



**Prospective Study of Infants with Mild Encephalopathy
(PRIME):
Neurodevelopmental Outcomes at 18-22 Months of
Untreated Infants**

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**Prospective Study of Infants with Mild Encephalopathy (PRIME):
Neurodevelopmental Outcomes at 18-22 Months**

Lina F Chalak*¹ MD MSCS; Kim-Anh Nguyen*², MD; Chatchay Prempunpong³, MD; Roy Heyne MD¹; Sudhin Thayyil⁴ MD; Seetha Shankaran⁵, MD; Abbot R Laptook⁶, MD; Nancy Rollins¹ MD; Athina Pappas⁵, MD; Louise Koclas² MD; Birju Shah⁶, MD; Paolo Montaldo⁴MD; Benyachalee Techasaensiri MD³; Pablo J Sánchez MD⁷; and Guilherme Sant’Anna² MD, PhD.

Affiliations: ¹University of Texas Southwestern Medical Center, Texas, Dallas, USA. ²McGill University Health Center, Montreal, Canada. ³Mahidol University, Bangkok, Thailand. ⁴Imperial College, London, UK. ⁵Wayne State University, Michigan, Detroit, USA. ⁶Brown University, Rhode Island, Providence. ⁷Center for Perinatal Research, Nationwide Children’s Hospital, The Ohio State University College of Medicine, Columbus, OH, USA.

Corresponding author: Lina F. Chalak, MD, MSCS. The University of Texas Southwestern Medical Center at Dallas, Department of Pediatrics, Division of Neonatal-Perinatal Medicine 5323 Harry Hines Boulevard, Dallas, Texas 75390 Phone: (214) 648-3903; Fax: (214) 648-2481. E-mail: lina.chalak@utsouthwestern.edu

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Abstract (200 words)

Background: Mild hypoxic-ischemic encephalopathy (HIE) has been associated with abnormal short term outcomes but contemporary neurodevelopmental outcomes are unknown.

Objective: To evaluate neurodevelopmental outcomes at 18-22 months in infants enrolled in the Prospective Research on Infants with Mild Encephalopathy (PRIME) cohort.

Study design: Multicenter, prospective study of newborns with fetal acidosis and/or needed resuscitation who had mild HIE at < 6h of age. Mild HIE was defined as ≥ 1 abnormal category using the modified Sarnat score and not meeting cooling criteria. Primary outcome was any disability; *Mild*: Bayley III cognitive score of 70-84 or ≥ 85 and Gross Motor Function Classification System (GMFCS) 1 or 2 or seizures; *Moderate*: cognitive 70-84 and GMFCS 2, seizures or hearing deficit; *Severe*: cognitive < 70, GMFCS 3-5, blindness or severe impairment.

Results: 43 completed all study follow up, 7 (16%) had disability: 1 (2%) cerebral palsy, 2 (5%) autism. Any Bayley score < 85 was seen in 17 (40%): 14 (32%) language, 7 (16%) cognitive, and 6 (14%) motor domain. Infants with disability were significantly more likely to have abnormalities on the discharge examination, MRI and longer hospital stay ($p < 0.001$).

Conclusions: In this contemporary cohort disability was noted in 16% of untreated mild HIE.

Neonatal hypoxic-ischemic encephalopathy (HIE) remains an important cause of neurodevelopmental impairment and death.(1) While newborns with moderate and severe HIE have been the focus of neuroprotective trials utilizing hypothermia therapy, infants with mild HIE usually have been excluded due to a perceived low risk of death or major disability based on data from the pre-hypothermia era. (2-7) In the original description of the Sarnat (8) scores, neonatal encephalopathy following asphyxia was determined based on serial clinical evaluations and electroencephalographic signals during the first week of age. In the current era of therapeutic hypothermia and due to the narrow therapeutic window, the assessment of encephalopathy has changed to a more focused neurological examination (i.e. modified Sarnat score) performed within the first six hours of age with or without the use of the amplitude-integrated EEG (aEEG). The short time window is particularly challenging to identify and define mild HIE. Recent reports suggest that infants with mild HIE may have hypoxic-ischemic injury detected on brain magnetic resonance imaging (MRI) and experience higher rates of disability at 5 years when compared to historical controls but contemporary prospective studies since the implementation of Bayley III are lacking to date. (9-12)

We reported the prior short term hospital outcomes of infants with mild HIE who were enrolled in the Prospective Research on Infants with Mild Encephalopathy (PRIME) study with abnormalities in 52% of the infants(13). The primary aim of this current study was to report the neurodevelopmental outcomes of these infants at 18-22 months and in a secondary analysis, associations between neonatal variables and 18-22 months' outcomes.

Methods

Study design and population

PRIME was a multicenter, international, observational cohort study conducted from December 2012 to October 2015 (NCT01747863) that started with four and extended to six academic centers (McGill University Health Center, Montreal, Canada, University of Texas Southwestern Medical Center, Dallas, USA, Brown University, Providence, USA, Wayne State University, Detroit, USA, London Imperial College, London, UK, Mahidol University, Bangkok, Thailand). As previously published (13) Newborns were eligible for enrollment in the first 6 hours of age if they were ≥ 36 weeks' gestational age, had severe perinatal acidosis or received delivery room resuscitation as defined by the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (3), and had abnormalities on the modified Sarnat examination performed by a certified examiner (3) consistent with mild HIE (*Table 1*).

Mild HIE was defined within 6 hours of age as the presence of any ≥ 1 abnormality in any of the six categories of the modified Sarnat scoring system but that did not meet the NICHD criteria for cooling, i.e. ≥ 3 categories as moderate or severe. Exclusion criteria included the inability to enroll at ≤ 6 hours of age, presence of a major congenital abnormality, severe growth restriction (birth weight < 1800 g), hypothermia therapy instituted at ≤ 6 hours of age, or a completely normal neurological examination. The Institutional Review Board of each center approved the study and written informed consent was obtained from parents.

Study procedures and data collection

All infants enrolled were treated according to the standard of care of each center; none of them received therapeutic hypothermia. As previously published (13), the short term outcomes of

PRIME were any abnormality: early aEEG (< 6h of age) or clinical seizures, brain MRI < 30 days of age or neurological exam at NICU discharge or transfer. The aEEG was recorded for $\geq$ 60 min by using the Olympic BrainZ Monitor® (Natus Medical Incorporated, USA) or the Olympic CFM 6000 (Natus Medical Incorporated, USA). aEEG Background patterns (14) were classified by two blinded examiners. An abnormal aEEG was defined by the presence of non-continuous background pattern (DNV, BS, CLV, or FT). (14-17)

MRI studies were obtained without sedation before discharge (T1 and T2 weighted sequences and diffusion weighted images) and were scored by an independent pediatric neuroradiologist blinded to the clinical outcomes using the NICHD scoring (18, 19): 0, normal MRI; 1a, minimal cerebral lesions only with no involvement of basal ganglia and thalami (BGT), anterior limb of the internal capsule (ALIC), posterior limb of the internal capsule (PLIC), or watershed (WS) infarction; 1b, more extensive cerebral lesions only with no involvement of BGT, ALIC, PLIC, or WS infarction; 2a, any BGT, ALIC, PLIC, or WS infarction noted without any other cerebral lesions; 2b, involvement of either BGT, ALIC, PLIC, or area of infarction and additional cerebral lesions; and 3, cerebral hemispheric devastation.

The standardized neurological exam was performed by certified investigators at $\leq$ 6 h of age, 24 ± 6 h of age and as close as possible to NICU discharge/transfer. The examination at discharge/transfer also included. Abnormal neurological discharge/transfer exam was defined as any abnormality on modified Sarnat or additional abnormalities in gag reflex, clonus, fisted hands, abnormal movement, and persistent asymmetric tonic neck reflex (ATNR) which have been reported to be associated with abnormal outcomes in asphyxiated newborns. (20)

Neurodevelopmental Outcomes

Infants were evaluated at 18 to 22 months of age with a detailed neurological examination performed by trained examiners to establish the diagnosis of cerebral palsy (CP). The Gross Motor Function Classification System (GMFCS) was used to classify the functional motor impairment (1, 21): a) Level 1 = walk but gait is not fluent; b) Level 2 = unable to walk but can sit on his/her own, creep or crawl and may pull to stand and take steps holding on to furniture; c) Level 3 = require low back support for sitting, can roll and creep and may crawl, d) Level 4 = head control present but requires trunk support for sitting; and e) Level 5 = requires adult assistance to roll. Psychometric testing was performed by one trained psychometrician at each site, blinded to the clinical details of the children, and included the Bayley III cognitive, language, and motor evaluations (mean 100[SD 15], cognitive scale range 55-155; language range 45-155; and motor range, 46-154). (22) Additional subscales in language (expressive and receptive) and motor scores (fine and gross) also were done. The English version of the Bayley Scales of Infant and Toddler Development®, Third Edition (Bayley-III®) III was administered at all sites except at McGill University. The French version was administered by one bilingual psychometrician per standard practice at McGill since French was the primary language. Data on growth, vision, and hearing were obtained. Families of those who did not return for follow-up were contacted by telephone with a scripted interview form to screen for neurological problems, developmental delay, hearing loss, or blindness.

The primary outcome was any disability at 18-22 months of age as defined by the NICHD Neonatal Research Network (23, 24) and determined as: a) Mild: cognitive score of 70-84 alone, or a cognitive score ≥ 85 and GMFCS Level 1 or 2, seizure disorder (without anti-

epileptic medication), or hearing deficit with ability to follow commands without amplification; b) Moderate: cognitive score from 70-84 and GMFCS Level 2, active seizure disorder (receiving anti-epileptic medication), or hearing deficit with the ability to follow commands after amplification; and c) Severe: cognitive score < 70, GMFCS Level 3-5, blindness, or hearing impairment with inability to follow commands in spite of amplification.

Secondary outcomes included the individual domains of the Bayley III scores (cognitive, language, or motor scores), cerebral palsy (CP), Gross Motor Function Classification Score (GMFCS), vision and hearing, as well as exploratory analysis of the associations of disability with early neonatal variables.

Statistical analysis

Data were described as mean ± standard deviation, number (%) or median [interquartile range, IQR] where appropriate. The neurodevelopmental outcomes assessed at 18-22 months of age were further categorized as “no disability” or “disability”. Comparisons between these two groups were made with Fisher’s Exact or Cochran Armitage trend tests for categorical variables and Student *t*-tests for continuous variables. Total Sarnat examination score was calculated by adding the score for each of the 6 main categories (0 normal, 1 mild, 2 moderate, 3 severe). Analysis was conducted with SAS 9.4 (SAS Institute, Cary, NC) and a two-sided *p*-value of <0.05 was considered statistically significant.

Results

Of the 63 infants enrolled, 51 (81%) were examined for neurodevelopment at a mean age of 19 ± 2 months and 43 (68%) completed all study the Bayley III evaluations (**Figure 1**). There were no differences in maternal and perinatal characteristics of study infants with a complete

follow up at 18-22 months and those with limited (n=8) or no follow-up (n=12). Of the 20 infants with incomplete follow-up (**Table 2**) 5 had neurological exam at the follow-up visit but no Bayley III and another 3 had telephone scripted interviews to exclude developmental delays, hearing loss or blindness. The remaining 12 infants were lost to follow –up.

Primary outcome. Among the 43 infants who completed the study neurodevelopmental assessment, 7 (16%) were diagnosed with disability (**Table 3a**): 4 mild (9%) and 3 (7%) severe. None of the infants died or had vision or hearing impairments, or seizures. Details of the initial examination and outcomes of the infants that developed disability are described in **Table 3b**. Of the 3 infants with severe disability, one had CP (mixed type) with a GMFCS Level 4 score, and 2 others were diagnosed with autism spectrum disorder (ASD). The 2 ASD cases also had a Bayley III composite motor score <70. The ASD diagnosis was subsequently confirmed by a developmental health care specialist at 36 months of age.

Secondary outcomes. Bayley scores < 85 occurred in 17 (40%) infants: 14 (33%) language, 7 (16%) cognitive and 6 (14%) motor. Bayley scores < 70 occurred in 4 infants (9%): 3 (7%) in all scores and 1 (2%) only in language scores. The correlations between the prior abnormal short term outcomes and current 18-22m outcomes are summarized (**Table 4**).

As shown in **Figure 2**, infants with disability were significantly more likely to have remaining abnormalities on the neurological exam performed at discharge when compared to infants without disability (5 of 7 (71%) versus 6 of 36 (17%); p=0.01). Other factors associated with disability in the explanatory analyses (**Table 5**) were lack of maternal education, total score of neurological abnormalities within 6 h, abnormal MRI at < 30 days (38% vs. 14%; p=0.04) and longer hospitalization (14 [8-19] vs 4 [2-6] days; p=0.003). There were no statistically significant differences for Apgar scores at 5 and 10 min, blood gas analysis within 1 hour after birth or in

outborn status (43% vs. 61%). The time of the initial neurological examination was similar in infants with or without disability (4 ± 1 h). No genetic or metabolic syndromes or other confounding diagnosis were observed in any of the infants diagnosed with disability.

Discussion

This contemporary prospective observational multicenter study reports the 18 to 22 month neurodevelopmental outcomes of infants with mild HIE diagnosed at < 6 hours of age and not treated with therapeutic hypothermia. Key findings were an overall rate of disability of 16%, out of which 7% were classified as severe. Bayley III scores < 85 were noted in 40% of the infants and mostly affected the language domain, while Bayley < 70 were seen in four (9%). Infants with disability were more likely to have abnormal MRI and neurological examination at discharge with longer hospital stays, characterizing a higher risk group of mild HIE.

The PRIME 18-22 month outcomes are timely, as national and international registries have started reported a consistent shift in therapeutic hypothermia practice including infants with mild HIE based on short term outcomes and despite the lack of contemporary long term outcomes or evidence of safety/ efficacy in those infants. (25,26) Indeed, a recent survey in the United Kingdom demonstrated that 75% of centers are offering hypothermia for infants with mild HIE, with significant variability in cooling practices. (27)

Before therapeutic hypothermia was established as the standard of care, death or major disability was not reported in infants with mild HIE. (2-7) Subsequent follow-up of some of these cohorts at 9-10 years of age revealed subtle developmental delays with IQ evaluations slightly lower (98.1 ± 12.3) when compared to controls (109.0 ± 12.0). (28) Subtle language and behavioral problems were also noted. A more recent cohort of infants with mild HIE (2003-

2005) diagnosed based on EEG performed at ≤ 6 hours, reported normal neurodevelopmental outcomes at 24 months in all 13 infants. (29) This mild HIE study based on EEG is defined differently from our cohort where mild HIE was based on the early neurological examination.

In another mild HIE study, lower IQ scores at 5 years of age (99 [94-112]) were also noted in 20 infants when compared to historical controls (117 [110-124]) (10)

Previous studies that included infants with mild HIE were performed before the time of implementation of Bayley III Scales of Infant Development, had varied definitions of fetal acidosis/perinatal asphyxia, used the highest level of encephalopathy in the first week (not within 6 hours) for their neurological assessment which precludes direct comparisons with the current investigation. Despite the multiple differences among studies, a recent systematic review of mild HIE has reported abnormal outcomes in 25% of infants. Abnormal outcome was defined by CP or any standardized neurodevelopmental test score > 1 standard deviation below the mean. (30)

The primary outcome in the current study was selected to provide the same definition of disability used by recent hypothermia neuroprotection trials performed by the NICHD Neonatal Research Network (23-24). The definition uses solely the cognitive scores of the Bayley III thereby mitigating any primary bias in language scores which can occur in multilingual settings. (34) The observed high incidence of Bayley language scores < 85 of the present study should therefore be interpreted in the context of using french translated Bayleys in the canadian group.

Although unexpected, 2 infants with disability in this cohort were diagnosed with autism spectrum disorder (ASD). Similarly, a large Australian cohort had reported 5% of ASD in 239 HIE survivors. (31) A comprehensive meta-analysis demonstrated inconclusive evidence that exposure to perinatal conditions may increase the risks of ASD. (32, 33) These observations if replicated in other HIE cohorts could merit further investigation.

A significantly high rate of abnormal examination at discharge was observed in the group who developed disability as compared to those with normal outcomes. Only one infant had seizures after 6 hours, while the exam trends improved in the remaining infants. This study is the first to correlate the short term outcomes of the MRI and the discharge neurological examination to the long term outcomes in infants with mild HIE, consistent with previously published associations in infants with moderate to severe HIE.(18-20)

Study limitations include the sample size and a higher than desired loss to follow up, despite the academic centers experience in dealing with follow-up attrition. To mitigate concerns of bias the investigators took further steps: first comparing the neonatal characteristics of infants with and without complete follow-up and then performing phone interviews of infants, tracking a total of 51 (81%) infants. Another limitation is that the rate of disability may be under estimated due to difficulties in measuring the true cognitive ability at the age of 2 years. Swedish population studies have demonstrated that the gap between healthy controls and those with sustained HIE widens over time suggesting that unrecognized disability may be greater than documented at 2 years of age.(35)

Given the lack of a uniform consensus on how best to classify mild HIE within 6 hours of birth, the study investigators purposefully selected a broad definition of all infants with an acute perinatal event, fetal acidosis and any neurological abnormality on the modified Sarnat score that did not qualify for cooling. While the definition included a broad spectrum of infants with HIE that did not meet the current cooling published criteria, it was systematically and uniformly applied across centers by centrally trained examiners and showed that increasing abnormalities detected on the modified Sarnat score were significantly associated with disability.

It is important to mention that despite these limitations, this is the largest and only contemporary cohort of untreated mild HIE infants diagnosed at < 6 h of age in the era of therapeutic hypothermia and systematically evaluated up to 18-22 months of age, which fulfills an important gap in knowledge. Other study strengths include a prospective design, use of blinded central readers to evaluate brain MRI, and standardized neurodevelopmental outcomes using Bayley III.

In conclusion, the PRIME multicenter cohort demonstrated a 16% rate of disability at 18-22 months of age in infants with mild HIE not treated with therapeutic hypothermia. Currently, it is unknown whether or not abnormal neurodevelopmental outcomes in mild HIE infants can be mitigated by neuroprotective strategies, and large multicenter trials are required. Importantly, since the rate and severity of disability in mild HIE patients was lower than reported for infants with moderate/severe HIE future trials being planned will likely need to enroll a higher number of infants. The PRIME prospective contemporary uncooled cohort provides important data needed for planning of future studies of neuroprotection in this at risk population. Future investigations should include larger populations and include long-term evaluations of neurodevelopmental outcomes up to 6-7 years.

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Contributors' statement page

All authors contributed to the conception or design of the work, data collection, approval of the version to be published and agree to be accountable for all aspects of the work. Specifically

Lina Chalak - conception or design of the work, drafting the first draft article, central interpretation of aEEG recordings, data collection, critical revision and final approval of the version to be published and agrees to be accountable for all aspects of the work.

Kim-Anh Nguyen - conception or design of the work, data collection, final approval of the version to be published and agrees to be accountable for all aspects of the work.

Chatchay Prempunpong - conception or design of the work, data collection, critical revision of the article, final approval of the version, and agrees to be accountable for all aspects of the work.

Roy Heyne- outcome data collection and analysis, critical revision of the article, final approval of the version to be published, and agrees to be accountable for all aspects of the work.

Nancy Rollins- MRI central blinded reader and MRI data analysis, critical revision of the article, final approval of the version to be published, and agrees to be accountable for all aspects.

Sudhin Thayyil - design of the work data collection, critical revision of the article, final approval of the version to be published, and agrees to be accountable for all aspects of work.

Athina Pappas - design of the work, central interpretation of aEEG recordings, data collection, and critical revision of the article, final approval of the version to be published, and agrees to be accountable for all aspects of the work.

Birju Shah-data collection, critical revision of the article and final approval of version to be published, and agrees to be accountable for all aspects of work

Louise Koclas data regarding outcomes, critical revision of the article and final approval of version to be published, and agrees to be accountable for all aspects of work

Pablo J. Sánchez - conception or design of the work, critical revision of the article, final approval of the version to be published, and agrees to be accountable for all aspects of the work.

Seetha Shankaran - conception or design of the work data collection, critical revision of the article, final approval of the version to be published, and accountable for all aspects of the work.

Paolo Montaldo data collection, critical revision of the article and final approval of version to be published, and agrees to be accountable for all aspects of work

Benyachalee Techasaensiri data collection, critical revision of the article and final approval of version to be published, and agrees to be accountable for all aspects of work

Abbot Laptook - conception or design of the work, data collection, critical revision of the article and final approval of version to be published, and agrees to be accountable for all aspects of work

Guilherme Sant’Anna - conception or design of the work, data collection, critical revision of the article and final approval of the version to be published, and agrees to be accountable for all aspects of the work.

References

1. Ferriero DM. Neonatal brain injury. *N Engl J Med*. 2004;351(19):1985-95.
2. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet*. 2005;365(9460):663-70.
3. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*. 2005;353(15):1574-84.
4. Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal encephalopathy. *N Engl J Med*. 2009;361(14):1349-58.
5. Zhou WH, Cheng GQ, Shao XM, Liu XZ, Shan RB, Zhuang DY, et al. Selective head cooling with mild systemic hypothermia after neonatal hypoxic-ischemic encephalopathy: a multicenter randomized controlled trial in China. *J Pediatr*. 2010;157(3):367-72, 72 e1-3.
6. Simbruner G, Mittal RA, Rohlmann F, Muche R. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics*. 2010;126(4):e771-8.
7. Jacobs SE, Morley CJ, Inder TE, Stewart MJ, Smith KR, McNamara PJ, et al. Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial. *Arch Pediatr Adolesc Med*. 2011;165(8):692-700.
8. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol*. 1976;33(10):696-705.
9. Walsh MC, Bell EF, Kandefer S, Saha S, Carlo WA, D'Angio C T, et al. Neonatal outcomes of moderately preterm infants compared to extremely preterm infants. *Pediatr Res*. 2017;82(2):297-304.
10. Murray DM, O'Connor CM, Ryan CA, Korotchikova I, Boylan GB. Early EEG Grade and Outcome at 5 Years After Mild Neonatal Hypoxic Ischemic Encephalopathy. *Pediatrics*. 2016.
11. DuPont TL, Chalak LF, Morriss MC, Burchfield PJ, Christie L, Sanchez PJ. Short-term outcomes of newborns with perinatal acidemia who are not eligible for systemic hypothermia therapy. *J Pediatr*. 2013;162(1):35-41.
12. Massaro AN, Murthy K, Zaniletti I, Cook N, DiGeronimo R, Dizon M, et al. Short-term outcomes after perinatal hypoxic ischemic encephalopathy: a report from the Children's Hospitals Neonatal Consortium HIE focus group. *J Perinatol*. 2015;35(4):290-6.
13. Prempunpong C, Chalak LF, Garfinkle J, Shah B, Kalra V, Rollins N, et al. Prospective research on infants with mild encephalopathy: the PRIME study. *J Perinatol*. 2018;38(1):80-5.
14. Hellstrom-Westas L, Rosen I. Continuous brain-function monitoring: state of the art in clinical practice. *Semin Fetal Neonatal Med*. 2006;11(6):503-11.
15. Hellstrom-Westas L. Continuous electroencephalography monitoring of the preterm infant. *Clin Perinatol*. 2006;33(3):633-47, vi.
16. Toet MC, Lemmers PM. Brain monitoring in neonates. *Early HumDev*. 2009;85(2):77-84.
17. de Vries LS, Hellstrom-Westas L. Role of cerebral function monitoring in the newborn. *Arch Dis Child Fetal Neonatal Ed*. 2005;90(3):F201-7.

18. Shankaran S, Barnes PD, Hintz SR, Laptook AR, Zaterka-Baxter KM, McDonald SA, et al. Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. *Arch Dis Child*. 2012.

19. Shankaran S, McDonald SA, Laptook AR, Hintz SR, Barnes PD, Das A, et al. Neonatal Magnetic Resonance Imaging Pattern of Brain Injury as a Biomarker of Childhood Outcomes following a Trial of Hypothermia for Neonatal Hypoxic-Ischemic Encephalopathy. *The Journal of pediatrics*. 2015;167(5):987-93 e3.

20. Shankaran S, Laptook AR, Tyson JE, Ehrenkranz RA, Bann CM, Das A, et al. Evolution of encephalopathy during whole body hypothermia for neonatal hypoxic-ischemic encephalopathy. *J Pediatr*. 2012;160(4):567-72 e3.

21. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. *Dev Med Child Neurol*. 1997;39(4):214-23.

22. 2006. Bayley scales of infant and toddler development. 3rd ed. Harcourt Assessment 2006.

23. Shankaran S, Laptook AR, Pappas A, McDonald SA, Das A, Tyson JE, et al. Effect of Depth and Duration of Cooling on Death or Disability at Age 18 Months Among Neonates With Hypoxic-Ischemic Encephalopathy: A Randomized Clinical Trial. *JAMA*. 2017;318(1):57-67.

24. Laptook AR, Shankaran S, Tyson JE, Munoz B, Bell EF, Goldberg RN, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Effect of Therapeutic Hypothermia Initiated After 6 Hours of Age on Death or Disability Among Newborns With Hypoxic-Ischemic Encephalopathy: A Randomized Clinical Trial. *JAMA*. 2017 Oct 24;318(16):1550-1560.

25. Kracer B, Hintz SR, Van Meurs KP, Lee HC. Hypothermia therapy for neonatal hypoxic ischemic encephalopathy in the state of California. *J Pediatr* 2014; 165(2):267–273.

26. Massaro AN, Murthy K, Zaniletti I, Cook N, DiGeronimo R, Dizon M et al. Short-term outcomes after perinatal hypoxic ischemic encephalopathy: a report from the Children's Hospitals Neonatal Consortium HIE focus group. *J Perinatol* 2015; 35(4):290–296.

27. Oliveira V, Singhvi DP, Montaldo P, Lally PJ, Mendoza J, Manerkar S, et al. Therapeutic hypothermia in mild neonatal encephalopathy: a national survey of practice in the UK. *Arch Dis Child Fetal Neonatal Ed*. 2017 Sep 23. [Epub ahead of print]

28. Robertson CM and Finer NN. Long-term follow-up of term neonates with perinatal asphyxia. *Clinics in Perinatology* 1993;20(2):483-500.

29. Murray, D.M., et al., Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years. *Pediatrics*, 2009. 124(3): p. e459-67.

30. Conway JM, Walsh BH, Boylan GB, Murray DM. Mild hypoxic ischaemic encephalopathy and long term neurodevelopmental outcome - A systematic review. *Early Hum Dev*. 2018.

31. Badawi N, Dixon G, Felix JF, Keogh JM, Petterson B, Stanley FJ, et al. Autism following a history of newborn encephalopathy: more than a coincidence? *Dev Med Child Neurol*. 2006;48(2):85-9. (2)

32. Gardener H, Spiegelman D, and Buka SL (2011). Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. *Pediatrics*. 2011 Aug;128(2):344-55.

33. Amirhossein-Modabbernia A, Mollon J, Boffetta P, and Reichenberg A. Impaired Gas Exchange at Birth and Risk of Intellectual Disability and Autism: A Meta-analysis. *Journal of Autism and Developmental Disorders*.

34. Goh SKY, Tham EKH, Magiati I, Sim L, Sanmugam S, Qiu A, et al. Analysis of Item-Level Bias in Bayley-III Language Subscales: Validity and Utility of Standardized Language Assessment in Multilingual Setting. *J Speech Lang Hear Res.* 2017;60(9):2663-71.(30)
35. Lindstrom K, Hallberg B, Blennow M, Wolff K, Fernell E, Westgren M. Moderate neonatal encephalopathy: pre- and perinatal risk factors and long-term outcome. *Acta Obstet Gynecol Scand.* 2008; 87(5):503-9

Figure 1. Flowchart of infants enrolled in PRIME. 5 Infants had incomplete Bayley evaluations and 12 (19%) infants were completely lost to follow up.

Figure 2. Evolution of neonatal encephalopathy among infants without disability (2a) and those with disability (2b) at 18-22 months.

For Review Only

Table 1. Modified Sarnat scoring system for categorizing encephalopathy.

Each of the six main categories is assigned a score based on severity. When findings are common or overlapped in a category (Spontaneous activity and Suck overlap for mild and moderate HIE), the score assigned is one that corresponds to the level of consciousness.

Category	Normal (0)	Mild(1)	Moderate (2)	Severe(3)
Level of consciousness	Alert (responsive to external stimuli)	Hyper-alert (responsive to minimal stimuli)	Lethargic	Stupor/Coma
Spontaneous activity	Normal	Normal or decreased	Decreased	None
Posture	Predominantly flexed	Mild flexion of distal joints	Distal flexion or complete extension	Decerebrate
Tone	Strong flexor tone in all extremities	Normal or slightly increased	a. Hypotonia (focal or general)	Flaccid
			b. Hypertonia	Rigid
Primitive reflexes				
Suck	Strong, easily illicit	Weak or Incomplete	Weak or incomplete and/or bite	Absent
Moro	Complete	Intact (low threshold)	Incomplete	Absent
Autonomic Nervous System (ANS)				
Pupils	Normal	Mydriasis	Myosis	Variable or Nonreactive
Heart rate	100–160 bpm	Tachycardia	Bradycardia	Variable
Respirations	Regular respirations	Hyperventilation	Periodic breath	Apnea or need ventilation

Table 2. Maternal and perinatal characteristics of the PRIME cohort.

	Complete Follow-up 18-22m (n=43)	Incomplete Follow-up (n=20)
Maternal Characteristics		
Race		
Black	5 (12)	6 (30)
White (non-Hispanic)	19 (44)	4 (20)
Other	19 (44)	10 (50)
High school graduate	36 (84)	14 (70)
Hypertension	1 (2)	2 (10)
PPROM >18 hours	7 (16)	3 (15)
Maternal pyrexia	4 (9)	1 (5)
Chorioamnionitis	4 (9)	3 (15)
Antepartum hemorrhage	4 (9)	3 (15)
Abnormal fetal heart rate	34 (79)	14 (70)
Meconium-stained amniotic fluid	21 (49)	10 (50)
Umbilical cord accidents	2 (5)	0
Shoulder dystocia	2 (5)	0
Cesarean delivery	22 (51)	9 (45)
Perinatal Characteristics		
Gestational age, weeks	39.5 ± 1.4	39.7 ± 1.4
Birth weight, grams	3386 ± 534	3148 ± 667
Male	25 (58)	15 (75)
Delivery room resuscitation		
Intubation	19 (44)	6 (30)
Epinephrine	1 (2)	0
Chest compression	6 (14)	2 (10)
Apgar scores		
1 minute	2 [1-3]	2 [1-4]
5 minutes	6 [3-6]	5 [3-7]
Umbilical cord blood - pH	7.0 ± 0.1	7.0 ± 0.2
Umbilical cord blood - base deficit, mmol/L	13 ± 5	14 ± 7
1st hour blood gas - pH	7.2 ± 0.2	7.2 ± 0.1
1st hour blood gas - base deficit	11 ± 6	11 ± 5

PPROM, prolonged premature rupture of membranes; SGA, small for gestational age. Results are expressed as n (%), mean ± standard deviation or median [IQR]. *p ≤ 0.05. Infants are listed as completed all evaluations at 18-22 months (n=43) and incomplete or lost to follow-up (n=20). * Infants with incomplete follow up had a neurological exam and evaluations performed at 18-22 months (n=5) or scripted phone interviews (n=3) but no Bayley III evaluations.

Table 3. Neurodevelopmental Outcomes using the Bayley Scales of Infant and Toddler Development (Bayley-III).

a. Cohort Results		(n=43)
Age at evaluation, months		19 [18-22]
Disability		7 (16 %)
Mild		4 (9%)
Moderate		0
Severe		3 (7%)
Cognitive score		95 [90 – 106]
≥ 85		36 (84%)
70-84		4 (9 %)
<70		3 (7%)
Language score		89 [79 – 100]
≥ 85		29 (67%)
70-84		10 (23%)
<70		4 (10%)
Language subtypes:		
Receptive communication		9 [7-10]
Expressive communication		8 [7-10]
Motor score*		100 [88 – 105]
≥85		37 (86%)
70-84		3 (7%)
<70		1 (2%)
Motor subtypes:		
Fine motor		10 [9-12]
Gross motor		9 [8-10]
Cerebral palsy		1 (2%)
Autism		2 (5%)

Disability at 18-22 months of age as defined by the NICHD Neonatal Research Network: a) Mild: cognitive score of 70-84 alone, or a cognitive score ≥ 85 and GMFCS Level 1 or 2, seizure disorder (without anti-epileptic medication), or hearing deficit with ability to follow commands without amplification; b) Moderate: cognitive score from 70-84 and GMFCS Level 2, active seizure disorder or hearing deficit; and c) Severe: cognitive score < 70, GMFCS Level 3-5, blindness, or hearing impairment. Results are expressed as n (%), mean ± standard deviation or median [IQR]. *Two cases with autism were excluded from the motor scores due to inability to complete testing.

b. Selected Details of the Seven Mild HIE infants with disability at 18-22 months

Cases with disability	1	2	3	4	5	6	7
Sarnat at admission –	score=	Posture=1	Posture=	LOC=1	All	Post=1	Posture=1
abnormal categories	1	Tone=2	1 and	ANS=1,	categories=	Tone=1,	Suck=1;
(mild=1, moderate=2	For all	Moro=2	SA=2	Tone	1 but	Suck=2	Tone=2
and severe=3)				Moro=2	ANS=3	ANS=3	ANS=3
Total # of abnormal							
categories	4	3	2	4	6	4	4
Short-term outcomes							
aEEG at <9h days of							
age	CNV	CNV	CNV	CNV	CNV	CNV	CNV
Brain MRI at <30d of							
age	2b	0	0	2b	1b	0	0
Neurological exam at	All= 1	normal	normal	Tone	Abnormal	Moro	Tone
discharge/transfer	LOC,			=2b	ATNR	=1	=1
	activity						
	Tone						
	ATNR						
Follow-up							
18-22 months							
Disability	Severe	Severe	Severe	Mild	Mild	Mild	Mild
Bayley III Score -							
Cognitive	<70	60	<70	75	80	75	80
Bayley III Score -							
Language	<70	56	<47	79	74	97	91
Bayley III Score -							
Motor	<70	58	<70	88	82	107	88
Cerebral Palsy*	Yes	No	No	No	No	No	No
GMFCS	4 ^a	0	0	1	1	0	0
Autism	-	Yes ^b	Yes ^b	-	-	-	-

Sarnat at admission included 6 categories that could be scored as Normal 0, mild=1, moderate=2 or severe=3 (Table 1). SA, spontaneous activity; LOC, level of consciousness; ANS, autonomic nervous system (all abnormalities were need of intubation: score 3); aEEG, amplitude integrated electroencephalogram; CNV, continuous normal voltage; MRI, magnetic resonance image; GMFCS, Gross motor function classification score: ^a mixed cerebral palsy. ^b Both Cases with a diagnosis of autism were followed beyond 36 months to confirm the diagnosis.

Table 4. Neurodevelopmental outcomes at 18-22 months of age in the 20 infants who had abnormal short-term outcomes by discharge from hospital

	Abnormal Brain MRI at < 30 days (n=8)	Abnormal Neurological exam at discharge/transfer (n=16)
Cognitive Bayley Score		
≥85	5 (62) [‡]	11 (69) [¶]
70-84	2 (25) [§]	4 (25)
<70	1 (13)*	1 (6)*
Disability		
No	5 (62)	11 (69)
Mild	2 (25)	4 (25)
Severe	1 (13)*	1 (6)*

Legend: Data expressed as n (%). Abnormal MRI NICHD scoring system:
[‡] = 1a (n=4) and 1b (n=1); [§] = 1b (n=1) and 2b (n=1) and * = 2b with diagnosis of cerebral palsy.
[¶]For abnormal neurological exam 1 also had abnormal MRI= 1b.

Table 5. Mild HIE Infants with and without Disability

	Disability (n = 7)	No Disability (n = 36)
Maternal age	29.3 ± 6.5	30.6 ± 5.6
Maternal Education level*		
Less than High School	1 (14)	6/35 (17)
High School – completed	5 (72)	5/35 (14)
College – incomplete	1 (14)	11/35(32)
College or post-graduation	0	13/35 (37)
Maternal illness		
Diabetes mellitus	1 (14)	2 (6)
Thyroid dysfunction	1 (14)	1(3)
Others [#]	1 (14)	8 (22)
Apgar Scores		
1min	3 ± 2	1± 1
5min	5 ± 2	5 ± 2
10min	7± 2	7 ± 2
Cord blood gas		
pH	6.9±0.1	6.9±0.2
PCO ₂	78±20	75±38
Base deficit	13±5	15±7
First hour blood gas		
pH	7.2±0.1	7.2±0.2
Base deficit, mmol/L	10±6	13±8
Age at exam, hours	4.6± 1	4.6± 1
Total Sarnat Exam score <6h*	3 (2-6)	6 (5-7)
Abnormal exam at discharge*	6(17%)	5(71%)
Abnormal MRI*	6(17%)	5(71%)
Hospital stay, hours*	98(57-171)	229 (101-544)

Legend: MRI, magnetic resonance image; PCO₂, partial pressure of carbon dioxide.

Results are expressed as n (%), mean ± SD (n), and median [interquartile range] *; p≤0.01

[#]2 diagnosis of anxiety/depression, 1 Psoriasis.

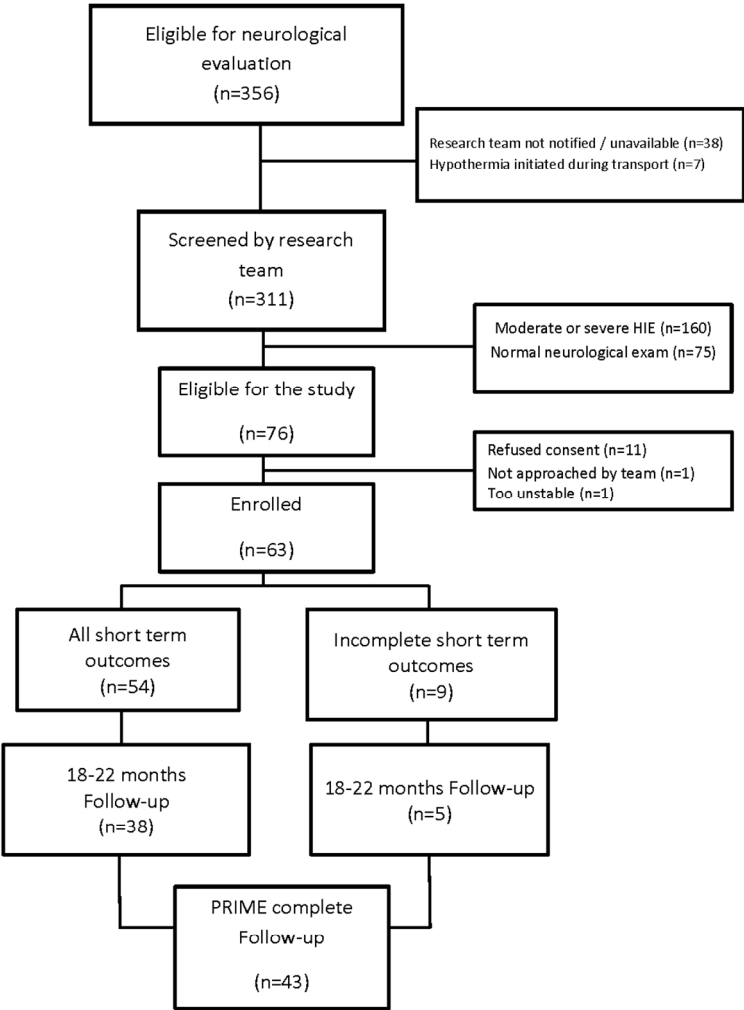
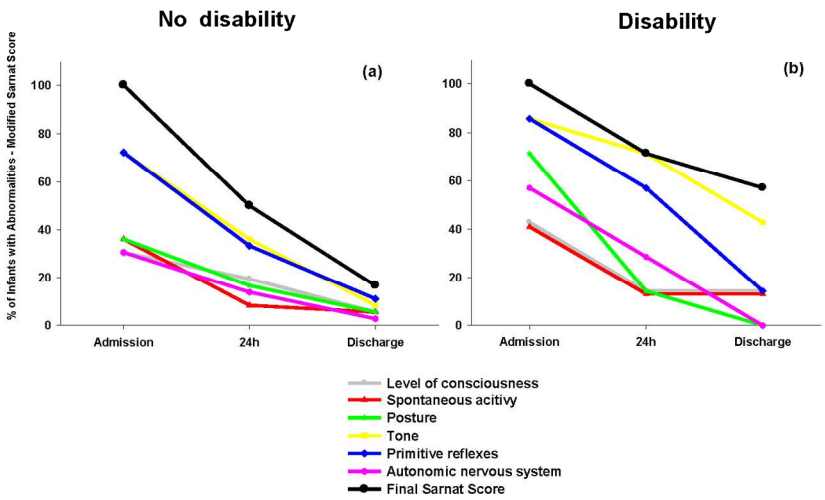


Figure 1. Patient enrollment

Flowchart of infants enrolled in PRIME. 5 Infants had incomplete Bayley evaluations and 12 (19%) infants were completely lost to follow up.

215x279mm (150 x 150 DPI)

Figure 2. Evolution of neonatal encephalopathy among infants without disability (2a) and those with disability (2b) at 18-22 months.



Evolution of neonatal encephalopathy among infants without disability (2a) and those with disability (2b) at 18-22 months.

215x279mm (200 x 200 DPI)