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Unanswered questions regarding therapeutic hypothermia for neonates with neonatal encephalopathy

Hemmen Sabir,¹,² Sonia L. Bonifacio,³ Alistair J. Gunn,⁴ Marianne Thoresen,⁵,⁶ Lina F. Chalak⁷; on behalf of the Newborn Brain Society Guidelines and Publications Committee*.

¹Department of Neonatology and Pediatric Intensive Care, Children's Hospital University of Bonn, Bonn, Germany
²German Centre for Neurodegenerative Diseases (DZNE), Bonn, Germany
³Department of Pediatrics, Stanford University, Palo Alto, California, USA
⁴Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland, Auckland, New Zealand
⁵Division of Physiology, Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
⁶Neonatal Neuroscience, Translational Medicine, University of Bristol, Bristol, United Kingdom
⁷Department of Pediatrics, Division of Neonatal-Perinatal Medicine, University of Texas Southwestern Medical School, Dallas, TX, USA

* Newborn Brain Society, PO Box 200783, Roxbury Crossing, MA 02120. Tel: +1 (844) 541 8709. Email: publications@newbornbrainsociety.org
Hemmen Sabir MD (Corresponding Author)
University of Bonn, Department of Neonatology and Pediatric Intensive Care, Children’s Hospital, Venusberg Campus 1, 53127 Bonn, Germany.
Phone.: +49-151-58280586. Email: hemmen.sabir@ukbonn.de

Sonia Lomeli Bonifacio MD
Division of Neonatal and Developmental Medicine, Department of Pediatrics Stanford University School of Medicine
750 Welch Road, Suite 315, Palo Alto, California, 94304, United States of America
Phone: 650-497-8800. Email: soniab1@stanford.edu

Alistair J. Gunn, MBChB, PhD,
Departments of Physiology and Paediatrics, The University of Auckland, Auckland 1142, New Zealand.
Phone: +64 373 7599. Email: aj.gunn@auckland.ac.nz .

Marianne Thoresen MD PhD
Division of Physiology, Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway. Neonatal Neuroscience, Translational Medicine, University of Bristol, Bristol, United Kingdom
Phone: +4790885956. Email: marianne.thoresen@medisin.uio.no

Lina Chalak MD MSCS
Professor of pediatrics. University of Texas Southwestern Medical Center, Dallas, TX 75390-9063, USA.
Phone: 214-648-3753; 214-648-3903 E-mail: Lina.chalak@utsouthwestern.edu
Abstract

Therapeutic hypothermia (TH) is now well established to improve intact survival after neonatal encephalopathy (NE). However, many questions could not be addressed by the randomized controlled trials. Should late preterm newborns with NE be cooled? Is cooling beneficial for mild NE? Is the current therapeutic time window optimal, or could it be shortened or prolonged? Will either milder or deeper hypothermia be effective? Does infection/inflammation exposure in the perinatal period in combination with NE offer potentially beneficial preconditioning or might it obviate hypothermic neuroprotection? In the present review, we dissect the evidence, for whom, when and how can TH best be delivered, and highlight areas that need further research.

Key words: newborn, neonatal encephalopathy, hypoxia-ischemia, therapeutic hypothermia, infection
Introduction

A decade has passed since therapeutic hypothermia (TH) became the standard treatment for newborns (≥ 36 weeks of gestation) with moderate to severe neonatal encephalopathy (NE) of presumed hypoxic-ischemic (HI) origin [1, 2]. The neuroprotective benefits of TH after HI were first demonstrated in small and large animals [3]. Subsequent large randomized controlled trials (RCTs) confirmed that TH reduced the risk of death or severe disability in moderate to severe NE by about 15% [4]. Since then, the risk of adverse outcome despite treatment with standard TH protocols has fallen from about 45% in the original trials to about 29% in a more recent RCT [5]. This is mainly due to a reduction in mortality, from 25% in the original trials to 10% in the more recent RCT [5]. The mechanism of this fall is unclear, but it may reflect earlier initiation of TH now that it is routine care, gained knowledge of standardized neuroprotective care of cooled asphyxiated newborns, or recruitment of a higher proportion of infants with milder NE.

Despite this progress, clinicians treating newborns with NE are often faced with situations that cannot be answered from the RCTs of TH or current clinical guidelines [6]. Should late preterm newborns with NE be cooled? Is cooling beneficial for mild NE? Is the current therapeutic time window optimal, or could it be shortened or prolonged? Will either milder or deeper hypothermia be effective? Does infection/inflammation exposure in the perinatal period, in combination with NE represent potentially beneficial preconditioning or might it reduce the efficacy of hypothermic neuroprotection? In the present review we dissect the evidence, for whom, when and how can TH best be delivered.
1. Therapeutic Hypothermia for late preterm newborns

TH improves rates of normal survival when implemented within 6 hours of postnatal age and maintained for 72 hours in term and near-term infants ≥ 36 weeks with moderate to severe NE [4]. Its direct application to preterm infants cannot be assumed without further studies because of 1) potentially greater susceptibility to some of the adverse effects of hypothermia such as intracerebral hemorrhage in preterm infants, 2) the complex and not fully understood etiology of encephalopathy in preterm infants [7] and 3) the possible impact of differences in neurological maturity. Data about diagnosis, frequency, severity, and outcome of NE in infants 33-35 weeks of gestational age are sparse. Important knowledge gaps include how NE manifests clinically in this age group, and whether TH confers neuroprotection for this group. These gaps need to be further studied as these infants were not included in any of the large RCTs of TH to date.

Preclinical evidence

The Vannucci-rat model of unilateral hypoxic-ischemic brain injury [8] has been used by many researchers worldwide to study the effect of hypoxia-ischemia on the developing brain. The original model uses 7-day-old rat pups (P7). In this model, TH was shown to be neuroprotective [9], leading to further studies in large animal models and clinical RCTs. The P7 rat brain’s maturation is comparable to a late preterm human brain at 34-36 weeks of gestation [10, 11]. Therefore, the P7 rat model widely approved hypothermic neuroprotection is comparable to late preterm infants.

In preterm fetal sheep, whose brain development is comparable to term newborns, global HI causes a potent neuroinflammatory response, characterized by excessive microglial activation, from 3 hours until at least 21 days post-HI [12, 13]. This is
associated with acute loss of pre-oligodendrocytes (OL) at 72 hours after the insult, and restoration of pre-OL numbers at 7 days and 14 days post-reperfusion, in association with increased restorative proliferation of oligodendrocyte progenitors [14-16]. At the same time, there is impaired survival of myelinating OLS at these time points, suggesting maturational arrest contributing to hypomyelination [15-18]. Studies of TH in preterm fetal sheep support a similar pathophysiologic profile as at term equivalent. In preterm-equivalent fetal sheep, cerebral cooling for 72 hours (with extradural temperature titrated to 29.5 ± 2.6°C) started 90 min after severe asphyxia was associated with basal ganglia and hippocampal neuroprotection, protection of immature OLS in periventricular and parasagittal white matter, and reduced overall microgliosis and apoptosis [14, 19]. In association with this histological improvement, higher EEG frequencies recovered faster, cephalic blood flow was restored, and the amplitude of stereographic seizures was reduced [14]. Brain structures that are frequently injured in term and preterm infants with acute NE were protected. Interestingly, in preterm fetal sheep, neuroprotection using head cooling, started after a 90 minutes delay and being prolonged for 3 days after severe asphyxia, was associated with potent, specific suppression of epileptiform transients in the early recovery phase, whereas hypothermia did not affect numbers of subsequent seizures during the 3 day cooling phase in fetal sheep [14]. Thus, increased epileptiform transients in the latent phase may be a useful biomarker of evolving neuronal injury in the immature brain [20]. Similar results were seen with mild whole body hypothermia in the same paradigm [21]. Early hypothermia at 30 minutes after asphyxia, but not delayed hypothermia started at 5 hours, reduced neuronal loss and microglial induction in the striatum, with faster recovery of spectral edge frequency, reduced seizure burden and
improved recovery of EEG amplitude (p<0.05). These results confirm that starting hypothermia as soon as possible after HI is vital in these models.

Clinical evidence

Clinical studies of TH are in progress for late preterm infants with evidence of NE (NCT01793129). Historical evidence, from before modern intensive care, suggested that hypothermia is associated with increased mortality in preterm newborns. For example, in 1958 Silverman and colleagues reported increased mortality in newborns under 1000 grams who were kept in incubators with an environmental temperature of 28.7°C and mean axillary temperature of 31°C in the first 5 days of life [22]. This was confirmed in subsequent RCTs [23, 24]. In recent retrospective cohort studies of very low birthweight infants, lower admission temperatures continue to be associated with greater risk of late-onset sepsis and mortality; albeit the direction of causality cannot be determined [25]. These safety concerns must preclude immediate application of TH to all preterm newborns with NE, until we have evidence from robust, pragmatic RCTs.

Nevertheless, there has been some clinical therapeutic creep; in one study 2.4% of late preterm infants at 33-35 weeks received TH [26]. The RCTs of TH largely excluded preterm infants < 36 weeks gestation, although the ICE trial enrolled 5 infants between 35 and 36 weeks [27]. In practice, most studies to date have been retrospective and do not describe the neurologic criteria for treating late preterm infants with NE [28-31]. Rao et al. reported a retrospective cohort study of 31 preterm infants treated with TH, 34-35 weeks gestational age, who met ICE trial criteria compared to 32 term newborns with NE [31]. Preterm infants had increased mortality compared to term infants, although the increase in mortality was thought to be related to severity of brain and multi-organ injury. Preterm infants had a higher incidence of hyperglycemia compared to term infants and more frequently white
matter and cortical brain injury compared to term infants, who showed relatively more frequent deep gray matter injury. No differences were observed in adverse effects (e.g. bradycardia or coagulopathy) between preterm and term infants.

Encephalopathy secondary to HI in preterm infants is physiologically complex as it involves a combination of asphyxia and brain specific maturational disturbances. The true incidence of encephalopathy due to HI in preterm infants is unclear [32]. Schmidt and Walsh reported an incidence of 8 per 1000 live births in 32-36 week infants over six years based on a 5 minute Apgar score <5, associated with fetal acidosis and any neurologic abnormality [33, 34]. In 2012, Parkland Memorial (Dallas, TX, USA) reported an NE incidence of 5 per 1000 live births with NE at 33 to 35 weeks’ gestation [35]. At that large inborn NICU at Parkland Memorial (14,000 births/year) where cord blood samples are routinely obtained following preterm delivery, 2.9% of 33 to 35 week infants had significant perinatal acidosis, 27% of those with acidosis had moderate-severe encephalopathy [35] using modified Sarnat scoring as in the NICHD cooling trials for term newborns [36]. The overall incidence was 5.9 per 1000 live births in 32-35 weeks’ gestation, compared with a 1-2% incidence in infants born at term [35].

It is important to establish reliable diagnostic criteria for NE in late preterm infants to support trials to assess the efficacy of TH in this vulnerable group. Due to changes in neurologic maturation, it is not clear whether the selection criteria used to identify term infants with NE are appropriate for preterm infants and if the brain injury patterns of NE observed in term infants will also be seen in the developing preterm brain. In particular, it may be more difficult to diagnose NE in the late preterm because compared to term, normal preterm infants have less well developed primitive reflexes, and maturational differences in tone and posture [37]. While the modified Sarnat exam has been used extensively to evaluate term newborns at risk
for encephalopathy, its inter-rater reliability in preterm infants is not well established [37, 38]. Pavageau et al. prospectively evaluated 86 late preterm newborns to determine the reliability of the neurologic exam between study investigators and other neonatologists [39]. The inter-rater kappa score was good to excellent (k>0.72) in most categories except for the Moro reflex and tone, which showed only fair agreement. This is physiologically a reflection of the developmental flexor posture gradually evolving to the upper extremities around 36 weeks’ gestation [40]. Similarly, the Moro reflex may be incomplete until 37 weeks’ gestation, so proximal flexion of the upper extremities was limited in preterm infants. This study suggests that the criteria used for identification of hypothermia candidates in term infants are applicable to 33-35 weeks preterm infants, except for the Moro reflex. The next step will be to await results of the NICHD Neonatal Research Network (NRN) late preterm cooling study (NCT01793129). An important difference in eligibility criteria between this study and TH studies in term infants is the requirement for an abnormal level of consciousness on the qualifying exam in order to avoid confounding by factors related to prematurity as listed above. This trial is designed to assess the safety and effectiveness of whole body hypothermia for 72 hours in late preterm infants (33 0/7 to 35 6/7 weeks GA ≥ 1500 grams birth weight) who present at <6 h postnatal age with moderate to severe NE. Subjects were randomized to either whole body hypothermia (33 to 34°C) or standard care (normothermic control group with esophageal temperature at or near 37.0°C). The primary outcome is death or moderate to severe disability at 18-22 months corrected age. The trial enrolled 168 infants, who are now undergoing two year follow up (personal communication) and we hope it will answer conclusively whether TH benefits late preterm infants.
Clinical practice recommendation

- Current AAP recommendations are ≥35 0/7 weeks as lowest gestational age appropriate for cooling. Internationally, some countries (including most European countries) use ≥ 36 0/7 as the lowest age suitable for initiation of cooling.

Ongoing research and future directions

- The NICHD Neonatal Research Network (NRN) late preterm cooling study (NCT01793129) is designed to assess the safety and effectiveness of whole body hypothermia for 72 hours in late preterm infants 33 0/7 to 35 6/7 weeks GA (best obstetrical estimate) and ≥ 1500 grams birth weight who present at <6 h postnatal age with moderate to severe NE.

2. Therapeutic hypothermia for mild NE

The large RCTs of TH excluded infants who had “mild” NE in the first 6 hours of life in order to select patients at the highest risk of unfavorable outcome, and so the potential benefit of TH for these infants is unknown. There is increasing evidence from cohort studies that infants with mild NE in the first 6 hours of life are at risk of disability. A meta-analysis of studies with standardized neurodevelopmental assessment at 18 months or older suggested that 86/341 (25%) of infants with “mild” NE in the first 6 hours of life had an adverse outcome by 18 to 24 months of life [41].

Preclinical Evidence

Although there is no published evidence of the effectiveness of TH in mild HI from large animal models, in P7 rats, TH following less severe HI is associated with substantially better neuroprotection than after severe HI [42]. Using the same animal model, it has been shown that TH significantly reduces the frequency of mild brain
injury, as compared to normothermia treatment (Figure 1). Moreover, it is reasonable to consider that early studies of TH in adult rodents used relatively mild ischemia leading to selective hippocampal injury. For example, early initiation of cooling within 1 hour and continued for 12 to 24 hours after brief ischemia in adult rodents substantially reduced CA1 necrosis at 10 and 30 days, and improved learning in an open field and T-maze after 6 months recovery [43, 44]. Delaying the start of TH to 4 hours after ischemia attenuated neuroprotection [44]. However, even when the start of TH was delayed until 6 h after reperfusion, neuroprotection could be almost completely restored by extending the interval of moderate TH to 48 hours plus slow rewarming over 6 hours [45].

Clinical evidence

Newborns with mild NE are at risk of developing neurodevelopmental disabilities, but no effective treatment has been established. Although the risk of mortality is lower in mild NE compared to those with moderate or severe NE, there is evidence for resulting cognitive and motor impairments. For example, in a recent prospective cohort of neonates with mild NE who were not treated with TH (PRIME study), 16% had moderate-severe disability and 40% had Bayley-III cognitive, motor, or language composite scores more than one standard deviation below the mean at 2 years of age [46]. In another cohort, up to 35% of neonates with untreated mild NE had difficulty with cognition and behavior at school age [47]. Despite a lack of evidence that TH can improve outcomes after mild HI, there has been a marked therapeutic drift. Recent cohort studies report that 13-56% of TH treated patients do not meet RCT criteria for moderate-severe NE and are given a diagnosis of mild NE [48, 49]. Across a variety of data registries, up to 75% of newborns with ‘mild NE’ are being treated with TH despite a lack of evidence regarding safety or efficacy [50].
Furthermore, widely varying definitions of mild NE are applied, and so observational data are inadequate to evaluate safety or efficacy [51]. A major impediment to evaluating contemporary observational data is lack of a uniform definition of the constellation of findings that should be used to define mild versus moderate-severe NE. In 1976, Sarnat and Sarnat identified that following perinatal HI brain injury, the severity of encephalopathy on exam is a continuum, reflecting the evolution of neuropathologic changes in the brain over the first days of life [52]. Variations of the neurological examination as described in the original Sarnat exam have since been used to grade the severity of clinical encephalopathy in suspected NE. The major RCTs of TH only sought to identify neonates with moderate-severe NE, and did not establish specific criteria for mild NE. In smaller descriptive cohorts of neonates with ‘mild NE’ treated with TH, there is often no clear definition of ‘mild NE’ [53]. Finally, in reports from large clinical registries, severity of NE is frequently determined by the most abnormal level of consciousness over the first 7 days after birth, rather than describing the severity of NE in the hours after birth [48, 49].

A cohort study from Ireland defined the severity of NE using a combination of clinical exam and EEG background features in the first 24 hours of life [41]. The more recent PRIME study defined mild NE by similar physiologic and laboratory criteria as per neonatal research network (NRN) trials and required >1 abnormal feature of the modified Sarnat exam - whether mild, moderate or severe - as long as exam did not meet existing NRN requirements of at least 3 moderate or severe abnormal findings [46]. In the PRIME study, mild NE was defined based on examination in the first 6 hours of life in order to address decisions regarding initiation of treatment in a timely manner. All infants identified with abnormal outcome at two years had 2 or more abnormalities using the PRIME exam criteria before 6 hours of life.
TH is relatively safe and easy to implement for those with moderate-severe NE. Adverse effects of TH reported in the meta-analyses include sinus bradycardia and thrombocytopenia, which often do not require medical intervention [54]. However, it is unknown if the relatively favorable safety profile observed is also true for mild NE. Neonates with mild NE may be less systemically ill than those who have suffered a more severe insult. Thus, TH for mild NE is likely to lead to more medical interventions, including greater exposure to sedating/analgesic medications to provide comfort and reduce shivering during treatment, need for respiratory support, use of central lines, exposure to inotropes, need for medical transport to centers that perform TH, separation of the neonate from its mother for care in an ICU setting, delayed initiation of enteral feeds and establishment of breastfeeding, and longer hospital stay [55]. Accurate information regarding these adverse effects and drawbacks of TH for mild NE are crucial to fully understand the balance of risks to potential benefits. It is biologically plausible that TH will reduce brain injury to a milder insult. However, the degree of benefit is unknown at this time and so it is unclear if the benefit will outweigh the risks.

There are few data from RCTs and observational cohorts on use of TH for mild NE. Several of the RCTs of TH for moderate-severe NE inadvertently included neonates with mild NE. A recent meta-analysis, identified 91 infants with mild NE (45 treated, 46 control) who were included in 4 RCTs of TH [51]. Abnormal outcome occurred in 13/45 (29%) of treated and 17/47 (38%) of control patients, with an odds ratio of 0.67 (95% CI 0.28-1.61). Overall, based on available RCTs and recent cohort studies, approximately 25% of patients with mild NE had an abnormal outcome at 2 years of age.

In a retrospective single center study of 167 neonates referred for potential cooling between 2008 and 2012, 38% (63/167) had mild NE defined by modified Sarnat
score and amplitude-integrated electroencephalogram. Of these neonates with mild NE, 40% (20/50) of the not cooled ones vs. 31% (4/13) of the cooled ones developed brain injury. [56] In another retrospective cohort study of 89 neonates treated with TH at a single center between 2013 and 2015 [53], 48 (54%) were identified as having mild NE, defined as $\geq$ to 1 abnormality on modified Sarnat exam, but not meeting criteria for moderate-severe NE. None of the patients with mild NE died; 26 (54%) infants with mild NE had abnormalities on MRI, including 2 with mild basal ganglia/thalamic injury, 2 with punctate white matter lesions, 4 with a single focal infarction, and 18 with a watershed pattern of injury. The predominance of the watershed pattern of injury in infants with mild NE in this study raised the concern that the pathophysiology in these patients represents partial or repeated HI [57], and speculatively might not be as amenable to TH because of a longer period of evolution of injury before birth. Conversely, it is reasonable to consider that in preclinical studies cell death evolves more slowly after milder HI injury, increasing the potential for treatment with TH. No long-term follow-up information was available from these cohorts.

In a recent case-control study of 194 infants with NE (defined using Vermont Oxford Network definition) treated with TH between 2007 and 2014, Rao et al. described 30 (15%) neonates with mild NE who were cooled and compared to 30 control children [31]. MRI evidence of injury was present in 13/30 (43.3%). The watershed pattern of injury was most common, present in 5/13 (39%). The next most common was the basal ganglia/thalami pattern in 3/13 (23%), 1 infant had global injury, and 4 had non-specific white matter injury. Half of the neonates had follow-up data at an average age of 29 months. Only half of the infants with follow-up had brain injury on MRI. There was no difference in Bayley-III cognitive, motor, or language composite scores
between mild NE patients and controls, suggesting that TH may be beneficial in this population.

The latest published study including cooled infants with mild NE is the MARBLE study [58], which was carried out between 2013 and 2016 and included 37 cooled newborns with mild encephalopathy. The primary objective was to correlate MR biomarkers with outcomes at 18-24 months. Of the 37 infants with mild NE, 1 had basal ganglia or thalamic injury, 20 had white matter lesions and 4 had cortical lesions. Long term outcome was available in 31 infants, of whom only one infant had cerebral palsy. Mean Bayley-III composite sores were 105 for cognition, 98 for language and 100 for motor outcome. Together these data support the hypothesis that TH may result in improved outcomes in neonates with mild NE, though this remains to be formally proven.

**Clinical practice recommendation**

- There is insufficient systematic evidence that TH will result in improved neurodevelopmental outcome, nor is there a clear picture of risks and drawbacks of TH for newborns with mild NE.
- The American Academy of Pediatrics Committee on the Fetus and Newborn has started to revise its 2014 recommendations that TH be used only for those infants with moderate-severe NE, but may be beneficial for those infants with mild NE.

**Ongoing research and future directions**

- The COMET study (NCT03409770) is enrolling patients with mild NE randomized to control or TH for 48 hours or 72 hours. The PRIME definition is used for enrolling <6 hours and the primary outcome is MRS measures of N-acetyl aspartate in the thalami.
• The TIME Study (NCT04176471), a RCT of TH for mild NE in California, has very similar entry criteria to the MARBLE and COMET studies. The primary aims of the TIME study are to determine feasibility, safety and short-term neurodevelopmental outcomes at 1 year of age.

• The COOL PRIME study is starting in 2021. The design is a comparative effectiveness non-randomized cooling therapy vs. normothermia as per standard of care at each center in 500 infants from 12 centers using the PRIME inclusion criteria. (Chalak, personal communications). The primary outcome is the 2-year Bayley outcome. Secondary outcomes include MRI/MRS, hospital stay, parental stress scores and a standardized clinical examination (Hammersmith neonatal neurological examination) trajectory changes.

3. The window of opportunity for cooling

Preclinical evidence – what is the optimal window of opportunity?

There is considerable preclinical evidence that TH must be started as early as possible within the latent phase for optimal benefit. This is highly consistent with evidence that cerebral oxidative metabolism fails progressively during the latent phase after moderate to severe HI in piglets [59], fetal sheep [14] and human infants [60, 61]. In near-term fetal sheep, partial neuroprotection was seen when the start of prolonged hypothermia was delayed until 5.5 hours after cerebral ischemia [62], with loss of electrophysiological and histological protection if hypothermia was delayed until 8.5 hours after reperfusion [63]. Similarly, in unanesthetized P21 rats, at the age of weaning, a mild 2-3°C, reduction in brain temperature for 72 hours soon after HI dramatically reduced cortical
infarction, whereas cooling delayed until 6 hours after the insult had an intermediate, non-significant effect [64]. In P7 rat pups, whereas hypothermia was not protective after prolonged HI for 150 min, immediate induction of hypothermia after a “moderate” (90 minutes) period of HI markedly reduced the area of cortical infarction [42]. Strikingly, in that study there was significant protection even when hypothermia was delayed until 3 hours after HI. This illustrates that the window of opportunity for TH may be dependent on the severity of initial HI, with a longer window for treatment possible after less severe insults.

Preclinical - What is the optimal depth of cooling?

In P7 rat pups, there is a u-shaped response curve for cooling after HI. The optimal temperature was 32-33.5°C compared with 37°C in controls. Deeper hypothermia (rectal temperatures of 18 to 30°C) was associated with attenuation of neuroprotection [65] and conversely pyrexia exacerbated brain injury [66]. This profile is highly consistent with the finding in piglets that neuronal loss was reduced by cooling by 3.5°C to 5°C but increased with cooling by 8.5°C [67].

Preclinical - What is the optimal duration of cooling?

The original RCTs of TH continued cooling for 72 hours, based on relatively limited animal evidence [3] that cooling started after 5.5 h, and continued until 72 h was still partially protective [62]. Subsequent studies have now shown in near-term fetal sheep that although cooling from 3 hours after ischemia until 48 hours is partially protective, it was substantially less effective for both recovery of EEG power and neuronal survival than cooling for 72 hours [68]. Conversely, in the same paradigm in near-term equivalent fetal sheep, when delayed hypothermia starting 3 hours after ischemia was prolonged from 3 to 5 days, there was no further improvement in electrophysiological recovery or neuronal survival or reduced induction of cortical microglia [69].
Clinical evidence

Since the International Liaison Committee on Resuscitation (ILCOR) recommended in 2010 that infants with moderate to severe NE should be treated with 72h of TH starting within 6h of birth [1], no new evidence has arisen to change this guidance. The important study of longer (3 vs 5 days) or deeper (33.5 vs 32°C) cooling was terminated early when interim analysis showed an apparent increase in early mortality and no improvement in survival without disability after either cooling longer or deeper alone or in combination [70]. There has been no RCT of the effect of starting cooling earlier than 6 hours of age, although there is some evidence from small cohort studies. For example in a small study of 76 infants recruited using the CoolCap/TOBY protocol, infants in whom hypothermia was started before 3 hours of age showed better psychomotor developmental index scores (Bayley Scales of Infant Development II) at 18 months than those who were cooled between 3 and 6 h; however, there was no association of timing of treatment on mental development index outcomes [71] (Figure 2).

Interestingly, recent cohort studies of TH report favorable outcomes of 71-75% % in infants treated with NE, including mortality of 10% [70, 72]. This mortality rate is significantly lower, as the 25% mortality described in the large RCTs [54]. It is likely that these improving outcomes in current practice in part reflect the cooling of a higher proportion of infants with less severe NE and earlier initiation of TH now that it is routine practice. Encouragingly, there is some evidence that the distribution of the severity of cerebral palsy (CP) has changed since the introduction of TH. By contrast with historical cohorts, in a population-based cohort in the UK, 12/18 children who developed CP after TH were independently ambulant by 24 months [73]. In this population (2.6 million), all regional hospitals and ambulances have servo-controlled cooling-devices and passive or active TH are started early at ≤1h [74]. Thus, it is
possible that the apparent reduction in CP by 24 months might be related to the initiation of early cooling within 1 hour of age.

Conversely, a recent randomized controlled trial of TH compared 85 infants receiving standard, normothermic (NT) care, with 83 infants who started TH more than 6 hours but less 24 hours after birth [75]. TH was started at a median of 16 hours after birth and continued for 96 hours. The rate of adverse outcomes in the TH and NT groups were similar, 24.4% vs 27.9%, with similar mortality of 11 vs 10%. There were no adverse effects of cooling when started within the first 24 hours.

Clinical practice recommendation

- The available data emphasizes the importance of focusing on improving early initiation of TH (Figure 2).
- There is no benefit from deeper or longer duration of cooling so it is important to follow the published protocols for TH by maintaining $T_{rec/es}$ at 33.5°C for 72 hours.
- There is no evidence that late cooling initiated more than 6 to 24 h after birth is beneficial.

4. Cooling in the context of Perinatal Infection

Perinatal infection and NE

The etiology of NE is multifactorial with different factors predisposing to the onset of NE, either alone or in combination, including periconceptional risk factors, maternal comorbidities, placental pathologies, neonatal stroke, metabolic disorders, genetic or epigenetic abnormalities, HI and perinatal infection [76]. Perinatal infection has been shown to be an independent risk factor of adverse neurological outcome in near-term newborns [77] and is suspected to alter the neuroprotective effect of TH in term, or
near-term newborns with NE [78]. Clinical manifestation of perinatal infection is often non-specific and overlaps with symptoms of non-infectious inflammation of different origin. Perinatal infection may lead to chorioamnionitis or funisitis, which not automatically leads to brain injury. As shown in newborn rodents, an inflammatory stimulus before hypoxia-ischemia can exacerbate HI brain injury [79, 80]. However, the time interval between the inflammatory stimulus and the onset of HI is crucial. Administration of a non-infectious inflammatory stimulus up to 6 hours before HI exacerbated injury [79] and produced short-term memory impairment [81], whereas a 24-h interval was protective [82].

Rao at al. reported that most newborns with NE (~ 98%) are automatically treated with antibiotics, as presumed perinatal infection might have led to NE [83]. However, the use of prophylactic antibiotic treatment is also controversial. It has been shown that antenatal prophylactic antibiotic treatment for inhibiting preterm labor with any macrolide or beta-lactam antibiotic was associated with increased risk for neonatal death and cerebral palsy [84]. Finally, changes in the neonatal microbiome secondary to antibiotics use might impact brain development [85].

Preclinical evidence- Effectiveness of cooling in the context of infection-sensitized NE

The question, whether TH is as beneficial in the context of infection-associated NE as it is with HI-associated NE, has not been fully answered yet. In P7 rodents, after infection-sensitized HI [80, 86, 87], TH following HI was not beneficial [80]. This has also been reported in a large animal model of infection-sensitized HI in newborn pigs [88]. However, the beneficial effect of TH in the setting of inflammation-sensitization and HI may be pathogen-dependent [2]. The preclinical models offer the possibility to establish clinical relevant biomarkers that might distinguish between infection-sensitized and non-infection-sensitized NE in the near future.
Clinical evidence - cooling in the context of infection-sensitized NE

After successful implementation of TH as standard neuroprotective treatment following perinatal asphyxia in industrialized countries, the transfer to low- and middle-income countries was not as successful. In 2007 Robertson et al. introduced TH in Uganda [89]. The authors found that it was feasible to cool newborns with NE in a low-resource setting. However, mortality tended to be greater in cooled newborns compared to non-cooled newborns. It is important to note that treatment was markedly delayed, and that infants were consistently hypothermic before randomization; in many cases infants needed to be rewarmed to achieve target temperatures of TH. This study has delayed further clinical implementation of TH in low- and middle-income countries, as it identified the need to better understand the differences in the etiology of newborns presenting with NE in low- and middle-income countries. Tann et al. have shown that perinatal infection and inflammation are independent risk factors for NE in low-resource settings [90]. Hakobyan et al. found that in a cohort of 1084 cooled asphyxiated newborns in the Netherlands and Belgium, 42 newborns were diagnosed with proven or probable early-onset sepsis [91]. Of these 42 newborns, 9 died and 5 showed impairments on follow-up. This was not different to infants without proven early-onset sepsis. Unfortunately, there are no large RCTs to resolve whether TH is beneficial in infants exposed to perinatal infection. More recently, TH was successfully implemented in neonatal units in India in the context of the HELIX trial [92]. The authors showed that cooling was feasible, but they caution about the lack of hypothermic neuroprotection and question the earlier onset of brain injury in low-resource settings.

Clinical practice recommendations

- Normothermia and avoidance of hyperthermia should remain for now the standard of care in low-resource settings.
Ongoing research and future directions

- Further studies are needed in low-resource settings before cooling should be routinely offered in these settings.

Conclusion

Despite the successful translation of TH to treat neonates with NE, its routine clinical use over the last decade has raised many new questions. Some of these still need to be answered in pre-clinical studies; others should be addressed in well-powered RCTs. Overall, we suggest that cooling is highly likely to be beneficial in late preterm infants with NE and term or near-term infants with mild NE. There is no evidence that late cooling initiated more than 6 to 24 h after birth is beneficial. Whether cooling is effective in inflammation-sensitized NE remains unclear and may be pathogen dependent. As many researchers worldwide are completing clinical studies, we will gain further knowledge in the coming years that will lead to better survival and clinical outcomes in infants with NE.

Practice Points

- Cool to a central body temperature of 33.5°C for 72 hours as soon as possible in term and near-term neonates with moderate and severe NE.

- TH for term and near-term newborns with mild NE remains debated with insufficient systematic evidence proving improved neurodevelopmental outcome with treatment, and without a clear picture of risks and drawbacks.

- There is evidence of no benefit from deeper or longer duration of cooling in term and near-term neonates with moderate and severe NE.
There is no evidence that late cooling initiated more than 6 to 24 h after birth is beneficial in term and near-term neonates with moderate and severe NE.

Normothermia and avoidance of hyperthermia should be the standard of care in low-resource settings.

Research Directions

- The NICHD Neonatal Research Network (NRN) late preterm cooling study (NCT01793129) is designed to assess the safety and effectiveness of whole body hypothermia started <6 h postnatal age for 72 hours in late preterm infants with moderate to severe NE at 33 0/7 to 35 6/7 weeks GA and <= 1500 grams birth weight.

- The COMET study (NCT03409770) is enrolling patients with mild NE randomized to control or TH within < 6 h for 48 hours or 72 hours. The primary outcome is MRS measured N-acetyl aspartate in the thalami.

- The TIME Study (NCT04176471), a RCT of TH for Mild NE in California seeks to determine feasibility, safety and short-term neurodevelopmental outcomes at 1 year of age.

- The COOL PRIME (NCT04621279) will start in 2021. The design is a comparative effectiveness non-randomized cooling therapy vs. normothermia as per standard of care at each center in 500 infants from 12 centers using the PRIME inclusion criteria [46]. (Chalak, personal communication), with 2-year follow-up.

- Further studies are needed in low-resource settings before cooling should be routinely offered in such settings.
Figures

**Figure 1: Beneficial effect of cooling following experimental mild hypoxic-ischemic brain injury.** Graph representing the cumulative hemispheric area loss one week after hypoxic-ischemic brain injury in two groups of P7 pups. 50% of pups subjected to normothermia (NT) had less than <10% area loss. 75% of pups subjected to hypothermia (TH) had <10% area loss (unpublished data, M Thoresen and JK Gundersen).

**Figure 2: Effect of delayed cooling on long-term motor outcome.** There was a step-wise reduction of median motor outcome scores in newborns with NE treated with TH dependent on initiation of cooling. Immediate cooling (initiated within the first 3 hours after birth) led to best motor outcome with 20-24 months [71]. PDI = psychomotor developmental index.

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References


hypomyelination but does not reduce seizure burden in preterm sheep exposed to global hypoxia-ischemia. Exp Neurol. 2013;250:293-303.


