Pediatric RESEARCH

SPECIAL ARTICLE

Should therapeutic hypothermia be offered to babies with mild neonatal encephalopathy in the first 6 h after birth?

Mohamed El-Dib[1], Terrie E. Inder[1], Lina F. Chalak[2], An N. Massaro[3], Marianne Thoresen[4] and Alistair J. Gunn[5]

Infants with moderate to severe neonatal encephalopathy (NE) benefit significantly from therapeutic hypothermia, with reduced risk of death or disability. However, the need for therapeutic hypothermia for infants with milder NE remains unclear. It has been suggested that these infants should not be offered therapeutic hypothermia as they may not be at risk for adverse neurodevelopmental outcome and that the balance of risk against potential benefit is unknown. Several key questions need to be answered including first, whether one can define NE in the first 6 h after birth so as to accurately distinguish infants with brain injury who may be at risk for adverse neurodevelopmental consequences. Second, will treatment of infants with mild NE with therapeutic hypothermia improve or even worsen neurological outcomes? Although alternate treatment protocols for mild NE may be feasible, the use of the current approach combined with rigorous avoidance of hyperthermia and initiation of hypothermia as early as possible after birth may promote optimal outcomes. Animal experimental data support the potential for greater benefit for mild HIE compared with moderate to severe HIE. This review will summarize current knowledge of mild NE and the challenges to a trial in this population.

Pediatric Research (2019) 85:442–448; https://doi.org/10.1038/s41390-019-0291-1

INTRODUCTION

The management of mild neonatal encephalopathy (NE) remains controversial. Historically, infants with mild NE were believed to have minimal risk for adverse neurodevelopmental outcomes, with studies reporting outcomes very similar to controls.[1–3] Thus, if infants had no adverse outcomes, then there was no reason for any intervention. Due to this and the potential for adverse effects of therapeutic hypothermia (TH), infants with mild NE were largely not included in the randomized controlled trials (RCTs) of TH. Because of the limited evidence of benefit from RCTs, the net risk to benefit ratio for infants with mild NE for treatment with TH remains unknown. Of concern, recent studies have shown an increased risk of mortality, brain injury, seizures, as well as adverse outcomes such as behavior problems, neurodevelopmental impairment, and lower intelligence quotient (IQ) in infants with mild NE.[4] As TH has a wide margin of safety, and many centers have gained expertise and comfort using TH, there has been widespread therapeutic creep, such that many centers are now treating milder cases of NE with TH.[5] Despite the safety of TH itself, TH may trigger significant shivering and apparent stress, leading to administration of sedatives that may have neurotoxic effects,[6] and so could compromise any benefit of treatment. Additionally, TH is associated with separation from their parents for 3 days, delayed oral feeding and establishment of breast feeding, and, at least potentially, increased use of invasive treatments such as central lines, inotropes, antibiotics, and respiratory support. Finally, critically for targeted, accurate delivery of care, there is no consensus on the accurate definition of mild NE within the first 6 h after birth.[5,7]

In April 2018, an expert panel met at the annual Neonatal Neurocritical Care Special Interest Group (www.NNCC-SIG.org) meeting to examine the evidence for considering TH for mild NE. In this article, the panel will review the topic in detail, addressing factors related to (a) neurodevelopmental outcomes in mild NE as evidence of the need to consider treatment; (b) current practices in mild NE; (c) existing preclinical evidence that mild NE could benefit from TH; (d) the definition of mild NE; and (e) a proposed framework for a possible RCT for mild NE.

EVIDENCE THAT MILD NE IS ASSOCIATED WITH WORSE NEURODEVELOPMENTAL OUTCOME

In the pre-hypothermia era, the Sarnat neurological staging system was used serially over the first week after birth to define the severity of NE. During the TH era, the Sarnat staging system including neurological examination and formal electroencephalogram (EEG) from 24 h onwards, was shifted in its application to use only the clinical examination within 6 h of birth, much earlier than originally proposed, to align with the known limited therapeutic window of TH. Moreover, we should also note that after 2006 there was a transition to the Bayley III edition to define neurodevelopmental outcomes.[8] It is essential to appreciate these changes in the definition of mild NE (related to the timing of evaluation) and of the developmental outcomes in order to interpret the outcomes mild NE.

[1]Department of Pediatric Newborn Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [2]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX, USA; [3]Department of Pediatrics – Neonatology Division, The George Washington University School of Medicine and Children's National Health Systems, Washington, DC, USA; [4]Neonatal Neuroscience, Translational Medicine, University of Bristol, Bristol, UK and [5]Department of Physiology, School of Medical Sciences, University of Auckland, Auckland, New Zealand
Correspondence: Alistair J. Gunn (aj.gunn@auckland.ac.nz)

Received: 26 October 2018 Revised: 20 December 2018 Accepted: 30 December 2018
Published online: 16 January 2019

PRE-HYPOTHERMIA ERA OUTCOMES

Robertson et al. evaluated the outcome of term infants at 3.5 years of age ($n = 69$) and reported that none of the infants with mild NE had a major handicap, defined by cerebral palsy, visual impairment, or cognitive delay. The Stanford-Binet Intelligence Scale was 101.5 (±14.0) and the intelligence score using Peabody Picture Vocabulary Test was 104.1 (±13.7).[1] At 8 years of age, school performance of the children with mild NE was similar to the control children.[2] Subsequently, Van Handel and colleagues prospectively examined the effects of mild NE ($n = 34$) on behavioral function at 9–10 years of age. The IQ was 98.1 ± 12.3 (mean ± standard deviation (SD)) in children with mild NE compared to 109 ± 12 in control children. The authors reported that social and attention problems scores were higher among children with mild NE.[9] From today's perspective, the major limitation is that the original full Sarnat assessment consisted of serial evaluations including EEG, which were repeated over several days, and so this is likely to represent a different cohort than in modern studies where infants are identified within 6 h of birth.

Odd and colleagues evaluated cognitive function at 8 years of age among children who were resuscitated after birth with or without NE. They found higher odds of lower IQ score in resuscitated children without NE (odds ratio 1.65 [95% confidence interval (CI) 1.13–2.43]) but the greatest difference was seen in infants who also developed NE (odds ratio 6.22 [1.57–24.65]).[10] The limitations of this study are that the neonatal records were assessed in retrospect to identify NE, and while cognitive testing was obtained as part of a prospective longitudinal study, 48% of eligible infants did not attend for testing. Murray et al. evaluated the cognitive outcome at 5 years of age of 22 infants with mild NE and reported lower full-scale IQ than normal controls [99 (94–112) vs. 117 (110–124), $p = 0.001$], verbal IQ [105 (99–111) vs. 116 (112–125), $p = 0.001$], and performance IQ [103 (98–112) vs. 115 (107–124), $p = 0.004$].[11]

A recent systematic review identified 20 studies that included infants with mild NE. The studies from before the hypothermia era reported retrospectively on long-term outcomes in 250 children following mild NE. The criteria for NE were variable and often not well defined. That review reported that abnormal outcome, defined as the presence of cerebral palsy or any standardized neurodevelopmental test score more than 1 SD below the mean at 18 months of age or older, occurred in 56 (22%) of children following mild NE.[4]

HYPOTHERMIA ERA OUTCOMES

Four RCTs of TH for moderate or severe neonatal NE enrolled infants with mild NE even though the studies were not powered or designed to target mild NE. Jacobs et al. tested whole-body hypothermia in moderate to severe NE but also allowed the inclusion of mild NE ($n = 40$). Among infants with mild NE receiving standard care, the rate of death or major sensorineural disability was 33% (8/24) compared with 25% (4/16) in hypothermic infants (relative risk 0.53, 95% CI 0.17–1.66).[12] Zhou et al. evaluated the role of selective head cooling among infants with moderate or severe NE ($n = 194$) and included cases with mild NE ($n = 39$). Abnormal outcomes occurred in 6 cooled and 7 controls with mild NE.[13] Battin et al. enrolled a total of 9 infants with mild NE (5 cooled and 4 control) and reported abnormal outcomes in 1 cooled and 2 controls.[14] The CoolCap trial enrolled 8 infants with mild NE (5 cooled and 3 controls) and found unfavorable outcomes in 2 cooled and 0 control infants.[15] Of note, the initial amplitude integrated EEG (aEEG) patterns of all 8 mild cases of NE showed moderately or severely abnormal background voltage inferring that the clinical assessment of Sarnat grade I is subjective and challenging on clinical evaluation alone. The RCT studies were summarized reporting an abnormal outcome in 29% of cooled vs. 37% of uncooled infants with mild NE, an odds ratio of 0.67 (95% CI: 0.28–1.61, $p = 0.59$). While there was a trend for benefit in favor of TH, none of these studies were powered to detect a clinical effect in mild NE.[4]

CONTEMPORARY OUTCOMES OF UNTREATED MILD NE

The PRIME Study (Prospective Research in Infants with Mild Encephalopathy) recently enrolled 54 infants with rigorously defined mild NE in the first 6 h after birth and reported neonatal intensive care unit (NICU) discharge outcomes of infants[7] and neurodevelopmental follow-up.[16] In that study, mild NE was defined as the presence of the National Institute of Child Health and Human Development (NICHD) criteria for evidence of hypoxia-ischemia during the perinatal period (the same as used in previous NICHD clinical trials) and ≥1 abnormality using the modified Sarnat criteria (but no evidence of three abnormal categories defining moderate or severe NE). This is the only recent study to date providing 18–24 months' outcomes prospectively using Bayley III assessment in a contemporary cohort of untreated infants with mild NE. Forty-three infants completed the full neurodevelopmental follow-up testing (of 63 infants enrolled and 51 infants who were reviewed at a mean of 19 months), 7 (16%) had disability, including 1 (2%) infant with cerebral palsy, and 2 (5%) who had autism. Bayley III scores < 85 were documented in 17 infants (40%): 14 (32%) language domain, 7 (16%) cognitive domain, and 6 (14%) in the motor domain. The 16% rate of disability after mild NE, and 40% with Bayley III scores < 85 is higher than would be expected for infants who did not have NE, but lower than reported in moderate to severe encephalopathy.[17]

Interestingly, in a recent prospective multicenter cohort study of magnetic resonance spectroscopy assessment of brain injury in NE,[18] of 30 infants with mild NE, all of whom were treated with TH, just 1 infant developed disability, and 20 had mild white matter injury on formal magnetic resonance imaging (MRI) assessment. Although this study was not controlled, these results are very encouraging for potential benefit of TH in this population.

In older studies, before the era of hypothermia, the severity of NE was defined in retrospect. For treatment, it is essential to define NE prospectively as early as possible in the first 6 h after birth, in a phase when the severity of neurological findings often changes over time. Thus, the large RCTs of TH excluded infants who had mild NE as assessed in the first 6 h after birth in order to improve their trial power since these infants were less likely to have an unfavorable outcome. This means that the real world clinical benefits of treating these infants with TH are unknown. Moreover, it is important to appreciate that in absence of TH, the severity of clinical signs of NE often increases over the first 2–3 days after birth[19,20] and that the original Sarnat and Sarnat classification, was based on retrospective assessment of neurological progress over the first week *plus* multimodal assessment, typically including formal EEG and imaging. Thus, potentially, some infants with observed disability after "mild" NE identified within 6 h of birth in contemporary prospective studies like the PRIME study may have been classified in the pre-hypothermia era as "moderate".

HYPOTHERMIA FOR MILD NE: THERAPEUTIC CREEP

Despite the exclusion of infants with mild NE from most hypothermia trials, therapeutic creep has been observed, so that TH has been extended to babies with mild NE in many NICUs. Reports from single centers as well as multicenter registries in the post-hypothermia era highlight the recognition by the clinical community that infants with mild NE are at risk for adverse outcomes. While it is not possible to be certain of the reasons behind this change, the use of TH in this population may reflect clinicians' assumption that TH must also be beneficial for mild NE, but also might reflect medicolegal concerns or misdiagnosis.

A report from McGill University in Canada described that among the 215 infants referred for cooling between 2008 and 2012, 79 (36%) had mild NE, of whom 13 (16%) were cooled.[21] In these 13 infants, TH was initiated based on amplitude integrated EEG findings consistent with moderate encephalopathy, despite mild staging based on neurological exam. Brain MRI was available in 50 non-cooled and all cooled infants. Infants who were not cooled had higher frequency of MRI-defined brain injury than patients who were cooled (40% vs. 31%). In another single-center report, Walsh et al. reported brain MRI findings for infants with encephalopathy treated with hypothermia at the Brigham and Women's Hospital between 2013 and 2015, based on institutional criteria that included cooling infants who were >34 weeks gestational age, with pH ≤ 7.1, base deficit (BD) ≥ 12 mEq/L, and any stage of NE.[22] Of the 64 cooled infants, 33 (51.5%) had mild NE. Almost 30% of these infants with mild NE had features of moderate/severe brain injury on MRI and a similar rate of MRI injury to infants with moderate NE. However, neurodevelopmental outcome at 2 years of age is unknown.

Data registries also provide evidence of therapeutic creep across centers. Data from the Vermont Oxford Network Neonatal Encephalopathy Registry (2006–2011) revealed that amongst 2457 enrolled cases from 99 centers, only 57% met standard eligibility criteria used in the randomized trials. Of the infants who did not meet eligibility criteria for cooling, 40% had neither moderate to severe NE nor seizures before initiation of hypothermia.[23] Azzopardi and colleagues reported data from the TOBY registry (2006–2011) that include over 2000 registered cases of NE treated with hypothermia in the United Kingdom.[24] Of the 513 cases that had Thompson encephalopathy score data recorded before initiation of cooling, lower scores (0–5) consistent with mild NE were recorded in 91 (18%) infants. Kracer and colleagues reported the use of TH for NE in the state of California using the California Perinatal Quality Care Collaborative and California Perinatal Transport System 2010–2012 datasets.[25] Of 829 cases with NE, 237 (28.5%) were mild. Overall, 50% of neonates with mild NE were treated with hypothermia, with an increasing trend over time (38% in 2010, 53% in 2011, and 55% in 2012). In the most recent registry report from 27 regional NICUs in the United States, the Children's Hospital Neonatal Consortium reported short-term outcomes of infants with NE born between 2010 and 2013.[26] Of the 945 infants with NE, 160 cases were mild. The majority (76%) received hypothermia, and brain MRI was normal in only 41% of cases of mild NE. Unfortunately, there are no neurodevelopmental outcome data in these registry cohorts. There is significant variation between MRI scoring systems and unless documented with outcome one cannot assume that mild-moderate changes on the MRI necessarily denotes poor outcome.[27]

These reports reflect the recognition that babies with mild NE assessed in the first 6 h after birth are at risk for adverse outcomes and that many clinicians, nationally and internationally, have adopted the practice of extending TH on a clinical basis to this population. These reports also highlight the potential for lack of equipoise in the clinical community that will need to be considered to support future therapeutic trials aimed to improve outcomes in babies with mild NE.

PRECLINICAL EVIDENCE OF ROLE OF HYPOTHERMIA IN MILD NE

Experimentally, the efficacy of neuroprotection with hypothermia is highly related both to the severity and nature of the insult and to the timing of initiation of cooling, its duration, and its depth.[28] These factors interact in complex ways to determine the outcome of any particular regime. After moderate to severe HI, optimal outcomes in humans and large animals are seen with a reduction in brain temperature of approximately 33.5 °C, induced as early as possible within the first 6 h after birth and continued for 72 h.[28] At present, the minimum duration of treatment for any given period of hypoxia-ischemia (HI) can only be determined empirically and there is little direct evidence for the optimal parameters for mild HI. Overall, as discussed next, the available animal evidence suggests that TH after mild injury is likely to show even better relative neuroprotection than after moderate to severe HI, if TH is applied within the same 6 h time window, depth (33.5 °C), and duration (72 h) as the current recommended cooling protocol.

WHAT DOES MILD HI INJURY LOOK LIKE IN EXPERIMENTAL MODELS?

The impact of HI around term on different areas of the brain is a function both of the nature of the insult and the susceptibility of different brain regions.[29] Broadly speaking, injury often follows basal ganglia or watershed predominant patterns. In fetal sheep, a watershed pattern is seen in multiple models, including pure cerebral ischemia,[30] prolonged partial umbilical cord occlusion,[31–33] and repeated brief cord occlusion.[34] By contrast, basal ganglia and thalamic damage is clinically associated with more severe or "sentinel" events at birth.[35] Consistent with this, in near-term fetal sheep this pattern is seen after prolonged complete umbilical cord occlusion.[36]

Within these models of HI injury, it is notable that short insults such as 10 min of cerebral ischemia and the same duration of complete umbilical cord occlusion are associated with highly selective hippocampal injury, particularly affecting the cornu ammonis regions 3 and 1/2.[37,38] Both insults lead to a highly similar pattern of transient suppression of EEG activity and few or no secondary electrographic seizures. As the insults are continued for longer, more regions are affected, and seizures become more prominent. Twenty minutes of cerebral ischemia leads to profound hippocampal damage and moderate injury of the parasagittal cortex, followed by marked but discrete secondary seizures, and 30 min leads to parasagittal laminar necrosis and delayed onset of status epilepticus.[38] Umbilical cord occlusion continued until fetal arterial blood pressure was <8 mm Hg was associated with severe basal ganglia damage and all fetuses developed seizures, including status epilepticus in approximately half.[36] These patterns, seen across multiple models, suggest that predominantly hippocampal damage will be seen after mild HI without significant delayed seizures, whereas thalamic or cortical damage with multiple electrographic seizures are features of moderate to severe injury.

MORE SEVERE INSULTS LEAD TO MORE RAPID EVOLUTION OF PROGRAMMED CELL DEATH

Programmed cell death, involving activation of multiple pathways, including programmed necrosis, apoptotic, and autophagy, plays a key role in the delayed evolution of HI injury after resuscitation.[28] Data support that the initiation of neuronal degeneration occurs more slowly after a shorter, less severe period of HI compared with severe insults leading to infarction.[39] The reader should note that DNA fragmentation and classic ischemic cell change represent the terminal events of this cascade and so their appearance only means that cell death is now irreversible.

EARLY INITIATION OF COOLING AFTER HYPOXIA-ISCHEMIA

Consistent with the evidence of slow evolution of programmed cell death in milder insults, early initiation of cooling after brief ischemia in adult rodents was highly protective. For example, after 3 min of cerebral ischemia in adult gerbils, cooling to 32 °C for 12 h that was initiated at 1 h, attenuated abnormal open field behavior and substantially reduced CA1 necrosis at 10 and 30 days, but was only partially effective after 5 min of occlusion.[40] By contrast, extending the duration of hypothermia to 24 h provided near total

protection after 5 min of ischemia. Learning in an open field and T-maze were improved by hypothermia after 6 months recovery.[41] Moreover, in adult gerbils, when the delay before initiating a 24 h period of cooling was increased from 1 to 4 h after ischemia, neuroprotection in the CA1 field of the hippocampus after 6 months of recovery fell from 70 to 12%.[41] However, neuroprotection was almost completely restored by extending the interval of moderate hypothermia (a reduction in body temperature of up to 5 °C) to 48 h or more, even when the start of cooling was delayed until 6 h after reperfusion.[42]

Similarly, in 7-day-old rat pups, hypothermia was found to be notably protective after a "moderate" duration of HI (90 min),[43] such that immediate induction of hypothermia after moderate HI significantly reduced the area of cortical infarction ($P < 0.05$), with a linear loss of effect with greater delay in starting cooling, at least up to 6 h of delay. By contrast, even immediate hypothermia did not improve outcomes after very prolonged HI (150 min).

It is important to contrast the finding of neuroprotection with a relatively short duration of just 6 h of moderate hypothermia in P7 rats[44] but not in older rodents or large animals.[45] Potentially, the apparent protection with such a short duration of TH might reflect more rapid rate of brain development in rodents than larger animals. A more likely explanation is that rectal temperatures of healthy nesting neonatal rats in the vivarium are significantly lower than in later life (median of 35.4 °C at P7 vs. ~38 °C in adults).[46,47] Thus, P7 rats may be exposed to continuing mild hypothermia after the acute study period, and so the exact duration of hypothermia cannot be compared with human or large animal studies or adult rodent studies with rigorous temperature control.

It is important to appreciate that these experimental protocols do not, and are not intended to, reflect the complex nature of human NE. Keeping this caveat in mind, these findings as a whole support that early treatment of infants with milder HIE with TH applied in the most effective manner using the current approach is likely to be effective.

HOW SHOULD WE DEFINE MILD NE?

Although mild NE is commonly cited, the exact definition of mild NE is variable. In addition, in mild NE, as in all forms of NE, we cannot be absolutely sure of the underlying etiology of the NE, which may be more complex than just HI and so might not benefit from TH. For example, there is some evidence that TH may be less helpful in infants with preceding infection/inflammation.[48,49] Nevertheless, others reported, in a retrospective cohort study of 1084 infants treated with TH for NE, that the majority of 42 infants with NE and early-onset sepsis had a favorable outcome.[50] As we consider trials of TH in infants with milder NE, our aim should be to correctly identify infants with brain injury from hypoxia-ischemia who may benefit most from TH. On the other hand, we must not ignore or downplay the stress of being hypothermic, the multiple interventions associated with intensive care including sedation, respiratory support, intravenous lines, blood pressure support, antibiotics, separation from parents for at least 3 days, and delayed oral feeding to more mildly affected infants since such interventions could have net adverse effects.

To assist in defining the presence of mild NE, particularly associated with evidence of hypoxia and ischemia, an array of markers may assist. While neurological clinical examination is the cornerstone for the classification of the presence and severity of NE,[19,20] this approach has notable limitations. The neurological state is dynamic and may evolve over hours, and so a baby with an early normal examination may evolve over a few hours of age into mild NE while a baby with mild NE may evolve into more moderate NE.[51,52] Although the Sarnat clinical criteria are widely used, another neurological scoring system, the Thompson score has been used in clinical trials for infant selection.[53,54] In addition, because of the changes over time of the neurological examination, timed and standardized clinical evaluation will help strengthen its validity,[20,55,56] and videotaping of the examination may be valuable for training and quality control.[55,56]

Standard biochemical markers

Standard biochemical markers of acid-base, including pH and BD, have been traditionally used to assess the severity of perinatal HI. The earliest metabolic or circulatory sign of reduced delivery of oxygen and substrate to the brain is reduced cardiac output leading to hypotension, and so reduced cerebral blood flow (CBF). In the adult brain, a fall in CBF of 30% is associated with reduced protein synthesis in the brain.[57] When CBF is reduced by 40–50%, sensory evoked potentials and EEG power are progressively suppressed. Reduced ATP and anoxic depolarization with cytotoxic edema develop when CBF is reduced by >70–90%—from that time to cell death is relatively short, as the ion pumps stop working. Standard biochemical changes following energy deprivation, inflammation, or generalized stress measurable in plasma include acidosis, lactate, glucose, C-reactive protein (CRP), lactate dehydrogenase (LDH), and more. Current cutoff values used as entry criteria for cooling therapy ranged from <7.0 to 7.15 for pH and >10 to 16 mmol/L for BD. However, the values used as significant criteria in the major RCTs were pH < 7.0 and BD ≥ 16. It has been suggested that a cutoff of pH ≤ 7.1 would be more sensitive in detecting infants with NE,[58] but at the cost of increasing the number of infants who would need to be formally examined for some hours after birth. In that study 25 infants with pH between 7.0 and ≤7.1 needed to be screened to identify one additional infant with moderate to severe NE who would otherwise have been missed, representing a 15% increase in infants correctly selected for treatment.[58] The impact of a higher pH threshold on screening for mild NE is unknown but it is plausible that less severe acidosis would capture a higher proportion of infants with milder NE.

The peak CRP value associated with NE has been shown to be delayed by 36 h and to stay high for longer in cooled babies.[59] Cooling itself increases CRP without any documented infection, as a nonspecific response to stress, just as CRP is slightly increased after surgery. Therefore, cooled infants tend to receive antibiotics for a longer period than standard care babies. Nevertheless, high CRP levels are associated with neonatal sepsis.[60]

Finally, LDH levels measured within 6 h after birth in infants with NE discriminated between acute and nonacute perinatal HI.[61] Moreover, increased LDH levels were associated with worse neurodevelopmental outcome at 18–24 months of age.[62]

More specific potential biomarkers

More specific potential biomarkers of neurological injury have been suggested including neuron-specific enolase, myelin basic protein, S-100β, glial fibrillary acidic protein, ubiquitin carboxyl-terminal hydrolase-L1, brain-derived neurotrophic factor, cleaved Tau, microRNA-21, and activin. Other categories of biomarkers include vascular markers, e.g. vascular endothelial growth factor; oxidative stress markers e.g., superoxide dismutase; inflammatory markers such as high-sensitivity CRP, interleukins, and tumor necrosis factor-α, as well as exosomes. Details of these biomarkers are described elsewhere.[63,64] The sensitivity and specificity of using these markers or combinations of them to select babies with mild NE for cooling are not yet known and these biomarkers have not been validated in large cohorts.

Amplitude integrated EEG

aEEG is a widely available real-time continuous cotside marker that displays brain activity and its changes over time. In Europe, the technique has been in routine use for infants with NE for 25 years. The background aEEG amplitude and pattern within 6 h of age are strong predictors of later neurodevelopmental outcomes

in normothermic children with perinatal NE.[65,66] In the setting of TH, early (<6 h) aEEG has limited positive predictive value (PPV). Thus, early aEEG is more useful for diagnosing/grading encephalopathy for recruitment than for outcome prediction after treatment.[67] Continuous aEEG, however, remains a strong predictor of outcome in HIE. The key features of the aEEG trace that predict long-term neurodevelopmental outcome in cooled children are the time it takes for the aEEG to recover to a normal background pattern and the time of onset of sleep-wake cycling.[67]

In a Norwegian, prospectively recruited, cohort of 47 TH infants in whom aEEG data were recorded, but not used for treatment selection, 15 infants had normal aEEG (continuous normal voltage (CNV)) on retrospective analysis. Of these, 3 infants had an unfavorable outcome (defined as a Bayley III score < 85 in any domain, with language scores of 76, 82, and cognition 81, respectively).[68] Of 14 infants with initial CNV who were cooled in a Bristol dataset,[69] all had good outcomes and normal MRI. Gagne-Loranger et al. used aEEG to select for TH in a cohort study. Thirteen babies with mild NE had abnormal aEEG and were cooled; 31% had abnormal MRI. By contrast, 9 babies with mild NE and normal aEEG who were not cooled had no brain injury on MRI.[21]

The CoolCap and TOBY trials included an abnormal aEEG within 6 h as one of three main criteria for TH.[54,70] In the TOBY study of 314 infants randomized to standard care or TH, the PPV of a severe aEEG assessed by the voltage and pattern methods within 6 h of birth for death or disability at 18 months of age was 0.63 and 0.59 respectively in non-cooled infants and 0.55 and 0.51 in cooled infants ($p > 0.05$).[71]

Magnetic resonance imaging

Finally, while injury on MRI is common in babies with mild NE, predictive patterns are typically not able to be identified until after the first 3 days of life.[27] Thus, its utility in the first 6 h after birth is limited by the timing of evolution of injury as well as by availability, and lack of studies looking at serial MRI. Having readily available MRI in NICUs to undertake serial MRIs in such babies may provide greater insight into early MRI as a selective tool.[72]

FRAMEWORK FOR A RCT

Since there is agreement on the growing evidence of abnormal neurodevelopmental outcomes of some infants with mild NE, and since it is plausible that hypothermia will be beneficial, a RCT seems prudent to provide definitive clinical evidence. In a survey sent by email to members of the Neonatal Neurocritical Care Special Interest Group (www.NNCC-SIG.org) who attended the 2018 annual meeting, of 52 respondents (from 45 different centers), 46 (88%) said they would support an RCT of neuroprotection in mild NE. When asked about what type of neuroprotective therapy to use, 43 (93%) supported TH and 31 (67%) supported using erythropoietin. While there is consensus on the need of a RCT, there was disagreement on the exact inclusion criteria for such study.

One approach to inclusion criteria for such an RCT would be to use the same strict inclusion criteria used in previous RCTs for evidence of exposure to HI but to be more inclusive of clinical signs of a lower severity of encephalopathy. This population would be very similar to the one studied in the PRIME study, which has characterized their outcomes.[16]

An alternate approach would be to broaden the acidosis inclusion criteria and add other complementary available biomarkers. Using a pH of 7.1 and BD of 10 mmol/L could provide a marker of a significant insult responsible for the presence of encephalopathy. Additionally, one could combine aEEG monitoring with a clinical neurological examination. It is important to agree on when and how to manage the start of passive and active cooling and the time window in which to undertake the entry examinations. We recommend filming the neurological examination of trial babies for later review and comparisons of interpretation between sites. We know from compelling preclinical evidence and indicative clinical findings that the earlier cooling is started, the greater the neuroprotective benefits.[28] In infants requiring resuscitation or fulfilling the blood gas entry criteria, slow passive cooling should be allowed, at the pace of their own metabolism with continuous monitoring of core temperature, should have serial standardized neurological examinations, and aEEG monitoring and optimally have a decision regarding starting active cooling within 3 h.[73]

In order to make valid comparisons we will need a detailed management protocol that minimizes differences in regional practices including intubation, sedation, EEG monitoring, therapy for seizures, and feeding. Finally, the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley III) underestimate the percentage of infants with delay compared to Bayley-II.[8] If the Bayley III scale is used for follow-up, using a cutoff of ≤90 (rather than the conventional 85) may be more appropriate to capture abnormal outcomes after mild NE. This would naturally increase the rate of adverse outcomes. In the PRIME study, 7 infants with mild NE had cognitive scores ≤ 85 (16%) and 12 had scores of ≤90 (23%). Seventeen of infants had at least one cognitive, motor, or language score ≤ 85 (40%) and 24 infants had at least one score of ≤90 (56%, unpublished data). It is evident that a range of study designs could reasonably be adopted.

We propose a framework consistent with that used in the PRIME study, as follows:

1. Infants with cord arterial pH ≤ 7.0 or BD ≥ 16 mmol/L, or pH ≤ 7.15 and BD of >10 mmol/L if intubated and or received bagging for 10 min, or APGAR scores < 5 would be assessed for presence of encephalopathy by trained observers as reported in the PRIME trial.[16] Mild NE would be defined as ≥1 abnormality using the modified Sarnat criteria but not meeting the criteria for moderate or severe NE. Potentially, continuous aEEG recordings could be started in all infants meeting the clinical criteria for mild NE; this addition is not critical, but would allow infants with moderate to severe NE to be identified within the first 6 h and offered TH as part of standard care.[53] Infants meeting the criteria and whose parents gave informed consent would be randomized to either standard care or TH for 3 days.
2. The primary outcome would be Bayley III neurodevelopmental scores in either cognition, motor, or language ≤ 90. Secondary outcomes would include time to recover sleep-wake cycling on the neonatal aEEG, MRI imaging, and moderate to severe disability at 2 years of age.[16]
3. Study size. Based on personal communication from the PRIME study 56% of infants with mild NE managed with normothermia would have at least 1 abnormal Bayley score if we use a cutoff ≤ 90. Based on this, an absolute reduction in risk of from 56 to 40%, with 80% power and with alpha 0.05, would require randomizing 152 infants per group. Allowing for 20% loss to follow-up, this suggests that a total 365 infants would need to be randomized. A slightly larger cohort would increase power for long-term follow-up. The greater incidence of impaired Bayley scores than disability after mild NE suggests that Bayley scores are the most appropriate end point. For example, if 14% of control infants show moderate to severe disability, then at least twice as many infants would need to be randomized to detect an absolute reduction of 7%.

Given the limitations of assessing language and cognition in infancy then Bayley III neurodevelopmental scores would only be the first outcome, supplemented by secondary outcomes. We

strongly suggest that infants should be followed to school age to allow definitive assessment of intellectual and academic performance.

CONCLUSIONS

There is compelling clinical and experimental evidence that TH initiated in the first 6 h after birth and continued 72 h is associated with long-term improved survival without disability in babies with moderate to severe NE.[17] Despite exclusion of babies with mild NE from most neuroprotection intervention trials to date, increasing evidence supports that this population is at risk for adverse neurodevelopmental outcomes. Formal trials of neuroprotective therapies in infants with mild NE are now essential to confirm the risk:benefit ratio for treatment of this larger group. Given that the definition of mild NE is still controversial and the ability to differentiate infants at high risk of disability from those who are truly "mildly" affected is poor, research into improving our tools (e.g. revised neurological examination, incorporation of EEG/aEEG, and biomarkers for risk stratification) is urgently needed. At present, a pragmatic RCT investigating the effect of TH on infants with mild NE seems prudent.

ACKNOWLEDGEMENTS

The work reported in this review was supported in part by The Norwegian Research Council, the Moulton Foundation UK, and Health Research Council of New Zealand and the Neurological Foundation of New Zealand.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Robertson, C. & Finer, N. Term infants with hypoxic-ischemic encephalopathy: outcome at 3.5 years. *Dev. Med. Child Neurol.* **27**, 473–484 (1985).
2. Robertson, C. M., Finer, N. N. & Grace, M. G. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. *J. Pediatr.* **114**, 753–760 (1989).
3. Finer, N. N., Robertson, C. M., Peters, K. L. & Coward, J. H. Factors affecting outcome in hypoxic-ischemic encephalopathy in term infants. *Am. J. Dis. Child.* **137**, 21–25 (1983).
4. Conway, J. M., Walsh, B. H., Boylan, G. B. & Murray, D. M. Mild hypoxic ischaemic encephalopathy and long term neurodevelopmental outcome—a systematic review. *Early Hum. Dev.* **120**, 80–87 (2018).
5. Chalak, L., Latremouille, S., Mir, I., Sanchez, P. J. & Sant'Anna, G. A review of the conundrum of mild hypoxic-ischemic encephalopathy: current challenges and moving forward. *Early Hum. Dev.* **120**, 88–94 (2018).
6. Wassink, G. et al. Analgesics, sedatives, anticonvulsant drugs, and the cooled brain. *Semin. Fetal Neonatal Med.* **20**, 109–114 (2015).
7. Prempunpong, C. et al. Prospective research on infants with mild encephalopathy: the PRIME study. *J. Perinatol.* **38**, 80–85 (2018).
8. Jary, S., Whitelaw, A., Walloe, L. & Thoresen, M. Comparison of Bayley-2 and Bayley-3 scores at 18 months in term infants following neonatal encephalopathy and therapeutic hypothermia. *Dev. Med. Child Neurol.* **55**, 1053–1059 (2013).
9. van Handel, M., Swaab, H., de Vries, L. S. & Jongmans, M. J. Behavioral outcome in children with a history of neonatal encephalopathy following perinatal asphyxia. *J. Pediatr. Psychol.* **35**, 286–295 (2010).
10. Odd, D. E., Lewis, G., Whitelaw, A. & Gunnell, D. Resuscitation at birth and cognition at 8 years of age: a cohort study. *Lancet* **373**, 1615–1622 (2009).
11. Murray, D. M., O'Connor, C. M., Ryan, C. A., Korotchikova, I. & Boylan, G. B. Early EEG grade and outcome at 5 years after mild neonatal hypoxic ischemic encephalopathy. *Pediatrics* **138**, e20160659 (2016).
12. Jacobs, S. E. et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch. Pediatr. Adolesc. Med.* **165**, 692–700 (2011).
13. Zhou, W. H. et al. Selective head cooling with mild systemic hypothermia after neonatal hypoxic-ischemic encephalopathy: a multicenter randomized controlled trial in China. *J. Pediatr.* **157**, 367–372 (2010).
14. Battin, M. R., Dezoete, J. A., Gunn, T. R., Gluckman, P. D. & Gunn, A. J. Neurodevelopmental outcome of infants treated with head cooling and mild hypothermia after perinatal asphyxia. *Pediatrics* **107**, 480–484 (2001).
15. Wyatt, J. S. et al. Determinants of outcomes after head cooling for neonatal encephalopathy. *Pediatrics* **119**, 912–921 (2007).
16. Chalak, L. F. et al. Prospective research in infants with mild encephalopathy identified in the first six hours of life: neurodevelopmental outcomes at 18-22 months. *Pediatr. Res.* **84**, 861–868 (2018).
17. Jacobs, S. E. et al. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst. Rev.* **1**, CD003311 (2013).
18. Lally, P. J. et al. Magnetic resonance spectroscopy assessment of brain injury after moderate hypothermia in neonatal encephalopathy: a prospective multi-centre study. *Lancet Neurol.* **18**, 35–45 (2019).
19. Sarnat, H. B. & Sarnat, M. S. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch. Neurol.* **33**, 696–705 (1976).
20. Thompson, C. M. et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr.* **86**, 757–761 (1997).
21. Gagne-Loranger, M., Sheppard, M., Ali, N., Saint-Martin, C. & Wintermark, P. Newborns referred for therapeutic hypothermia: association between initial degree of encephalopathy and severity of brain injury (What about the newborns with mild Encephalopathy on admission?). *Am. J. Perinatol.* **33**, 195–202 (2016).
22. Walsh, B. H. et al. The frequency and severity of magnetic resonance imaging abnormalities in infants with mild neonatal encephalopathy. *J. Pediatr.* **187**, 26–33.e21 (2017).
23. Soll, R. F. Cooling for newborns with hypoxic ischemic encephalopathy. *Neonatology* **103**, 261 (2013).
24. Azzopardi, D. et al. Implementation and conduct of therapeutic hypothermia for perinatal asphyxial encephalopathy in the UK—analysis of national data. *PLoS ONE* **7**, e38504 (2012).
25. Kracer, B., Hintz, S. R., Van Meurs, K. P. & Lee, H. C. Hypothermia therapy for neonatal hypoxic ischemic encephalopathy in the state of California. *J. Pediatr.* **165**, 267–273 (2014).
26. Massaro, A. N. et al. Short-term outcomes after perinatal hypoxic ischemic encephalopathy: a report from the Children's Hospitals Neonatal Consortium HIE focus group. *J. Perinatol.* **35**, 290–296 (2015).
27. Thayyil, S. et al. Cerebral magnetic resonance biomarkers in neonatal encephalopathy: a meta-analysis. *Pediatrics* **125**, e382–e395 (2010).
28. Wassink, G. et al. A working model for hypothermic neuroprotection. *J. Physiol.* **596**, 5641–5654 (2018).
29. Gunn, A. J. & Bennet, L. Fetal hypoxia insults and patterns of brain injury: Insights from animal models. *Clin. Perinatol.* **36**, 579–593 (2009).
30. Gunn, A. J., Gunn, T. R., de Haan, H. H., Williams, C. E. & Gluckman, P. D. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J. Clin. Invest.* **99**, 248–256 (1997).
31. Gunn, A. J., Parer, J. T., Mallard, E. C., Williams, C. E. & Gluckman, P. D. Cerebral histologic and electrocorticographic changes after asphyxia in fetal sheep. *Pediatr. Res.* **31**, 486–491 (1992).
32. de Haan, H. H., van Reempts, J. L., Vles, J. S., de Haan, J. & Hasaart, T. H. Effects of asphyxia on the fetal lamb brain. *Am. J. Obstet. Gynecol.* **169**, 1493–1501 (1993).
33. Ikeda, T. et al. Physiologic and histologic changes in near-term fetal lambs exposed to asphyxia by partial umbilical cord occlusion. *Am. J. Obstet. Gynecol.* **178**, 24–32 (1998).
34. de Haan, H. H., Gunn, A. J., Williams, C. E. & Gluckman, P. D. Brief repeated umbilical cord occlusions cause sustained cytotoxic cerebral edema and focal infarcts in near-term fetal lambs. *Pediatr. Res.* **41**, 96–104 (1997).
35. Okereafor, A. et al. Patterns of brain injury in neonates exposed to perinatal sentinel events. *Pediatrics* **121**, 906–914 (2008).
36. Drury, P. P. et al. Status epilepticus after prolonged umbilical cord occlusion is associated with greater neural injury in fetal sheep at term-equivalent. *PLoS ONE* **9**, e96530 (2014).
37. Mallard, E. C., Gunn, A. J., Williams, C. E., Johnston, B. M. & Gluckman, P. D. Transient umbilical cord occlusion causes hippocampal damage in the fetal sheep. *Am. J. Obstet. Gynecol.* **167**, 1423–1430 (1992).
38. Williams, C. E., Gunn, A. J., Mallard, C. & Gluckman, P. D. Outcome after ischemia in the developing sheep brain: an electroencephalographic and histological study. *Ann. Neurol.* **31**, 14–21 (1992).
39. Beilharz, E. J., Williams, C. E., Dragunow, M., Sirimanne, E. S. & Gluckman, P. D. Mechanisms of delayed cell death following hypoxic-ischemic injury in the immature rat: evidence for apoptosis during selective neuronal loss. *Mol. Brain Res.* **29**, 1–14 (1995).

40. Colbourne, F. & Corbett, D. Delayed and prolonged post-ischemic hypothermia is neuroprotective in the gerbil. *Brain Res.* **654**, 265–272 (1994).
41. Colbourne, F. & Corbett, D. Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. *J. Neurosci.* **15**, 7250–7260 (1995).
42. Colbourne, F., Corbett, D., Zhao, Z., Yang, J. & Buchan, A. M. Prolonged but delayed postischemic hypothermia: a long-term outcome study in the rat middle cerebral artery occlusion model. *J. Cereb. Blood Flow Metab.* **20**, 1702–1708 (2000).
43. Sabir, H., Scull-Brown, E., Liu, X. & Thoresen, M. Immediate hypothermia is not neuroprotective after severe hypoxia-ischemia and is deleterious when delayed by 12 h in neonatal rats. *Stroke* **43**, 3364–3370 (2012).
44. Bona, E., Hagberg, H., Loberg, E. M., Bagenholm, R. & Thoresen, M. Protective effects of moderate hypothermia after neonatal hypoxia- ischemia: short- and long-term outcome. *Pediatr. Res.* **43**, 738–745 (1998).
45. Colbourne, F., Sutherland, G. & Corbett, D. Postischemic hypothermia. A critical appraisal with implications for clinical treatment. *Mol. Neurobiol.* **14**, 171–201 (1997).
46. Wood, T. et al. Treatment temperature and insult severity influence the neuroprotective effects of therapeutic hypothermia. *Sci. Rep.* **6**, 23430 (2016).
47. Davidson, J. O. et al. How long is sufficient for optimal neuroprotection with cerebral cooling after ischemia in fetal sheep? *J. Cereb. Blood. Flow. Metab.* **38**, 1047–1059 (2018).
48. Osredkar, D. et al. Hypothermia is not neuroprotective after infection-sensitized neonatal hypoxic-ischemic brain injury. *Resuscitation* **85**, 567–572 (2014).
49. Mir, I. N. et al. Placental pathology is associated with severity of neonatal encephalopathy and adverse developmental outcomes following hypothermia. *Am. J. Obstet. Gynecol.* **213**, 849 e841–847 (2015).
50. Hakobyan, M. et al. Outcome of infants with therapeutic hypothermia after perinatal asphyxia and early-onset sepsis. *Neonatology* **115**, 127–133 (2018).
51. Murray, D. M. et al. The predictive value of early neurological examination in neonatal hypoxic-ischaemic encephalopathy and neurodevelopmental outcome at 24 months. *Dev. Med. Child Neurol.* **52**, e55–e59 (2010).
52. Shalak, L. F., Laptook, A. R., Velaphi, S. C. & Perlman, J. M. Amplitude-integrated electroencephalography coupled with an early neurologic examination enhances prediction of term infants at risk for persistent encephalopathy. *Pediatrics* **111**, 351–357 (2003).
53. Azzopardi, D. V. et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N. Engl. J. Med.* **361**, 1349–1358 (2009).
54. Gluckman, P. D. et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* **365**, 663–670 (2005).
55. Shankaran, S. et al. Evolution of encephalopathy during whole body hypothermia for neonatal hypoxic-ischemic encephalopathy. *J. Pediatr.* **160**, 567–572 e563 (2012).
56. Perez, J. M., Golombek, S. G. & Sola, A. Clinical hypoxic-ischemic encephalopathy score of the Iberoamerican Society of Neonatology (Siben): a new proposal for diagnosis and management. *Rev. Assoc. Med Bras. (1992)* **63**, 64–69 (2017).
57. Hossmann, K. A. Pathophysiology and therapy of experimental stroke. *Cell. Mol. Neurobiol.* **26**, 1057–1083 (2006).
58. Vesoulis, Z. A. et al. Re-examining the arterial cord blood gas pH screening criteria in neonatal encephalopathy. *Arch. Dis. Child. Fetal Neonatal Ed.* **103**, F377–F382 (2018).
59. Chakkarapani, E., Davis, J. & Thoresen, M. Therapeutic hypothermia delays the C-reactive protein response and suppresses white blood cell and platelet count in infants with neonatal encephalopathy. *Arch. Dis. Child. Fetal Neonatal Ed.* **99**, F458–F463 (2014).
60. Celik, I. H. et al. What are the cut-off levels for IL-6 and CRP in neonatal sepsis? *J. Clin. Lab. Anal.* **24**, 407–412 (2010).
61. Karlsson, M. et al. Lactate dehydrogenase predicts hypoxic ischaemic encephalopathy in newborn infants: a preliminary study. *Acta Paediatr.* **99**, 1139–1144 (2010).
62. Thoresen, M. et al. Lactate dehydrogenase in hypothermia-treated newborn infants with hypoxic-ischaemic encephalopathy. *Acta Paediatr.* **101**, 1038–1044 (2012).
63. Graham, E. M., Burd, I., Everett, A. D. & Northington, F. J. Blood biomarkers for evaluation of perinatal encephalopathy. *Front. Pharmacol.* **7**, 196 (2016).
64. Mir, I. N. & Chalak, L. F. Serum biomarkers to evaluate the integrity of the neurovascular unit. *Early Hum. Dev.* **90**, 707–711 (2014).
65. Hellstrom-Westas, L., Rosen, I. & Svenningsen, N. W. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. *Arch. Dis. Child. Fetal Neonatal Ed.* **72**, F34–F38 (1995).
66. al Naqeeb, N., Edwards, A. D., Cowan, F. M. & Azzopardi, D. Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. *Pediatrics* **103**, 1263–1271 (1999).
67. Thoresen, M., Hellstrom-Westas, L., Liu, X. & de Vries, L. S. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. *Pediatrics* **126**, e131–e139 (2010).
68. Skranes, J. H. et al. Amplitude-integrated electroencephalography improves the identification of infants with encephalopathy for therapeutic hypothermia and predicts neurodevelopmental outcomes at 2 years of age. *J. Pediatr.* **187**, 34–42 (2017).
69. Liu, X., Jary, S., Cowan, F. & Thoresen, M. Reduced infancy and childhood epilepsy following hypothermia-treated neonatal encephalopathy. *Epilepsia* **58**, 1902–1911 (2017).
70. Azzopardi, D. et al. Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. *Arch. Dis. Child. Fetal Neonatal Ed.* **94**, F260–F264 (2009).
71. Azzopardi, D. Predictive value of the amplitude integrated EEG in infants with hypoxic ischaemic encephalopathy: data from a randomised trial of therapeutic hypothermia. *Arch. Dis. Child. Fetal Neonatal Ed.* **99**, F80–F82 (2014).
72. Walsh, B. H. & Inder, T. E. MRI as a biomarker for mild neonatal encephalopathy. *Early Hum. Dev.* **120**, 75–79 (2018).
73. Thoresen, M. et al. Time is brain: starting therapeutic hypothermia within three hours after birth improves motor outcome in asphyxiated newborns. *Neonatology* **104**, 228–233 (2013).