induced by a phase changing
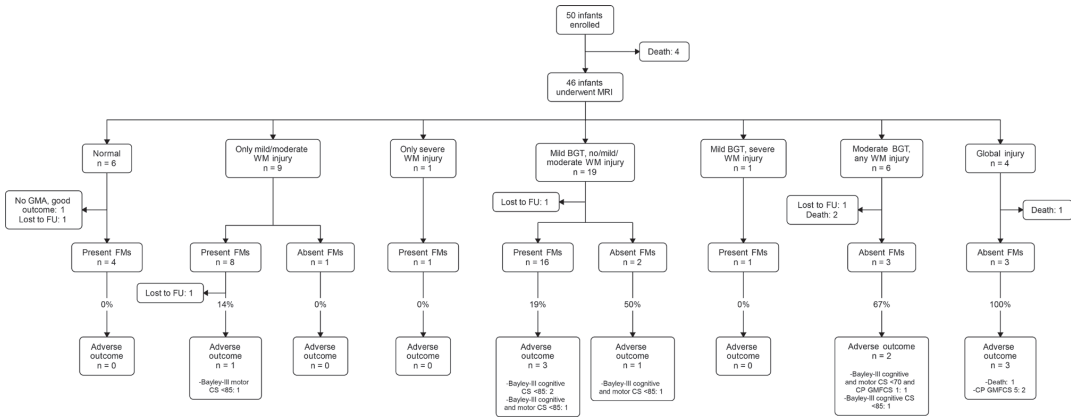
Original research

Figure 2 Flow chart showing patterns of brain injury, present or absent fidgety movements (FMs) and outcomes. BGT, basal ganglia and thalami; CP, cerebral palsy; CS, composite score; FU, follow-up; GMA, general movements assessment; GMFCS, Gross Motor Function Classification System; WM, white matter.

material-based cooling device (MiraCradle Neonate Cooler, Pluss Advanced Technologies, India) or standard care with normothermia. Full description of the trial is published.[5] Written parental consent was obtained after giving the parents an information leaflet and oral explanation.

Early assessments

All infants underwent cerebral MRI at 5 ± 1 days of age with a 3.0 T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands; software V.3.2.3.1) using a standard protocol.[5] The clinical images were reviewed by one neonatologist (MM-B) with experience in neonatal neuroimaging and scored in accordance with Rutherford *et al*.[19] Images were classified as normal/mildly abnormal (BGT score normal or mild and/or WM score normal, mild or moderate) or moderately/severely abnormal (moderate/severe BGT score and/or absent posterior limb of the internal capsule (PLIC), and/or severe WM score).[19] Images were also categorised in seven patterns of brain injury: normal (no signs of abnormalities), only mildly/moderately abnormal WM, only severely abnormal WM, mildly abnormal BGT with normal or mildly/moderately abnormal WM, mildly abnormal BGT with severely abnormal WM, moderately abnormal BGT with any WM score and global brain injury (severely abnormal BGT, WM and cortex with absent PLIC and brainstem abnormalities).

At 10 to 15 weeks post-term age, a video for GMA was recorded using a standardised set-up with a digital camera (Sanyo VPC-HD2000, SANYO Electric, Osaka, Japan). The infants were filmed once in active wakefulness laying on a mattress in supine position. The video was analysed independently by two certified GMA observers in accordance with Prechtl's method.[18] FMs were classified in accordance with their temporal organisation as continuous, intermittent or sporadic based on their presence and interspersed pauses.[20] The category exaggerated was used if the FMs were exaggerated in speed and amplitude. FMs were defined as 'absent' if they were not observed, and 'present' if present in a continuous, intermittent, sporadic or exaggerated pattern. If disagreement occurred, the observers reassessed the video and reached consensus.

Neurodevelopmental outcome

At 18 months, an experienced developmental paediatrician and a clinical psychologist performed a complete neurological examination, the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III),[21] and clinical assessment of vision and hearing. CP and CP subtype were diagnosed in accordance with Surveillance of Cerebral Palsy in Europe[22] and gross motor function was classified using the Gross Motor Function Classification System (GMFCS).[23] Adverse outcome was defined as
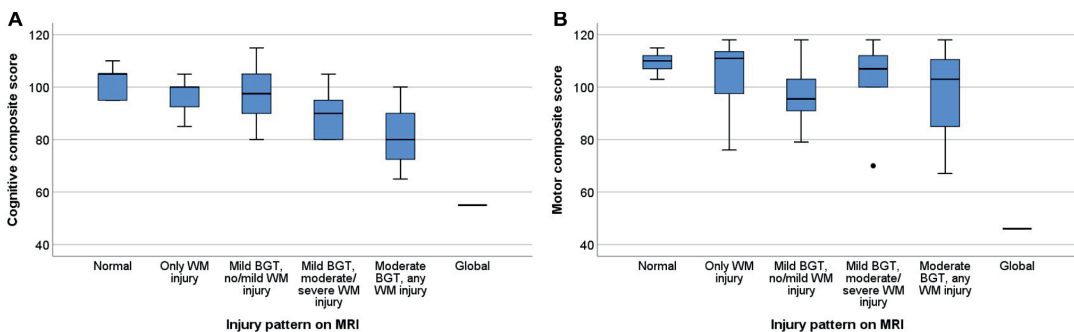

Figure 3 Bayley-III cognitive (A) and motor (B) composite score (median, first and third quartile and range) according to injury pattern on neonatal MRI. Bayley-III, Bayley Scales of Infant and Toddler Development, third edition; WM, white matter; BGT, basal ganglia and thalami.
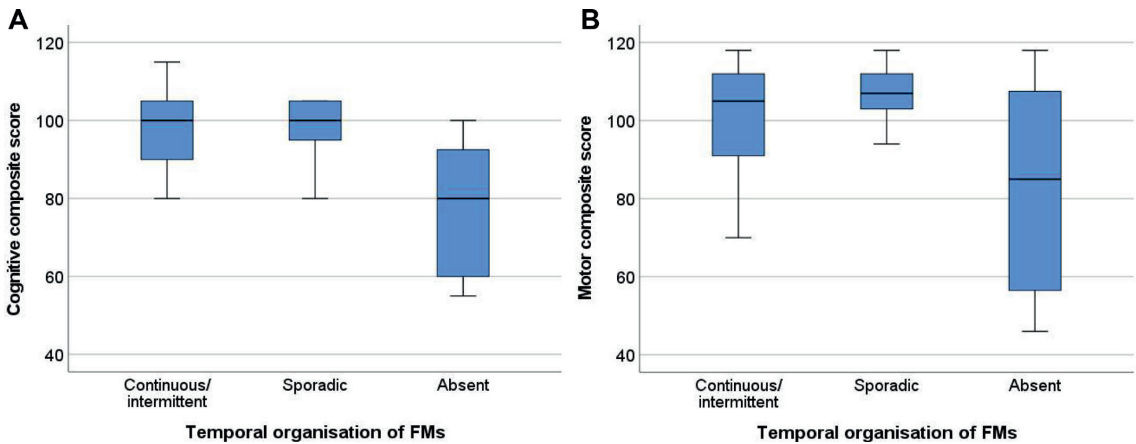
Figure 4 Bayley-III cognitive (A) and motor (B) composite score (median, first and third quartile and range) according to temporal organisation of fidgety movements (FMs) on general movements assessment. One infant with exaggerated FMs is not included in the box plot but had Bayley-III cognitive and motor composite score of 95 and 85, respectively. Bayley-III, Bayley Scales of Infant and Toddler Development, third edition.

a Bayley-III cognitive and/or motor composite score (CS) <85 (−1 SD),[24 25] a diagnosis of CP GMFCS level 3–5, impaired sensory/communication outcomes (blindness or deafness), ongoing seizure disorder or death related to associated causes. The Bayley-III language CS was not used in the analysis due to the infants' young age and the subjectivity of their non-English application. One infant had a normal Griffith Mental Development Scales (GMDS) assessment at 48 months and was assigned a Bayley motor and cognitive CS of 105 and 107, respectively, due to higher scores found in testing of typically developing children.[26 27] Another infant's outcome was based on phone assessment and classified as normal in the binary analysis of outcome.

All assessors of MRI, GMA and outcomes were blinded to the intervention and the results of former examinations.

Statistics

Data were analysed using SPSS V.25 (IBM, Chicago, Illinois, USA). Clinical characteristics and outcomes were summarised with absolute numbers (percentages) for categorical variables and means (SD) or medians (IQR) for continuous variables. Group differences in outcomes were analysed by χ^2 tests, Fisher's exact tests, linear-by-linear associations, Student's t-tests or Mann-Whitney U tests, as appropriate. Sensitivity, specificity,
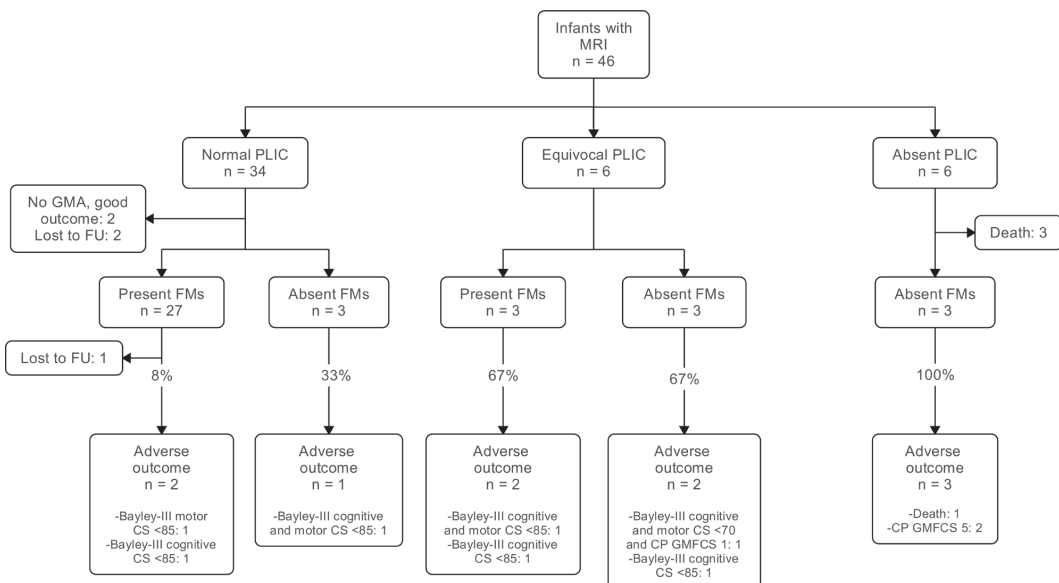
Figure 5 Flow chart showing involvement of posterior limb of internal capsule (PLIC), present or absent fidgety movements (FMs) and outcomes. CS, composite score; CP, cerebral palsy; FU, follow-up; GMA, general movements assessment; GMFCS, Gross Motor Function Classification System.

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Table 2 Predictive ability of MRI abnormalities and general movements assessment for adverse outcome*

	Sensitivity	Specificity	PPV	NPV	Accuracy
Moderately/Severely abnormal MRI†	61.5 (31.6 to 86.1)	90.0 (73.5 to 97.9)	72.7 (45.6 to 89.5)	84.4 (72.9 to 91.6)	81.4 (66.6 to 91.6)
Moderate/Severe BGT injury	61.5 (31.6 to 86.1)	96.7 (82.8 to 99.9)	88.9 (52.6 to 98.3)	85.3 (74.4 to 92.1)	86.1 (72.1 to 94.7)
Absent PLIC	46.2 (19.2 to 74.9)	100.0 (88.4 to 100.0)	100.0	81.1 (72.2 to 87.6)	83.7 (69.3 to 93.2)
Equivocal/Absent PLIC	76.9 (46.2 to 95.0)	93.3 (77.9 to 99.2)	83.3 (55.9 to 95.2)	90.3 (77.5 to 96.2)	88.4 (74.9 to 96.1)
Absent FMs	60.0 (26.2 to 87.8)	89.3 (71.8 to 97.7)	66.7 (38.0 to 86.7)	86.2 (74.3 to 93.1)	81.6 (65.7 to 92.3)
Sporadic or absent FMs	70.0 (34.8 to 93.3)	57.1 (37.2 to 75.5)	36.8 (24.4 to 51.3)	84.2 (66.3 to 93.6)	60.5 (43.4 to 76.0)

Data are % (95% CI).
*Adverse outcome defined as death, cerebral palsy with Gross Motor Function Classification System level 3–5 or Bayley-III cognitive and/or motor composite score <85.
†Moderately/Severely abnormal MRI defined as moderate/severe BGT score and/or absent PLIC and/or severe WM score.
BGT, basal ganglia and thalami; FMs, fidgety movements; NPV, negative predictive value; PLIC, posterior limb of the internal capsule; PPV, positive predictive value.

positive predictive value (PPV), negative predictive value (NPV) and the predictive accuracy for adverse outcome were calculated for moderately/severely abnormal MRI, moderate/severe BGT injury, absent PLIC, combined absent and equivocal PLIC, absent FMs and combined absent and sporadic FMs. The CIs for the predictive values were calculated using a free online statistical calculator from MedCalc (MedCalc Software, Ostend, Belgium).[28] There were no corrections made for multiple comparisons. Differences with p values <0.05 were considered statistically significant.

RESULTS

Fifty neonates were recruited, 25 in the cooled and 25 in the non-cooled group (figure 1). Demographics, neonatal characteristics and outcomes according to treatment group are shown in online supplemental eTable 1 and 2. Forty-six infants who survived until day 4 underwent MRI at a median age of 5 days (range 4–7), and 12 (26%) of these had moderately/severely abnormal MRI (table 1). The most frequent pattern of brain injury was mildly abnormal BGT with normal or mildly/moderately abnormal WM (online supplemental eTable 1). A video recording for GMA was obtained in 39 infants at a median post-term age of 12 weeks (range 8–16), and 9 (23%) infants had absent FMs. Two came for GMA before 10 weeks, and both had intermittent FMs. Assessment of outcome using Bayley-III was available in 37 infants at a median age of 18 months (range 16–21), and another infant had Bayley-III score estimated based on a normal assessment with GMDS. Three infants were lost to follow-up (one with completely normal MRI, one with moderate WM injury and present FMs and one with moderate BGT and severe WM injury).

Seventeen of 47 (36%) infants with available follow-up data had adverse outcome, 5 cooled and 12 non-cooled (21% vs 52%, p=0.025). Eight infants died of reasons related to HIE, seven in the neonatal period and one at 12 months. The nine survivors with adverse outcomes included two with CP GMFCS level 5 (both had Bayley-III scores <70), and seven who had a Bayley-III motor and/or cognitive CS <85 (one with bilateral hearing loss, and one with CP GMFCS level 1 and Bayley-III scores <70).

Prediction of outcome
MRI and outcome
Adverse outcome was significantly more common among infants with moderately/severely abnormal than normal/mildly abnormal MRI (8 of 11 and 5 of 32, respectively; p<0.001, table 1). The eight infants with moderately/severely abnormal MRI and adverse outcome had either a global brain injury pattern or moderate BGT injury with absent or equivocal PLIC (figure 2). All five infants with normal/mildly abnormal MRI and adverse outcome had either mild BGT injury with normal or equivocal PLIC and/or moderate WM injury (online supplemental eTable 3).

Bayley-III cognitive CS was significantly lower in infants with moderately/severely abnormal MRI compared with those with normal/mildly abnormal MRI (median (IQR) 80 (55–100) vs 95 (90–105), respectively, p=0.030). Infants with moderately/severely abnormal MRI also had lower Bayley-III motor CS than infants with normal/mildly abnormal MRI, but this difference was not significant (median (IQR) 100 (46–115) vs 107 (91–112), p=0.335). The Bayley-III cognitive and motor CS according to injury patterns on MRI are shown in figure 3.

GMA and outcome
Six of nine (67%) infants with absent FMs had adverse outcome: one died, two developed severe CP and three had Bayley-III motor and/or cognitive CS <85. The remaining three infants with absent FMs had normal outcome. Most infants with present FMs (25 of 29) had normal outcome, and the four infants with adverse outcome all had Bayley-III motor and/cognitive CS 70–85 and none of them developed CP. Ten (26%) infants had sporadic FMs, of whom one had adverse outcome (Bayley-III cognitive CS <85).

Infants with present FMs had significantly higher Bayley-III cognitive CS than those with absent FMs (median (IQR) 100 (91–105) vs 80 (58–94), respectively, p=0.001). The Bayley-III motor CS was also higher, although not significant, in infants with present versus absent FMs (median (IQR) 107 (94–112) vs 85 (51–110), p=0.091). The Bayley-III cognitive and motor CS for the different temporal classifications of FMs are shown in figure 4.

MRI, GMA and outcome
Different patterns of brain injury in relation to GMA and outcome are shown in figure 2. All infants with a completely normal MRI and available follow-up assessments had present FMs and favourable outcome. One infant with mild BGT and WM injury had exaggerated FMs and was diagnosed with CP GMFCS level 1 (normal Bayley-III scores). All six infants with moderate BGT injury or global brain injury who came for GMA had absent FMs, and five of them had an adverse outcome (figure 2). All six infants with absent PLIC either died or survived with severe CP (figure 5). Sensitivity, specificity, PPV, NPV and accuracy of MRI and GMA for adverse outcomes are shown in table 2.

Four infants had either moderately/severely abnormal MRI or absent FMs, and a normal outcome (online supplemental etable4). Two had severe WM injury as predominant injury pattern, and the other two had absent FMs but a normal/mildly abnormal MRI. In addition, one cooled infant with both moderate BGT injury, equivocal PLIC and absent FMs had a normal outcome.

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DISCUSSION

This substudy of an RCT on TH for HIE in a low-resource setting found that both neonatal MRI and GMA are closely associated with the combined outcome of death and adverse neurodevelopment at 18 months. The patterns of brain injury, with the majority of infants having BGT injury with or without WM injury, is similar to studies from high-income settings.[19 29 30] Normal/Mildly abnormal BGT, normal PLIC and present FMs were all highly predictive of a normal outcome in this study. The GMA at 10–15 weeks post-term age may be an alternative predictive tool in settings where MRI is not available.

The BGT injuries in our study were mainly mild. Severe BGT injury was less common compared with the National Institute of Child Health and Human Development (NICHD) trial and the Total Body Hypothermia (TOBY) trial,[19 30] and this is most likely due to the low proportion of infants with severe HIE in our study. We found a similar distribution of WM injury to the TOBY trial.[19] Another Indian HIE trial reported predominantly WM injury and more than half of infants with moderate/severe HIE had normal BGT.[13] WM injury is generally thought to be caused by more prolonged asphyxia or other causes of neonatal encephalopathy.[16] Patterns of brain injury in HIE infants in different low-resource settings should be further explored.

Our results confirmed a close association between early spontaneous movements and neurodevelopmental outcome. Normal FMs at 2–3 months post-term age is a well-known predictor of a normal motor outcome.[17 31] The association between FMs and cognitive outcome found in this study has also been reported by others.[32 33] A normal outcome in 9 of 10 infants with sporadic FMs is in accordance with a large multicentre study where only 8% of infants with sporadic FMs developed CP.[34]

Moderate/Severe BGT abnormalities, equivocal/absent PLIC and absent FMs had the highest predictive accuracy for adverse outcome in this study. Ferrari et al found that the site and severity of brain injury on neonatal MRI and the results of GMA are closely correlated and has comparable accuracy in predicting motor outcome in non-cooled HIE infants.[35] Similar predictive accuracy of MRI has been found in substudies of both the TOBY trial and the Infant Cooling Evaluation (ICE) trial.[19 36] Lally et al,[13] however, reported low sensitivity, but high specificity of MRI abnormalities in their study from South India, but only a small proportion of infants in that study had BGT injury.

In low-resource settings, MRI is often unavailable and expensive, while smartphones and internet access are cheap and available even in remote areas.[37] A video recording for GMA can easily be performed using a simple video camera or even a smartphone. GMA is both time-efficient and cost-efficient and could be evaluated by remote assessment, which is especially important for LMICs. We suggest that in low-resource settings GMA is a good supplement, or even an alternative, to MRI to direct interventions and further follow-up in babies with HIE.

Although this study was not powered to detect differences in neurodevelopmental outcome between treatment groups, we found a reduction in adverse outcomes in cooled infants. Together with our previous report on reduction in several MRI biomarkers, we therefore state that TH for moderate HIE is feasible and may be neuroprotective in a low-resource setting.[5] However, implementation of TH in LMICs should be limited to tertiary NICUs and requires development of guidelines and training programmes.

Limitations of this study include a small sample size and short duration of follow-up. This applies in particular to infants with severe WM injury and normal outcome, who are at risk of later cognitive/behavioural disabilities.[38] However, we had good follow-up rate.

Another limitation is the low proportion of infants with severe HIE, which is most likely due to selection bias.

CONCLUSION

In conclusion, we report that both neonatal MRI and GMA are strongly associated with neurodevelopmental outcome at 18 months in infants with HIE admitted to a tertiary care NICU in South India. GMA at 10–15 weeks post-term age has similar predictive accuracy as neonatal MRI and is therefore a feasible low-cost predictive tool in low-resource settings. The patterns of brain injury and incidence of adverse outcomes should be further explored in these settings.

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Original research

researchers who provide a methodologically sound proposal for use in achieving the goals of the approved proposal.

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Supplementary online content

1

eTable 1. Demographics and neonatal characteristics according to treatment group*

	Therapeutic hypothermia (n=25)	Standard care (n=25)	Total (n=50)
Inborn, n/N (%)	13/25 (52.0)	17/25 (68.0)	30/50 (60.0)
Male gender, n/N (%)	17/25 (68.0)	16/25 (64.0)	33/50 (66.0)
Gestational age (weeks), mean (SD)[†]	39.1 (1.3)	39.2 (1.4)	39.1 (1.3)
Birth weight (g), mean (SD)	2911 (483)	2960 (553)	2935 (515)
Small for gestational age, n/N (%)[‡]	7/25 (28.0)	7/23 (30.4)	14/48 (29.2)
5-min Apgar score, n/N (%)			
0-3	5/22 (22.7)	1/21 (4.8)	6/43 (14.0)
4-6	13/22 (59.1)	12/21 (57.1)	25/43 (58.1)
7-10	4/22 (18.2)	8/21 (38.1)	12/43 (27.9)
Cord/blood<60min pH, mean (SD)[§]	6.81 (0.12)	6.93 (0.18)	6.89 (0.17)
Cord/blood <60min base excess, mean (SD)[¶]	-19.6 (3.2)	-16.5 (4.6)	-17.7 (4.4)
HIE stage at randomisation, n/N (%)			
Moderate	24/25 (96.0)	24/25 (96.0)	48/50 (96.0)
Severe	1/25 (4.0)	1/25 (4.0)	2/50 (4.0)
Thompson score, median (IQR)			
At randomisation	9 (8-10)	9 (8.5-12)	9 (8-11)
Day 7/at discharge[**]	2 (1-3.25)	2 (2-3.25)	2 (2-3)
Injury pattern on MRI, n/N (%)			
Normal	4/23 (17.4)	2/23 (8.7)	6/46 (13.0)
Only mild/moderate WM injury	4/23 (17.4)	5/23 (21.7)	9/46 (19.6)
Only severe WM injury	0	1/23 (4.3)	1/46 (2.2)
Mild BGT, no/mild/moderate WM injury	13/23 (56.5)	6/23 (26.1)	19/46 (41.3)
Mild BGT, severe WM injury	1/23 (4.3)	0	1/46 (2.2)
Moderate BGT, any WM injury	1/23 (4.3)	5/23 (21.7)	6/46 (13.0)
Global	0	4/23 (17.4)	4/46 (8.7)

[*]Percentages are based on the number of infants for whom data were available. HIE, hypoxic-ischaemic encephalopathy; WM, white matter; BGT, basal ganglia and thalami.
[†]Data were unavailable for two infants in the standard care group.
[‡]Small for gestational age defined as birth weight less than the 10th percentile according to the Intergrowth 21st chart.
[§]Data were unavailable for 13 infants in therapeutic hypothermia group and seven in standard care group. Cord pH was not available in outborn infants.
[¶]Data were unavailable for 14 infants in therapeutic hypothermia group and eight in standard care group. Cord base excess was not available in outborn infants.
[**]Data were unavailable for three infants in therapeutic hypothermia group (two died) and seven in standard care group (five died).

eTable 2. General movements assessment and outcomes according to treatment group*

	Therapeutic hypothermia (n=25)	Standard care (n=25)	Total (n=50)	P value
Fidgety movements				
Absent (-)	3/21 (14.3)	6/18 (33.3)	9/39 (23.1)	0.255†
Sporadic (+/-)	4/21 (19.0)	6/18 (33.3)	10/39 (25.6)	
Intermittent (+)	12/21 (57.1)	6/18 (33.3)	18/39 (46.2)	
Continual (++)	1/21 (4.8)	0	1/39 (2.6)	
Exaggerated	1/21 (4.8)	0	1/39 (2.6)	
Adverse outcome‡	5/24 (20.8)	12/23 (52.2)	17/47 (36.2)	0.025
Death	2/24 (8.3)	6/23 (26.1)	8/47 (17.0)	0.105
Bayley-III cognitive composite score				0.019
<70	0	3/16 (18.8)	3/38 (7.9)	
70-84	2/22 (9.1)	3/16 (18.8)	5/38 (13.2)	
≥85	20/22 (90.9)	10/16 (62.5)	30/38 (78.9)	
Bayley-III motor composite score				0.074
<70	0	3/16 (18.8)	3/38 (7.9)	
70-84	2/22 (9.1)	1/16 (6.3)	3/38 (7.9)	
≥85	20/22 (90.9)	12/16 (75.0)	32/38 (84.2)	
Cerebral palsy	1/22 (4.5)	3/16 (18.8)	4/38 (10.5)	0.159
GMFCS				
Level 1	1/22 (4.5)	1/16 (6.3)	2/38 (5.3)	
Level 5	0	2/16 (12.5)	2/38 (5.3)	

*Percentages are based on the number of infants for whom data were available. Bayley-III, Bayley Scales of Infant and Toddler Development, third edition; GMFCS, gross motor function classification system.
†P value based on Fisher exact test on absent versus present fidgety movements.
‡Adverse outcome defined as death, cerebral palsy with GMFCS level 3-5 or Bayley-III cognitive and/or motor composite score <85.

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eTable 3. Normal/mildly abnormal MRI[*] with adverse outcome[**]

No	Rando-misation	BGT	PLIC	WM	Fidgety movements	Bayley-III cognitive CS	Bayley-III motor CS	CP
1	Non-cooled	Mild	Normal	Normal	Absent	80	79	No
2	Cooled	Normal	Normal	Moderate	Intermittent	100	76	No
3	Cooled	Mild	Equivocal	Moderate	Intermittent	80	70	No
4	Cooled	Mild	Equivocal	Normal	Intermittent	80	89	No
5	Non-cooled	Mild	Normal	Moderate	Sporadic	80	118	No

BGT, basal ganglia and thalami; PLIC, posterior limb of the internal capsule; WM, white matter; Bayley-III, Bayley Scales of Infant and Toddler Development, third edition; CS, composite score; CP, cerebral palsy.
[*]Normal/mildly abnormal MRI defined as normal/mild BGT score and/or normal/mild/moderate WM score.
[**]Adverse outcome defined as death, cerebral palsy with gross motor function classification system (GMFCS) level 3-5 or Bayley-III cognitive and/or motor composite score <85.

eTable 4. Moderately/severely abnormal MRI[*] and/or absent fidgety movements with normal outcome[**]

No	Rando-misation	BGT	PLIC	WM	Fidgety movements	Bayley-III cognitive CS	Bayley-III motor CS	CP
6	Cooled	Normal	Normal	Mild	Absent	90	112	No
7	Cooled	Mild	Normal	Mild	Absent	95	91	No
8	Cooled	Mild	Normal	Severe	Intermittent	95	100	No
9	Non-cooled	Normal	Normal	Severe	Intermittent	100	115	No
10	Cooled	Moderate	Equivocal	Normal	Absent	100	118	No

BGT, basal ganglia and thalami; PLIC, posterior limb of the internal capsule; WM, white matter; Bayley-III, Bayley Scales of Infant and Toddler Development, third edition; CS, composite score; CP, cerebral palsy.
[*]Moderately/severely abnormal MRI defined as moderate/severe BGT score and/or absent PLIC and/or severe WM score.
[**]Normal outcome defined as survival without cerebral palsy with gross motor function classification system (GMFCS) level 3-5 or Bayley-III cognitive and/or motor composite score <85.

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This paper is submitted for publication and is therefore not included.

Paper III

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