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Neonatal encephalopathy: Update on therapeutic hypothermia and other novel therapeutics

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Abstract

Neonatal encephalopathy (NE) is a major cause of neonatal mortality and morbidity worldwide. Multiple randomized controlled trials (RCTs) have investigated induced hypothermia for newborns with NE, which has led to therapeutic hypothermia (TH) becoming a standard treatment for newborns 36 weeks of gestation with NE related to intrapartum hypoxic events. In term and late preterm infants with moderate to severe encephalopathy, TH improves survival and neurodevelopmental outcomes at 18 months of age. Available long-term follow up data of these neonates from the RCT cooling trials through middle childhood has demonstrated that TH can increase survival without increasing major disability, rates of an IQ score < 70, or cerebral palsy (CP) in surviving children. However, surviving neonates with severe NE remain at significant risk of death or severe neurodevelopmental impairment despite being cooled, emphasizing the need for additional adjunctive treatment strategies. This review discusses the evidence supporting TH for term or near term neonates with NE, including findings of recent long-term outcome studies, as well as novel adjunctive therapies to augment neurodevelopmental outcomes for neonates with NE who receive TH.

Keywords

dexmedetomidine; erythropoietin; melatonin; N-acetylcysteine; neurodevelopment; stem cells; umbilical cord milking; xenon

Introduction

Intrapartum hypoxic events are a major cause of neonatal mortality responsible for approximately one in five of all neonatal deaths worldwide, causing an estimated 717,000 deaths in 2010.¹ Intrapartum-related hypoxic events (“birth asphyxia”) may result in neonatal encephalopathy (NE), defined as a disturbance of neurological function evident in the first days after birth in a newborn, characterized by a subnormal level of consciousness,

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depressed tone and reflexes, with or without seizures and often with impaired respiration and feeding abilities (both of presumed central origin).² NE is characterized as mild, moderate, or severe based on the Sarnat scoring system.³ Neonates with moderate to severe NE who survive are at risk for motor disabilities and long-term neurodevelopmental impairments (NDI), including cognitive, neuropsychological, educational, and behavioral problems.^{4, 5} While preterm infants are at even higher risk of NE than term infants, this review will be restricted to discussion of term and near term infants.

Throughout this article, we will use the terms *NE* and *hypoxic ischemic encephalopathy* (HIE) interchangeably in order to describe the published studies discussed in this review. While debate continues over which of these terms to use,^{6, 7} HIE specifically refers to encephalopathy associated with intrapartum injury from hypoxia and ischemia, mechanisms that can be difficult to prove, whereas *NE* is a broader term denoting a syndrome of neurologic disturbance due to an intrapartum hypoxic insult or other causes.²

Considerable research has been conducted in the past decade on NE as demonstrated by the co-word analysis study by Haung et al., which identified 1892 scientific studies (1568 articles and 324 reviews) that included the co-occurrence of keywords related to HIE published between January 2005 to December 2014 in the Web of Science database.⁸ Multiple randomized controlled trials (RCTs) have investigated induced hypothermia for newborns with NE, which has led to therapeutic hypothermia (TH) becoming a standard treatment for newborns 36 weeks of gestation with NE related to intrapartum hypoxic events.^{9, 10} While TH appears to be effective at improving outcomes, neonates with severe NE remain at significant risk of death or severe NDI despite being cooled, emphasizing the urgent need for additional adjunctive treatment strategies.

This review discusses the evidence supporting TH for term or near term neonates with NE, including findings of recent long-term outcome studies. Clinical strategies and novel adjunctive therapies to augment neurodevelopmental outcomes for neonates with NE who receive TH are also discussed.

Clinical trials on the benefits of therapeutic hypothermia for NE

Numerous RCTs have investigated the benefit of TH for improving outcomes of newborns with NE.¹¹⁻²¹ TH methods for NE include whole-body and selective head cooling, with both methods demonstrating similar effects regarding long-term neurologic outcomes based on meta-analysis.²²

A recent Cochrane systematic meta-analysis review by Jacobs et al. included 11 RCTs, comprising 1505 term and late preterm infants with moderate to severe encephalopathy and evidence of intrapartum asphyxia. This Cochrane review demonstrated TH resulted in improvement of the primary outcome measure of less death and better neurodevelopmental outcomes for survivors.²³ Findings from 8 of the 11 studies (1344 infants) demonstrated TH decreased the combined outcome of mortality or major neurodevelopmental disability to 18 months of age (46%; 312/678 vs. 61%; 409/666 in controls) (typical RR 0.75, 95% CI 0.68 to 0.83; typical RD -0.15, 95% CI -0.20 to -0.10).¹¹⁻¹⁸ The number needed to treat (NNT) to benefit 1 newborn is 7 (95% CI 5 to 10).

Secondary outcomes of the Cochrane review included mortality, major neurodevelopmental disability, adverse effects of cooling, and additional indicators of neurodevelopmental outcome (e.g. severity of EEG abnormality, seizures, MRI findings). Eleven studies (1468 infants) supported a decreased mortality with TH (25%; 186/736 vs. 34%; 250/732 in controls) (typical RR 0.75, 95% CI 0.64 to 0.88; typical RD -0.09, 95% CI -0.13 to -0.04), for a NNT of 11 (95% CI 8 to 25).¹¹⁻²¹ Findings from 8 studies (917 infants) demonstrated that TH decreases neurodevelopmental disability in infants who survived (26%; 130/495 vs. 39%; 166/422 in controls) (typical RR 0.77, 95% CI 0.63 to 0.94; typical RD -0.13, 95% CI -0.19 to -0.07), for a NNT of 8 (95% CI 5 to 14).¹¹⁻¹⁸ Based on the available RCT studies, TH appears to increase survival without increasing major disability in survivors.

Meta-analysis of adverse effects of cooling

Significant adverse effects that can occur as a result of TH include sinus bradycardia (heart rate <80 beats/minute), which occurred in 5% (62/1292) of infants (RR 11.59, 95%CI 4.94 to 27.17; RD 0.09, 95% CI 0.07 to 0.11), for a NNT of 11,^{11-16, 18, 21} and thrombocytopenia (platelet counts < 150 × 10⁹/L), which occurred in 31% (438/1392) of infants (RR 1.21, 95% CI 1.05 to 1.40; RD 0.06, 95% CI 0.02 to 0.10), for a NNT of 17 (95% CI 10 to 50).¹¹⁻¹⁸ Based on the Cochrane meta-analysis review, TH was not associated with a significant increase in major cardiac arrhythmia, hypotension (MAP < 40 mmHg), or need for blood pressure support with inotropic agents.

One challenge for neonatal care providers assessing newborns with suspected intrapartum hypoxic events is that not all babies with brain injury will be symptomatic after birth. Some newborns who may appear depressed at birth seem to adequately recover, but then become symptomatic, manifesting seizure activity after the six-hour post-birth window when TH is typically offered. Recognizing and treating these at-risk neonates remains a challenge and will require further research to establish reliable biomarkers to accurately identify these neonates in a timely manner.

Long-term outcomes

There are a limited number of long-term follow-up studies assessing the sustained benefits of TH on reducing death and cognitive or motor disabilities later in childhood. At the time of this review, long-term outcome data were available on 379 of the 1505 infants (25.2%) included in the Cochrane meta-analysis review.²³⁻²⁶ Shankaran et al. reported long-term outcome data on 190 of 208 (91.3%) child participants in the US National Institute of Child Health and Human Development (NICHD) study¹³ who were subsequently assessed at the ages of 6 to 7 years.²⁴ The primary outcome of death or an IQ < 70, which had been significant at 18 to 22 months of age, was lower in the TH group (46 of 97 children; 47%) compared to the control group (58 of 93 children; 62%), but no longer significant (RR in TH group, 0.78; 95% CI, 0.61 to 1.01). Death remained significantly lower in the TH group (28% vs. 44% of controls; P = 0.04) as did death or severe disability (41% vs. 60% of controls; P = 0.03). There was no significant difference in moderate or severe disability (35% of TH group vs. 38% of controls, P = 0.87) or CP rates (17% of TH group vs. 29% of controls, P = 0.14). Based on this study, it appears TH increases survival without increasing major disability, rates of an IQ score < 70, or CP in surviving children.

Another follow-up study, which assessed children at the ages of 6 to 7 years, was published by Azzopardi et al. who reported data from 127 of 325 (39%) newborn infants originally enrolled in the European Total Body Hypothermia for Neonatal Encephalopathy (TOBY) RCT trial.²⁵ At 18 months, children in the TOBY trial who had received TH had reduced risks of CP and better Mental Developmental Index and Psychomotor Developmental Index scores based on Bayley Scales of Infant Development II.¹⁴ At 6 to 7 years of age children who had received TH (75 of 145; 52%) had a significantly higher frequency of survival with an IQ score of 85 or higher, compared to children not treated with TH (52 of 132; 39%), which was the primary study outcome (RR 1.31; $P = 0.04$).²⁵ The percent of children who died in the TH group (29%) and the control group (30%) were similar. However, in children who survived, normal neurologic outcomes were more common in the TH group (65 of 145, 45%) compared to the control group (37 of 132, 28%); RR 1.60 (95% confidence interval, 1.15 to 2.22). The risk for CP was significantly lower in surviving children from the TH group (21%) compared to the control group (36%). Children from the TH groups also had less moderate or severe disability (22%) than the control group (37%).

Similar to the TOBY trial, follow-up of children from the Cool Cap trial demonstrated that 18-month neurodevelopmental assessments are predictive of long-term functional outcomes. Guillet et al. reported outcomes on 62 (32 cooled; 30 controls) of 135 (46%) surviving children enrolled in the Cool Cap trial who were reassessed at 7 to 8 years of age.²⁶ Using a WeeFIM instrument to qualitatively measure self-care, mobility, and cognitive function, this follow-up analysis demonstrated that disability status at 18 months was strongly associated with WeeFIM ratings at 7–8 y of age ($P < 0.001$) supporting a sustained treatment effect of TH for NE. However, this follow-up study had insufficient power to determine whether treatment with TH affected long-term outcome.

Cooling duration and depth

The standard of care for full-term neonates with moderate or severe NE is to initiate TH within 6 hours of birth for a 72 hour duration to a depth of 33.0°C to 34.0°C.²⁷ However, not all neonates with moderate, and most with severe NE do not benefit from TH. Since animal studies suggest that extending TH beyond 72 hours and below 33.5°C may provide increased neuroprotection,²⁸ a trial was conducted in human neonates to determine whether TH for a longer duration and at a greater depth might show further benefit.

The NICHD trial, by Shankaran et al., studied the effects of longer and deeper cooling in full-term neonates with moderate or severe HIE.²⁹ The trial was stopped early, due to safety and futility concerns, with 364 neonates enrolled (of 726 planned). Analysis of the predefined secondary outcomes, safety and NICU deaths, was done by marginal comparisons of 72 hours vs. 120 hours duration and 33.5°C depth vs. 32.0°C depth (predefined secondary outcomes). Both longer and deeper cooling were associated with increased mortality. Death rates increased from 7% (7 of 95 neonates) to 16% (15 of 96 neonates) when cooling at 33.5°C was extended from 72 to 120 hours. Similarly, death rates increased from 14% (13 of 90 neonates) to 17% (14 of 83 neonates) when cooling at 32.0°C was extended from 72 hours to 120 hours. The adjusted RR for NICU deaths for the 120 hour group vs. 72 hour group was 1.37 (95%CI, 0.92-2.04) and for the 32.0°C group vs

33.5°C group was 1.24 (95%CI, 0.69- 2.25). Future analyses will determine the primary outcome of death or disability at 18 to 22 months. Although this trial was stopped early, available results suggest that more is not necessarily better regarding the effect of duration and depth of TH on survival.

Cooling newborns who do not meet standard cooling criteria

Current clinical standards for TH were derived from RCTs that used strict study entry criteria. In order to practice evidence-based medicine, most centers offering TH for neonates with NE adhere to treatment protocols based on similar criteria developed by published RCTs.⁹ However, like many practices in medicine, therapeutic drift has occurred such that patients not quite meeting entry criteria for cooling are still treated, or cooling protocols are not strictly followed. For example, in the TOBY trial, in 2.2% (29/1331) of infants, cooling was initiated >12 postnatal hours, and 65% of subjects (887/1368) were cooled to below 33°C with 4% (61/1368) reaching temperatures below at 31°C at some point. Allowing initiation of TH up to 12 hours after birth for neonates with suspected NE has been advocated given the potential for benefit, the low risk of TH, and limited alternative treatment options.³⁰

An observational study by Smit et al. analyzed prospective data on 165 infants collected over a 6-year period in a regional cooling center (St Michael's Hospital, Bristol, UK) to compare complications and outcomes between infants who were cooled despite not fulfilling the standard inclusion and exclusion criteria as set out in the CoolCap/TOBY protocol (n = 36, 21%) and infants who fulfilled the standard entry criteria (n = 129).³¹ Cooled infants not meeting standard entry criteria included infants cooled >6 postnatal hours (n=11), late preterm infants (n=6), and infants with postnatal collapse (n=10), major cranial hemorrhage (n=5), congenital cardiac disease (n=2), and surgical conditions (n=2). In the 11 infants cooled > 6 postnatal hours, cooling was started at a median age of 7.8 h (IQR 6.8–8.5 h), and target temperature was reached at a median time of 10.5 h (IQR 10–15 h). The mean gestation age of the 6 late preterm that received TH was 34⁺⁶ weeks (range 34⁺⁰ to 35⁺⁴). In the infants not fulfilling, compared to those fulfilling the cooling entry criteria, 11% (4 of 36) versus 16% (20 of 129) died and 33% (9 of 27 assessed) versus 29% (22 of 76 assessed) had a MDI and/or PDI score <70. A combined poor outcome of death or disability at 18 months occurred in 44% of infants cooled not fulfilling the entry criteria compared 45% who did meet entry criteria. All 5 infants with major cranial hemorrhage had coagulation abnormalities and 80% (4 of 5) died or had disability.

While the study by Smit et al. provides insight into patients not fulfilling the standard TH treatment criteria, this small study was observational, which precludes the ability to make any strong conclusions about patients that deviate from current evidence-based practices. Based on the multiple available RCTs, TH (33.5°C to 34.5°C) for 72 hours appears effective and safe when used to treat newborns 36 weeks gestational with moderate to severe NE. It is not advisable to deviate from this proven protocol as such deviations may result in increased adverse outcomes, as shown by Shankaran et al. investigating longer duration and deeper TH.²⁹

When caring for critically ill infants with suspected brain injury, the practitioner is often placed in a time-sensitive conundrum of trying to decide who to offer TH to and who not to treat. In these scenarios, the desire to do good may lead to harm, emphasizing the importance of understanding infrequent, but serious risks that may exist by treating newborns outside of standard TH guidelines, risks that typically require well-designed, properly powered studies to be revealed.

Adjunctive neuroprotective treatments plus therapeutic hypothermia

Erythropoietin (Epo)—Epo, a glycoprotein originally identified for its role in erythropoiesis, has remarkable neuroprotective and reparative effects in the central nervous system.³²⁻³⁷ Epo functions by binding to its homodimeric cell surface receptor (EpoR). EpoRs are expressed by multiple cell types in the central nervous system,^{38, 39} including neuronal progenitor cells,³⁵ subsets of mature neurons,⁴⁰ astrocytes,⁴¹ oligodendrocytes,⁴¹⁻⁴³ microglia⁴⁴ and endothelial cells.³⁵ Epo is expressed in brain, primarily by astrocytes. In the setting of hypoxia-ischemia, EpoR expression is rapidly upregulated, with Epo production increasing only if significant hypoxia is prolonged. If Epo is available to bind to the upregulated receptor, cell survival is promoted, but in the absence of Epo, the pathway of programmed cell death predominates.^{80,87} This creates an important rationale for exogenous Epo administration, given that upregulation of Epo may take several hours, whereas brain injury can occur after brief but catastrophic insults which are insufficient to stimulate an increase in endogenous Epo synthesis.⁸⁸

Pre-clinical studies of Epo neuroprotection following hypoxic-ischemic brain injury show robust histologic and functional evidence for benefit.^{43, 45-54} Epo has both early and late beneficial effects. Early benefits include anti-apoptotic and anti-inflammatory effects,⁵⁵⁻⁵⁹ while later effects include increased neurogenesis, oligodendrogenesis, and angiogenesis, all of which contribute to tissue remodeling after hypoxia-ischemia.⁶⁰⁻⁶⁴ In nonhuman primates, Epo reduces the combined outcome of death or CP, and improves neurologic function in animals undergoing TH for HIE.⁶⁵ While insufficiently powered to prove efficacy, phase I and II clinical trials of Epo neuroprotection raise no safety concerns, and suggest that infants with HIE treated with multiple doses of Epo during the first week of life have better neurologic outcomes.^{66, 67} The High-Dose Erythropoietin for Neuroprotection in Neonatal Encephalopathy (NEAT) and Neonatal Erythropoietin And Therapeutic Outcomes in Newborn Brain Injury (NEATO) trials (<https://clinicaltrials.gov> NCT00719407 and NCT 01913340) have demonstrated safety, feasibility,⁶⁸ and beneficial outcomes as measured by early MRI, biomarkers, 6 month, one and 2 year outcomes (even among infants with significant brain injury seen on MRI).⁶⁹(Wu, in press, Pediatrics) Epo is commercially available and safe in infants. Several phase III trials are now in the planning or early stages of execution internationally (PAEAN, HEAL).

Xenon—Xenon, a noble gas that crosses the placenta and the blood brain barrier, binds to N-methyl-D-aspartate glutamate receptors to inhibit function, thus decreasing neuronal apoptosis.^{70, 71} Significant benefit was demonstrated in preclinical studies of HIE,⁷² so a multicenter Total Body hypothermia plus Xenon (TOBY-Xe) trial was conducted in the UK. Ninety two infants (36–43 weeks) were enrolled, 46 of whom were randomly assigned to

cooling only and 46 to xenon plus cooling.⁷³ The primary outcomes were assessment of reduced thalamic lactate to N-acetyl aspartate (NAA) ratios measured with magnetic resonance spectroscopy and preserved fractional anisotropy in the posterior limb of the internal capsule determined by MRI within 15 days of birth. Lactate to NAA ratios have been demonstrated to be good predictive imaging biomarker of neurodevelopmental outcomes.⁷⁴ Changes in fractional anisotropy, a measure of brain connectivity derived from the diffusion tensor imaging that assesses the degree of regional anisotropic diffusion, correlate well with subsequent outcomes in neonates with HIE.⁷⁵ The TOBY-Xe trial was underpowered to detect changes in the lactate to NAA ratios, but well powered to detect changes in fractional anisotropy. Although no serious adverse events were recorded, no significant magnetic resonance differences were detected between groups. Based on the magnetic resonance results of 37 infants in the cooling only group and 41 in the cooling plus xenon group, early TH plus treatment with 30% xenon for 24 hours begun >6 hours after birth combined are not likely to improve clinical outcomes compared to TH alone for newborns with NE. Multiple factors that may impact inhaled xenon treatment outcomes, including the timing, dose, and duration of treatment, need further study. Study results of the CoolXenon3 Study (<https://clinicaltrials.gov> NCT02071394) combining TH with 18 hours of xenon inhalation in cooled infants with HIE are pending.

Melatonin—Melatonin (N-acetyl-5-methoxytryptamine), a neurohormone derived from the amino acid tryptophan and secreted by the pineal gland, is a strong antioxidant capable of scavenging free radicals and stimulating several antioxidative enzymes including glutathione, glutathione reductase, peroxidase, and superoxide dismutase.⁷⁶ Melatonin can directly stimulate cellular membrane G protein-coupled high-affinity melatonin receptors (melatonin receptors 1 and 2) that activate numerous second messenger cascades, which vary in cell, tissue, and species- specific ways, and also induce receptor-independent intracellular activities by targeting calcium- binding proteins, cytoskeletal and scaffold proteins, and components of mitochondrial signaling.⁷⁷ Melatonin's safety profile and antioxidant, anti-inflammatory and anti-apoptotic properties have made it an attractive neuroprotective candidate for treating neonates with NE.^{78, 79}

Postnatal neuroprotective benefits of melatonin have been demonstrated in an HIE piglet model, in which intravenous melatonin plus TH significantly improved cerebral energy metabolism based on proton magnetic resonance spectroscopy studies, reduced apoptosis in deep brain structures, and decreased microglial activation in the cortex at 48 hours post-injury.⁸⁰ In uncooled full term human newborns with NE (n =10), oral melatonin (8 doses of 10 mg each separated by 2-hr intervals) administration within the first 6 hours after delivery reduced serum malondialdehyde, a lipid peroxidation product, and nitrite/nitrate levels at 12 and 24 hours compared to untreated, uncooled controls (n = 10), suggesting a role for melatonin in reducing oxidative damage.⁸¹ In a recent prospective trial by Aly et al, involving 45 term newborns, 30 with HIE and 15 healthy controls, compared to TH alone (n = 15), melatonin (10 mg/kg daily × 5 enteral doses) plus TH (n =15) was associated with decreased seizures per EEG and decreased white matter abnormalities on MRI after 2 weeks of age and improved survival without neurological or developmental abnormalities at 6 months of age (p<0.001).⁸²

Melatonin remains an attractive potential antenatal neuroprotectant that could be administered to pregnant mothers since it appears safe, crosses the placenta,⁸³ and crosses the blood brain barrier (based on adult rat studies).⁸⁴ Further research is needed to clarify the mechanisms by which the pleiotropic neurohormone melatonin may regulate neuronal cell survival, brain tissue homeostasis, and neuroprotection in neonates with NE.

2-Iminobiotin (2-IB)—The biotin (Vitamin B7) analogue 2-iminobiotin (2-IB) is a combined neuronal and inducible (but not endothelial nitric oxide synthase inhibitor) that has been demonstrated to improve neuroprotection in animal models of HIE.⁸⁵⁻⁸⁷ While the exact mechanism of action has yet to be defined, 2-IB potentially protects against hypoxic–ischemic brain damage by preventing nitric oxide or peroxynitrate-induced mitochondrial damage.⁸⁶ Interestingly, after cerebral hypoxia-ischemia, the 2-IB treatment response appears to be gender-dependent, with neuroprotection demonstrated in female, but not male immature P3 rats.^{88, 89}

A small Phase 2 pilot trial (n=6) of neonates 36 and <44 weeks gestation with moderate to severe HIE has been completed in Turkey ([https://clinicaltrials.gov NCT01626924](https://clinicaltrials.gov/NCT01626924)). Study subjects were enrolled within 6 hours after birth and received 6 pulse doses of 2-IB within 20 hours. The primary study outcomes include the basal ganglia Lac/NAA ratio as measured by single or multiple voxel magnetic resonance spectroscopy and the composite endpoint of survival at 48h with a normal aEEG. To explore the short-term safety and tolerability of 2-IB and the pharmacokinetic profile of 2-IB when given on top of therapeutic hypothermia, a related phase 2 trial, the 2-STEP study, will be performed in the Netherlands (<http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=5221>).

Physiologic Placental Transfusion—Delayed cord clamping (DCC) and umbilical cord milking (UCM) are two methods of augmenting re-distribution of blood from the placenta back to the newborn after fetal delivery. In preterm infants, both DCC (delaying cord clamping for 30 to 60 secs) and UCM (stripping the unclamped umbilical cord toward the infant) have been associated with decreased hypotension requiring volume expanders and a decreased need for inotrope support following delivery, as well as decreased intraventricular hemorrhage of all grades.^{90, 91} DCC and UCM have not been studied in the context of newborns 36 weeks' gestation with NE. The current practice in depressed newborns suspected of having an intrapartum hypoxic event is to immediately clamp the umbilical cord, making DCC, which is typically performed over 1 to 3 minutes following delivery in term infants, an unlikely option for healthcare providers eager to initiate neonatal resuscitation.

However, in depressed newborns with NE, it is not clear if immediate cord clamping has a beneficial or adverse effect on an already compromised cardiovascular system. Immediate cord clamping may not be ideal in newborns with NE who have inadequate pulmonary ventilation, are severely bradycardic, and who are at risk for hypovolemic shock. UCM, which can be performed in <10 secs, may provide a beneficial strategy to augment cardiac preload by promoting placental to fetal transfusion. While clinical trials studying potential benefits of placental transfusion strategies may be challenging to conduct in newborns with NE, UCM techniques with unclamped or immediately clamped and milked cords may

provide a strategy to increase placental transfusion and allow for prompt neonatal resuscitation^{92, 93} Studies looking at the role of placental transfusion as the first therapeutic intervention after delivery of a newborn (e.g., <https://clinicaltrials.gov> NCT02287077) with NE are warranted.

Stem Cells—Stem cell therapies appear to hold significant potential for newborns with NE based on animal data demonstrating neuroprotection from hypoxic ischemic brain injury.⁹⁴ Umbilical cord blood (UCB) and tissue derived (Wharton jelly) cells, which contain endothelial progenitor cells,⁹⁵ mesenchymal stem cells,^{96, 97} and UCB-mononuclear cells,⁹⁸ are an attractive therapy given their ease of acquisition from cord blood at birth. Neural stem-like cells have been derived from human UCBs,⁹⁹ however, whether these UCB-derived progenitors may be effectively differentiated into functional neuron-like cells in human newborns with brain injury remains unknown.

In recent study, Cotton et al. reported on 23 infants 35 weeks gestation with NE who received TH fresh autologous UCB cells.¹⁰⁰ This study demonstrated safety and feasibility of UCB cell administration in cooled newborns with NE, but was not able to provide definitive conclusions regarding long-term neurodevelopmental outcomes or mortality. Current ongoing clinical trials (<https://clinicaltrials.gov> NCT01962233 and NCT00593242, NCT01506258) will further inform us of potential clinical outcomes. Future research will hopefully improve our understanding of stem cell-associated cellular mechanisms that may be involved in neuronal protection, rescue, or repair in the context of TH. While stem cell therapies for NE hold promise, numerous preclinical questions need to be addressed regarding the optimal stem cell type, delivery route, therapeutic window, safety profile, and short- and long-term outcomes before stem cell therapy

N-acetylcysteine (NAC)—NAC, a membrane-permeable cysteine precursor, is a free radical scavenger and major contributor to cellular maintenance of glutathione that has shown promise as an emerging treatment of adult vascular and nonvascular neurological disorders.¹⁰¹ In a neonatal rodent model (P7 rats) of hypoxic ischemic brain injury, NAC plus TH reduced brain volume loss with increased myelin expression and improved reflexes.¹⁰² Further research in a rabbit model of CP has shown that conjugating NAC to a polyamidoamine dendrimer, a synthetic biomimic of globular proteins that can function as a drug delivery vehicle, is more effective than systemically administered free NAC.¹⁰³ Information on the NAC-associated neuroprotection for neonates with NE is lacking.

Clinical management strategies

Along with seizures, neonates with NE frequently demonstrate cardiovascular instability evidenced by hypotension, metabolic acidosis, and pulmonary hypertension. Unlike the more standardized TH guidelines, clinical approaches regarding anti-epileptic use, fluid resuscitation, pressor support (including hydrocortisone treatment), the use of base replacement (e.g. sodium bicarbonate), ventilation strategies, oxygen saturation targets, inhaled nitric oxide use, and blood transfusion parameters may all influence outcomes in the setting of TH.

Additionally, the use of medications to provide sedation and prevent shivering in cooled newborns with NE needs to be studied. The current use of morphine during TH is not evidence-based and may not be ideal due to its side effect profile (e.g., respiratory depression, urinary retention, constipation) and because it does not specifically prevent shivering. Dexmedetomidine (DEX) and clonidine, both α_2 -adrenergic receptor agonists, are promising alternative sedatives because they specifically prevent shivering with suppressing respirations. DEX reduces inflammation,^{104, 105} does not produce abnormal brain histology (neonatal rats),¹⁰⁶ and produces neuroprotection in animal models of HIE.¹⁰⁷⁻¹⁰⁹ Currently, trials to assess the pharmacokinetics and safety of DEX (Cool DEX study, NCT02529202) and clonidine (NCT02252848) in newborns with NE during TH are underway.

Summary

Preventing NE related brain injury remains a monumental problem, since there is currently no way to predict whom NE will affect or when they will be affected. While animal models often demonstrate effective prenatal pharmacological treatments, this approach is often not feasible since NE is a post-injury event diagnosis. Whether safe and effective treatments (e.g., melatonin) could be prophylactically administered to all pregnant mothers requires further investigation. Deciding which infants need cooling, how long to cool, and the method of cooling remain important unanswered questions. In addition to further analysis of existing data (e.g., meta-analyses), to better understand the pathophysiology and how to treat NE, ongoing research is needed to assess biomarkers and different therapeutic interventions.

A tremendous amount of effort and sound research led to the establishment of TH as the standard of care for neonates with NE, yet many questions remain unanswered regarding this treatment. Although neonates with NE typically present with a common phenotype, which can be classified by Sarnat scoring, a myriad of acute and chronic *in utero* events may contribute to this phenotype. Our inability to identify the diverse and complex events that precede the manifestation of NE in human newborns is a contributing factor that may explain why effective neuroprotective treatments in animal models may not translate into clinical practice. Countless newborns had died or been devastated by NE and millions worldwide remain at risk for death and disability from NE despite the capabilities of modern day medicine. The concerted efforts of researchers and funding agencies to develop effective therapeutic strategies that can be combined with TH is needed to mitigate this perpetual tragedy.

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Best Practices Box**What is the current practice?**

Therapeutic hypothermia is the standard treatment for newborns < 36 weeks of gestation with moderate to severe neonatal encephalopathy related to intrapartum hypoxic events.

Best Practice/Guideline/Care Path Objective(s)**What changes in current practice are likely to improve outcomes?**

Therapeutic hypothermia plus adjunctive neuroprotective treatments, (e.g., erythropoietin, melatonin, stem cell therapies) and optimal clinical practice strategies are needed to improve survival and outcomes of newborns with moderate and severe neonatal encephalopathy.

Is there a Clinical Algorithm?**Major Recommendations**

Newborns < 36 weeks of gestation with moderate to severe neonatal encephalopathy should receive therapeutic hypothermia (33 °C to 34 °C) for 72 hours duration based on results from multiple RCTs.

Clinical Algorithm(s)**Rating for the Strength of the Evidence****Bibliographic Source(s)****Summary Statement**

Surviving neonates with severe neonatal encephalopathy remain at significant risk of death or severe neurodevelopmental impairment despite being cooled, emphasizing the need for well-studied additional adjunctive treatment strategies.

Key Points

1. Although limited in number, the available long-term follow-up studies of neonates with neonatal encephalopathy (NE) treated with therapeutic hypothermia (TH) demonstrate sustained benefits through middle childhood.
2. Neonates with severe NE remain at high risk for death and disability despite treatment with TH, emphasizing the need for adjunctive neuroprotective treatments.
3. Clinical trials of erythropoietin neuroprotection have raised no safety concerns, and suggest that erythropoietin treatment plus TH may improve neurologic outcomes in neonates with NE.
4. While preclinical trials appeared promising, the benefits of xenon, melatonin, and stem cell therapies in neonates with NE treated with TH need to be clarified by human clinical trials.
5. Further studies investigating clinical management strategies in neonates with NE, such as umbilical cord milking and sedative, anti-epileptics, and pressor medication choices are needed to optimize outcomes.