Review

Challenges in developing therapeutic strategies for mild neonatal encephalopathy

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Abstract

There is increasing evidence that infants with mild neonatal encephalopathy (NE) have significant risks of mortality, brain injury and adverse neurodevelopmental outcomes. In the era of therapeutic hypothermia, infants need to be diagnosed within 6 hours of birth, corresponding with the window of opportunity for treatment of moderate to severe NE, compared to the retrospective grading over 2 to 3 days, typically with imaging and formal electroencephalographic assessment in the pre-hypothermia era. This shift in diagnosis may have increased the apparent prevalence of brain damage and poor neurological outcomes seen in infants with mild NE in the era of hypothermia. Abnormal short term outcomes observed in infants with mild NE include seizures, abnormal neurologic examination at discharge, abnormal brain magnetic resonance imaging and difficulty feeding. At 2 to 3 years of age, mild NE has been associated with an increased risk of autism, language and cognitive deficits. There are no approved treatment strategies for these infants as they were not included in the initial randomized controlled trials for therapeutic hypothermia. However, there is already therapeutic creep, with many centers treating infants with mild NE despite the limited evidence for its safety and efficacy. The optimal duration of treatment and therapeutic window of opportunity for effective treatment need to be specifically established for mild NE as the evolution of injury is likely to be slower, based on preclinical data. Randomized controlled trials of therapeutic hypothermia for infants with mild NE are urgently required to establish the safety and efficacy of treatment. This review will examine the evidence for adverse outcomes after mild NE and dissect some of the challenges in developing therapeutic strategies for mild NE, before analyzing the evidence for therapeutic hypothermia and other strategies for treatment of these infants.

Key Words: asphyxia; electroencephalogram; erythropoietin; mild hypoxic ischemic encephalopathy; neonatal encephalopathy; neurological examination; neuroprotection; Sarnat score; therapeutic hypothermia

Introduction

Neonatal encephalopathy (NE) is one of the main causes of neonatal death and mortality worldwide. In the developed world, moderate to severe NE affects approximately 1 to 3/1000 live births (Smith et al., 2000). Therapeutic hypothermia is now standard care for full term or near-term neonates with moderate to severe NE (Gunn and Thoresen, 2019). Multiple large randomized controlled trials (RCTs) have shown that it significantly improves survival without disability into mid-childhood (Jacobs et al., 2013). However, many infants have mild encephalopathy in the first 6 hours of life, as shown by hyperalertness, agitation and hypertonia. These infants were not included in the RCTs due to perceived low risk of adverse outcomes and so do not meet current criteria for treatment with therapeutic hypothermia, and the clinical benefit of treating these infants with therapeutic hypothermia (TH) has not been established,

Worryingly there is mounting evidence to suggest that infants classified with mild NE have material risks of brain injury and adverse neurodevelopmental outcomes (Gagne-Loranger et al., 2016; Walsh et al., 2017; Conway et al., 2018). Despite the lack of evidence from RCTs, there is increasing therapeutic drift, so that many centers offer TH to babies with mild NE (Oliveira et al., 2018). Given the pressure to treat these babies, studies are urgently required to determine the safety, efficacy and optimal duration of treatment for infants with mild HIE (Lodygensky et al., 2018).

This review will examine the evidence for adverse outcomes after mild NE and acknowledge some of the challenges in developing therapeutic strategies, before analyzing the evidence for therapeutic hypothermia and other potential treatment strategies for these infants.

Database Search Strategy

Studies cited for this review were found on PubMed and Google Scholar up to December 10, 2020, using the following keywords: mild hypoxic ischemic encephalopathy, neonatal encephalopathy, perinatal asphyxia, hypoxia, therapeutic hypothermia, erythropoietin, biomarker, neurological examination, Sarnat score, magnetic resonance imaging, amplitude integrated electroencephalogram.

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How Neonatal Encephalopathy Is Diagnosed in the Era of Therapeutic Hypothermia and the Implications for Mild Neonatal Encephalopathy

It is important to appreciate that with the introduction of routine use of TH for the treatment of moderate-severe NE, there has been a change in how infants are diagnosed. Before TH, the grading of encephalopathy was made in retrospect, and included daily neurological assessment, most often using the Sarnat staging system, which involved both comprehensive neurological examinations but also electroencephalographic (EEG) monitoring and often brain imaging, over the first week of life (Sarnat and Sarnat, 1976). With the introduction of TH the diagnosis of NE had to be made within 6 hours of birth to enroll infants for treatment within its therapeutic window, and used only the clinical Sarnat criteria (with or without a period of amplitude integrated EEG monitoring) (Shalak et al., 2003).

This need to diagnose and assess the severity of NE in the first 6 hours of life is associated with a number of practical challenges. Firstly, the short time frame for diagnosis does not account for the fact that NE is a dynamic process in which the severity can progress over days from mild to moderate and even to severe (Shalak et al., 2003; Murray et al., 2010). Indeed, a recent, small retrospective study found that 64% (n = 7) of infants diagnosed with mild NE at less than 6 hours went on to develop moderate NE during TH (Perretta et al., 2020). Second, during the first few hours after birth, symptoms of NE can be subtle and difficult to discern. At this time, other factors such as maternal sedation or analgesics/anesthetics may confound the diagnosis.

Moreover, there is no uniform definition of mild NE. Although the modified Sarnat scoring system remains the most widely used neurological test to grade NE (Chalak et al., 2019a), other methods such the Thompson scoring system are also popular (Mendler et al., 2018). Indeed, Sarnat et al. (2020) have recently proposed a multicenter study to re-evaluate and refine the criteria for the Sarnat grading system. This proposal reflects the importance of the change in diagnostic criteria since the introduction of TH, and aims to correlate the scale with new technologies including advanced neuroimaging, continuous EEG with video monitoring and amplitude integrated EEG (aEEG). It should also be highlighted that neurological examination is subjective and often neurological signs can change over hours. In order to address these discrepancies there have been calls for videotaping of examinations for training and quality control (El-Dib et al., 2019; Chawla et al., 2020).

This lack of a consensus definition of mild NE within the first 6 hours has been acknowledged in proposed studies to investigate therapeutic strategies for mild NE (El-Dib et al., 2019). The following sections will discuss some tools that may aid in the definition and identification of infants with mild NE who are at risk of disability (**Figure 1**).

Was Mild Neonatal Encephalopathy Associated with Poor Outcomes in the Pre-Hypothermia Era?

The original studies by Robertson and colleagues comparing the outcomes of infants with mild, moderate and severe NE, showed that infants with mild NE had near normal outcomes. For example at 3.5 years of age no infants with mild NE (n = 66) had major handicap compared to 21.3% of infants with moderate NE (n = 94) and 100% of infants with severe NE (n = 7) (Robertson and Finer, 1985). At 8 years of age children with mild neonatal encephalopathy (NE) associated with birth asphyxia did not have neurological impairment and had school performance scores comparable to a control group of children (intelligence quotient (IQ) of infants with mild NE 106 compared to control of 112) in comparison to infants with moderate or severe NE that had significantly lower scores than control children (Robertson et al., 1989).

Subsequent studies suggested that children with mild NE (n = 34) showed a subtle increase in social and behavioral problems at 9–10 years of age and significantly lower IQ than that of control children (van Handel et al., 2012). This study also suggested that children with mild NE performed worse in tests of long term memory than control children (although not significant when corrected for the lower IQ of these children) and had a higher frequency of social behavior problems than control children. Similarly, Murray et al. reported significantly lower full scale IQ in children with mild NE (n = 22) than control children (n = 30) at 5 years of age (P < 0.001), verbal IQ (P < 0.001) and performance IQ (P = 0.004) (Murray et al., 2016). Further, there were no significant differences in any IQ measure between mild and moderate NE.

Overall these studies found that mild NE was not associated with death or major disabilities, but that there may be some more subtle developmental, attention and behavior issues. This formed the basis of exclusion of these infants from subsequent RCTs for TH. Interestingly, a contemporaneous systematic review of the outcome of 250 children with mild NE suggested that 22% (n = 56) had an abnormal outcome at ≥ 18 months of age defined as cerebral palsy or neurodevelopmental score ≥ 1 standard deviation (Conway et al., 2018).

What are the Outcomes of Mild Neonatal Encephalopathy in the Post-Hypothermia Era?

Of concern, recent evidence from the post-hypothermia era suggests that infants with mild NE do in fact have a significant risk of abnormal outcomes. Indeed abnormal outcomes can be observed within the first week of life with a recent study showing that 20% (n = 12) of infants with perinatal acidemia and mild NE who were not eligible for TH went on to have an abnormal short term outcome at one week of age defined as death (n = 1), seizures (n = 5), abnormal neurologic examination at discharge (n = 7), abnormal brain magnetic resonance imaging (MRI) (n = 6), gastrostomy tube feeding (n = 1) or inability to nipple all feeds (n = 8) (DuPont et al., 2013).

The largest recent prospective study so far that investigated the outcomes after mild NE is the PRIME (Prospective Research in Infants with Mild Encephalopathy) study. This study used the National Institute of Child Health and Human Development (NICHD) criteria with mild NE being defined as having perinatal acidosis and ≥ 1 abnormality using the modified Sarnat criteria (but no evidence of three moderate or severe abnormalities in three categories) within the first 6 hours of life (Prempunpong et al., 2018). The first part of this study investigated the rate of abnormalities until discharge from the neonatal intensive care unit (NICU). 52% (n = 28) had ≥ 1 abnormality in the early amplitude-integrated electroencephalography aEEG (n = 4), brain MRI (n = 9) or neurological exam at discharge (n = 22). The follow up study at 18–22 months of age showed that 16% (n = 7) were diagnosed with disability including 2% (n = 1) with cerebral palsy and 5% (n = 2) with autism (Chalak et al., 2019b). Bayley Scales of Infant and Toddler Development, Third Edition (BSITD-III) scores < 85 were observed in 40% (n = 17): 32% (n = 14) in language, 16% (n = 7) in cognitive and 14% (n = 6) in motor domain.

A recent large cohort study prospectively enrolled infants with mild NE classified using the modified Sarnat score and measured the cognitive outcome of these infants at 2 to 3 years of age (Finder et al., 2019). Cognitive, language and motor development measured by BSITD-III showed that infants with mild NE (n = 134) had significantly lower cognitive

composite scores compared to healthy control infants (n = 152). This study also found no significant difference in the mean cognitive composite scores between untreated children with mild NE (n = 47) and infants with moderate NE (n = 52) treated with TH.

Biomarkers

An ideal biomarker would rapidly determine not only if the infant has mild NE but also the long term prognosis and risk of bad outcomes. In terms of aiding the decision on whether or not to treat an infant, a biomarker that provided early feedback on whether the treatment was likely to reduce brain damage would be ideal for clinical decision-making (**Figure 1**).

The most commonly used biochemical markers are pH and base deficit (BD) from arterial cord blood samples. The majority of RCTs used cut off values for TH treatment of a pH < 7.0 or a base deficit of ≥ 16. More recent evidence suggests that increasing the pH to ≤ 7.1 is less specific, but would capture more infants with possible NE (Vesoulis et al., 2018). Increasing the pH threshold would increase the number of infants that need to be screened for possible TH therapy and likely increase the number of detected infants with mild NE. Although the extremes of BD measurements (e.g. BD > 18 mM or conversely BD < 10–12 mM) correlate well with high and low risk of encephalopathy, respectively, intermediate BD values have variable rates of severity and rates of NE (Low et al., 1997; Wayenberg, 2005), and so have limited use in identifying mild NE.

There is also the potential that biomarkers of neurological injury may be useful. For example, S100 calcium-binding protein B, neuron specific enolase, glial fibrillary acidic protein, ubiquitin carboxyl-terminal hydrolase L1, brain derived neurotrophic factor, tau, activin, and interleukins (IL) and many others, as reviewed (Mir and Chalak, 2014; Graham et al., 2016). For example IL-1, IL-6 and neuron specific enolase are associated with long-term neurodevelopmental outcomes (Ramaswamy et al., 2009) and abnormal neurological outcomes at 15–18 months of age have been associated with increased plasma concentrations of glial fibrillary acidic protein, IL-1 and IL-6 at 6–24 hours (Chalak et al., 2014). Currently, however, there is a lack of information regarding these biomarkers in relation to mild NE and therefore further analysis is required.

Continuous Electroencephalographic and Amplitude Integrated Electroencephalographic Monitoring

Continuous video EEG monitoring is considered the gold standard to assess neonatal brain activity (Murray et al., 2009). However, it requires a large number of electrodes to be placed on the neonate's head and specialist interpretation by a neurophysiologist. Therefore, many centers prefer to use amplitude integrated EEG (aEEG), as it is a simple, real time beside tool that is easily interpreted and shows changes in brain activity over time and is widely used for the detection of seizures. In infants with moderate to severe NE there is a good correlation between early aEEG findings and short and long term outcomes (Bjerre et al., 1983; al Naqeeb et al., 1999; ter Horst et al., 2004). Following this aEEG has been used in conjunction with neurological scoring to determine whether an infant has moderate/severe NE and therefore is eligible for TH (e.g. see the CoolCap and TOBY trials) (Gluckman et al., 2005; Azzopardi et al., 2008).

In a case control study, continuous multichannel video EEG segments were obtained at 6, 12, 24 and 48 hours of age and neurological outcome was assessed at 24 months. Mild NE was defined as a continuous background pattern with mild abnormal activity such as mild asymmetry, mild voltage

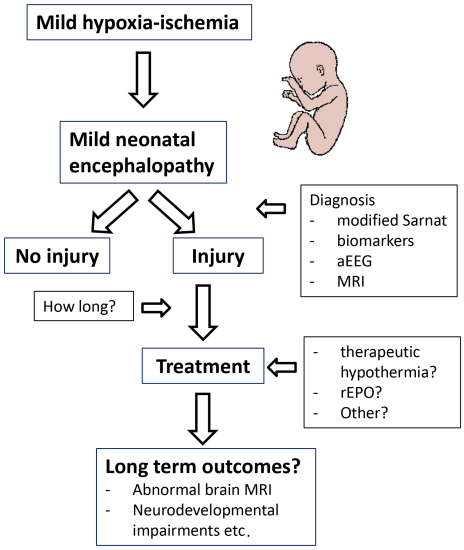

Figure 1 | **The evolution of mild neonatal encephalopathy.**
aEEG: Amplitude-integrated electroencephalography; MRI: magnetic resonance imaging; rEPO: recombinant erythropoietin.

depression or poorly defined sleep-wake cycle. Abnormal outcome was defined as death, cerebral palsy or a Griffiths quotient of < 87. They found that normal or mildly abnormal EEG results at 6, 12 or 24 hours had a 100% positive predictive value for a normal outcome and a negative predictive value of 67% to 76% at 2 years of age (Murray et al., 2009).

In contrast, the ability to predict outcomes for infants with mild NE using aEEG monitoring within 6 hours of birth (and therefore within the window for treatment with TH) appears to be limited. For example the PRIME study, which enrolled infants with mild NE, found that 24 (48%) of the 50 infants with a normal aEEG (taken at a median age of 5.5 hours after birth) had an abnormal outcome at discharge (defined as abnormal neurological examination and or MRI) (Prempunpong et al., 2018). This follows other studies that have shown the positive predictive value for determining the outcome of infants is more accurate at 24 and 48 hours of age (Murray et al., 2016). It should also be reiterated that the majority of infants with mild NE appear to display either normal background activity or potentially slightly broader bands of activity, which can be subtle and difficult to detect (Eken et al., 1995; Azzopardi, 2015). The usefulness of aEEG therefore in defining infants with mild NE is likely to be complimentary to that of neurological examination.

Magnetic Resonance Imaging

Using MRI, mild NE injury has been predominately within the watershed region, impacting the cortex and white matter (Walsh and Inder, 2018). The frequency of injury determined using MRI in mild NE infants according to the PRIME study was 17% (9/54) (Prempunpong et al., 2018). This is slightly lower than another study by Gagne-Loranger et al. (2016) which suggested that 40% (20/50) of non-cooled mild NE infants developed brain injury; the difference likely relating to differences in the grading of injury. However due to the time period of the evolution of this injury, MRI has limited predictive ability until after the first three days of life in normothermic infants (Walsh et al., 2017). Therefore, MRI is unlikely to be helpful for recruitment, but studies of serial MRI measurements may provide greater insight into future uses of this tool.

Therapeutic Creep

Despite the lack of RCTs to support the use of TH for mild NE, many centers report therapeutic creep, such that TH is now

often offered to infants with mild NE. For example at a single site in Canada the number of term newborns referred to the NICU for possible TH, 36% (*n* = 79) had mild NE and 16% (*n* = 13) of these infants went on to receive TH (Gagne-Loranger et al., 2016). This follows similar trends also reported from data registries. Analysis of the TOBY Cooling Register showed that 18% (*n* = 91) of infants with a Thompson encephalopathy score conducted prior to cooling had a score consistent with mild NE (Azzopardi et al., 2012). Analysis of the Children's Hospital Neonatal Database in the United States comprised of 27 regional NICUs showed that of the 160 infants with mild NE, 122 (76%) had received TH (Massaro et al., 2015).

Since the initiation of TH for moderate-severe NE, there has been increasing use of TH for treatment of infants with mild NE. Using the California Perinatal Quality Care Collaborative and California Perinatal Transport System linked 2010–2012 datasets, Kracer et al. (2014) showed that 237 mild NE cases 50% of infants were treated with hypothermia, with an increasing trend over the years with 38% in 2010, 53% in 2011 and 55% in 2012. Similarly, a recent study analyzing the trends of TH for treatment of mild NE in infants in the Netherlands, reported that between February 2008–November 2012, 7% of cooled infants received mild NE compared to 28% between and November 2012–July 2017 (Parmentier et al., 2020).

The reasons for this drift are not specifically known but in a recent survey of 48 centers in the UK that offered cooling to babies, 36 (75%) offered cooling to infants with mild NE. From the survey, it was suggested that the most common reason for offering cooling was due to concern that mild NE may progress to moderate NE and the window of opportunity to start TH would be missed (Oliveira et al., 2018). Of the centers offering cooling to these infants 25 (69%) justified giving TH for mild NE as it very difficult to grade NE soon after birth and 17 (47%) cited concern over the risk of long-term adverse neurological problems. One of the more worrying aspects that of this survey was that 1/3 of the centers that cooled infants with mild NE discontinued cooling therapy prior to 72 hours if there was rapid clinical improvement. This is despite research suggesting that shorter duration of cooling is associated with suboptimal neuroprotection in preclinical models (Davidson et al., 2018) .

Evidence for Benefit of Therapeutic Hypothermia in Mild Neonatal Encephalopathy

Hypothetically treatment with TH after mild NE could be associated with even better relative neuroprotection than more severe insults. This is partially due to the fact that milder insults are known to be associated with a slower progression of cell death. For example in 21 day old rats mild (15 minutes) unilateral hypoxic ischemic injury resulted in slower progression of cell death than severe (60 minutes) injury (Beilharz et al., 1995). Consistent with this, TH started 1 hour after 3 minutes of cerebral ischemia in adult gerbils, for 12 hours attenuated abnormal open field behavior and significantly reduced CA1 necrosis, but was only partially effective after 5 minutes of cerebral ischemia (Colbourne and Corbett, 1994). Similarly, hypothermia started immediately after moderate hypoxia-ischemia for 90 minutes in 7-day-old rat pups significantly reduced area loss compared to rats who had hypothermia started immediately after a more severe 150 minutes of hypoxia-ischemia (Sabir et al., 2012).

Current clinical data on the use of TH in infants with mild NE is inconclusive due to the lack of randomized clinical trials. Montaldo et al. (2019) showed that infants that were cooled (*n* = 32) had less white matter injury on MRI and improved thalamic metabolic indicators compared to non-cooled infants (*n* = 15). At 2 years there were no adverse outcomes for infants that were cooled, compared to mild disability in 2 out of the 14 (14.3%) of cases in the non-cooled group. However, mean cognitive, motor and language scores at 2 years were similar between the two groups. In another small study, there was a lower incidence of brain injury on MRI in infants who were treated with whole body cooling [4/13 (31%)] compared to those who did not receive cooling [20/50 (40%)] (Gagne-Loranger et al., 2016). In line with this, a recent retrospective study suggested that mild NE infants had lower odds of brain injury on MRI compared to non-cooled infants, although TH was associated with longer hospital stays and longer requirement for respiratory support (Goswami et al., 2020). Overall, as reported by a recent meta-analysis there is insufficient evidence to conclude whether TH significantly reduced adverse outcomes (Kariholu et al., 2020).

It should also be noted that TH requires admission to intensive care, invasive treatments, separation of the infants from their parents and delayed oral feeding (El-Dib et al., 2019). No serious side effects of TH have been reported, however, mild bradycardia and thrombocytopenia have been observed. Moreover, there is limited evidence to suggest that cooling in the absence of NE can increase apoptosis in pre-clinical models (Koo et al., 2017). In contrast, prolonged cooling for 3 or 21 days in adult rats was not associated with altered behavior, brain cell morphology or cell death (Auriat et al., 2012). Therefore fully cross matched pre-clinical studies and RCTs are urgently needed to provide evidence-based guidance on the optimal treatment of infants with mild NE (El-Dib et al., 2019; Chawla et al., 2020). There are currently two clinical trials listed on the clinical trials register that have not yet started recruiting that will examine therapeutic hypothermia for mild NE (the "TIME" study and the "COOLPRIME" study).

Other Treatment Strategies for Mild Neonatal Encephalopathy

As well as TH other neuroprotective strategies are being investigated for the treatment of moderate and severe NE for use either alone or adjunct with TH. Currently the only therapy other than TH with evidence in studies of mild NE is recombinant erythropoietin (rEPO). rEPO has been shown to improve both histological and functional outcomes in preclinical studies of moderate to severe NE (Robertson et al., 2012). rEPO has been shown to have a range of potentially neuroprotective effects including reducing apoptosis, excitotoxicity, inflammation, oxidative stress and cell swelling (Kumral et al., 2004, 2006; Spandou et al., 2004; Sun et al., 2005; Brissaud et al., 2010; Lan et al., 2016). In addition, rEPO may have neurorestorative effects by promoting neurovascular repair, neurogenesis and oligodendrocyte proliferation and maturation (Iwai et al., 2007, 2010). Phase II clinical trials of rEPO as a monotherapy or in combination with TH have confirmed a high safety profile for this drug and suggest improved neurodevelopmental outcomes in infants with moderate NE (Zhu et al., 2009; Wu et al., 2012, 2016; Malla et al., 2017). One small study that enrolled infants with mild or moderate NE, showed that the rEPO treated group had statistically lower serum nitric oxide levels, fewer neurodevelopmental abnormalities and improved EEG background at follow-up (Elmahdy et al., 2010). These studies suggest that rEPO may be a useful treatment option for mild NE. The results of current phase III trials of rEPO with TH are eagerly awaited.

Further preclinical research should not be limited to just TH but should also include a range of other potentially neuroprotective compounds to establish the best treatment strategy for mild NE. Proposed therapies include xenon, melatonin, magnesium sulfate, connexin hemichannel mimetic peptides and insulin-like growth factor (Davidson et al., 2012, 2018; Zhou et al., 2020).

Conclusion

This review has dissected evidence that infants with mild NE in the first hours of life are at substantial risk for adverse neurodevelopmental outcomes and thus that there is significant need for effective neuroprotective therapies. It highlights several major limitations. First, the lack of a well validated marker of mild NE. This likely contributed to significant variation in outcomes between studies, and in the future will make it difficult to recruit an homogenous population for clinical trials of neuroprotection. Thus, further research into clinical and biochemical biomarkers that can reliably identify the subset of infants with mild NE who will go on to develop disability is urgently needed. Second, there is a lack of appropriate pre-clinical or clinical studies of either TH or other potential neuroprotective strategies. Thus, although TH is increasingly being used in the clinics to treat mild NE there is no strong evidence for benefit. Thus we propose that to improve the outcome of mild NE, research needs to focus on both improving the diagnosis of mild NE and rigorously testing possible therapeutic strategies, including TH.

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