Evolution of Encephalopathy during Whole Body Hypothermia for Neonatal Hypoxic-Ischemic Encephalopathy

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Objective To examine the predictive ability of stage of hypoxic-ischemic encephalopathy (HIE) for death or moderate/severe disability at 18 months among neonates undergoing hypothermia.

Study design Stage of encephalopathy was evaluated at <6 hours of age, during study intervention, and at discharge among 204 participants in the National Institute of Child Health and Human Development Neonatal Research Network Trial of whole body hypothermia for HIE. HIE was examined as a predictor of outcome by regression models.

Results Moderate and severe HIE occurred at <6 hours of age among 68% and 32% of 101 hypothermia group infants and 60% and 40% of 103 control group infants, respectively. At 24 and 48 hours of study intervention, infants in the hypothermia group had less severe HIE than infants in the control group. Persistence of severe HIE at 72 hours increased the risk of death or disability after controlling for treatment group. The discharge exam improved the predictive value of stage of HIE at <6 hours for death/disability.

Conclusions On serial neurologic examinations, improvement in stage of HIE was associated with cooling. Persistence of severe HIE at 72 hours and an abnormal neurologic exam at discharge were associated with a greater risk of death or disability. *(J Pediatr 2012;160:567-72)*.

Clinical encephalopathy has been examined over the past 3 decades as an early predictor of neurologic and developmental outcome among term infants with intrapartum distress or hypoxia-ischemia. The Sarnat evaluation of staging of encephalopathy by clinical examination correlates well with subsequent neurodevelopmental impairment in infancy and childhood.[1-4] Other scoring systems adapted from the Sarnat evaluation are also used to predict outcome.[5,6]

Hypothermia to a target temperature of 33-34° C for 72 hours has currently been shown to reduce death or disability at 18 months or increase the rate of survivors without disabilities.[7-11] These trials have all included moderate/severe stage of encephalopathy[1] as eligibility criteria at study random assignment at <6 hours of age with 3 trials having the additional criteria for abnormal amplitude integrated electroencephalography.[7,9,11] The major trials have established a relationship between the stage of hypoxic-ischemic encephalopathy (HIE) at <6 hours of life and early childhood outcome. Relatively less is known about the relationship between stage of encephalopathy at other times in the neonatal period and later outcome. In the Cool Cap trial, infants with persistent moderate encephalopathy on day 4 had a more favorable prognosis after selective head cooling compared with standard care.[12] The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN) randomized controlled trial (RCT) data of serial neurologic examinations provided us an opportunity to examine the evolution of encephalopathy as a clinical biomarker and its relationship with outcome. Neurologic examinations over the first few days may serve as a marker of brain injury after hypoxia-ischemia as well as response to treatment. We hypothesized that the evolution of encephalopathy over the first 3 days may be a better predictor of death or disability than the stage of HIE at <6 hours of age and that failure to improve the stage of encephalopathy over the 72-hour intervention would be associated with a higher frequency of death or disability compared with those without persistent encephalopathy, and finally that the neurologic status at discharge would be a better predictor of outcome than the stage of HIE at <6 hours of age.

AUC	Area under the receiver operating characteristic curve
HIE	Hypoxic-ischemic encephalopathy
NICHD	National Institute of Child Health and Human Development
NRN	Neonatal Research Network
RCT	Randomized controlled trial

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Methods

This is a secondary analysis of the NICHD NRN RCT of whole body hypothermia for term infants with HIE. As part of the eligibility criteria, a modified Sarnat neurologic examination was performed at ≤6 hours of age. Additional examinations were performed at 24, 48, and 72 hours during the study intervention period and at discharge by a certified physician examiner. The certification process was as follows: each NRN site principal investigator was considered the gold standard examiner after orientation and review of the examination with the study subcommittee. Additional physician examiners reviewed the definitions of the components of the examination from the study manual of procedures and then performed neurologic examinations on 3 term infants, including 2 infants with abnormal findings, independent of the site principal investigator and within 1 hour of the examination performed by the principle investigator. A hard copy of the examinations was sent to the study Data Coordinating Center at RTI International, Research Triangle Park, North Carolina. The study lead investigator (S.S.) compared the examinations of the physician examiners with the site principal investigator and a physician was certified if all 3 examinations achieved concordance with that of the site principal investigator regarding stage of HIE.

Whole body hypothermia was performed for 72 hours at 33.5°C as described previously.[8] For study eligibility, encephalopathy was defined as the presence of moderate or severe encephalopathy in at least 3 of the following 6 categories: (1) level of consciousness: moderate (lethargy), severe (stupor or coma); (2) spontaneous activity: moderate (decreased), severe (no activity); (3) posture: moderate (distal flexion, complete extension), severe (decerebrate); (4) tone: moderate (hypotonia), severe (flaccid); (5) primitive reflexes, suck: moderate (weak), severe (absent) or Moro: moderate (incomplete), severe (absent); and (6) autonomic nervous system, pupils: moderate (constricted), severe (deviated, dilated or nonreaction to light) or heart rate: moderate (bradycardia), severe (variable) or respiration: moderate (periodic breathing), severe (apnea) (**Figure 1**; available at www.jpeds.com). Additional neurologic findings (presence of clinical seizures, increased tone, sustained clonus, fisted hand, abnormal movements, and absence of gag reflex) were noted during the evaluations at 24, 48, and 72 hours (±12-hour window). The discharge evaluation (performed within 3 days of discharge) was expanded to include the presence of an asymmetric tonic neck reflex and need for gavage or gastrostomy tube feeds. The presence of any of these findings was coded as abnormal.

All surviving infants were evaluated at 18-22 months of age by certified examiners trained to reliability and unaware of treatment assignment. Severe disability was defined as any one of the following: Bayley II Mental Developmental Index <70, Gross Motor Function Classification System level 3-5, hearing impairment requiring hearing aids, or blindness. Moderate disability was defined as Mental Developmental Index 70-84 and one or more of the following: Gross Motor Function Classification System level 2, hearing impairment with no amplification, or a persistent seizure disorder.

This study was performed in the 16 centers of the NICHD NRN. The institutional review board of each participating center approved the main trial protocol, and written informed consent was obtained from the parents of eligible infants prior to random assignment of study intervention.

Logistic and ordered logistic regression models were used for bivariate comparisons of HIE stage and length of time in HIE stage by treatment group (hypothermia vs control). In addition, a series of logistic regression models were conducted using SAS PROC GLIMMIX (SAS Institute, Cary, North Carolina) to examine the primary outcome, death or disability, by HIE stage, or length of time in HIE stage, after controlling for treatment group and HIE stage at random assignment. Goodness-of-fit between models was compared by the area-under-the-curve c statistic. Clinical center was included as a random effect to account for clustering of infants within center. Any comparisons regarding stage of HIE at the different time points were focused on the modified Sarnat evaluation. The exposure of infants to medications that may influence the neurologic examination (anticonvulsants, analgesics/sedatives, and neuromuscular blocking agents) was noted at the different time points and compared between the 2 groups. Medication use was not dictated by the study protocol but was per usual care at the clinical centers. Serum concentrations of medications were not obtained or documented as part of the study protocol.

Results

Of the 208 infants who participated in the RCT, 3 infants (control group) were lost to follow-up. One infant (hypothermia group) qualified for the study with clinical seizures but was missing a neurologic exam at randomization that would have assigned the infant to either moderate or severe stage of encephalopathy. Therefore, this analysis included a total of 101 infants in the hypothermia group and 103 infants in the control group. The number of infants who died or had missing examinations included the following: hypothermia group at 24 hours: 3 died, 3 missing exams; at 48 hours: 8 died, 4 missing exams; at 72 hours: 13 died, 3 missing exams and at discharge: 19 died and 5 missing examinations. In the control group, at 24 hours: 3 died, 3 missing exams; at 48 hours 8 died, 5 missing exams; at 72 hours, 10 died, 4 missing exams and at discharge: 28 died and 9 missing examinations. Among study infants, 64 of 174 infants had their 72 hour exam performed after the study intervention ended. Of these 64 infants, 27 were in the hypothermia group and the neurologic examination was performed within 4.3 ± 3.6 hours of initiation of rewarming, and in the control group, 37 infants had their examination performed 5.4 ± 6.6 hours after the 72-hour study intervention period.

Exposure to medications prior to study baseline, at baseline and at 24, 48, and 72 hours of study intervention

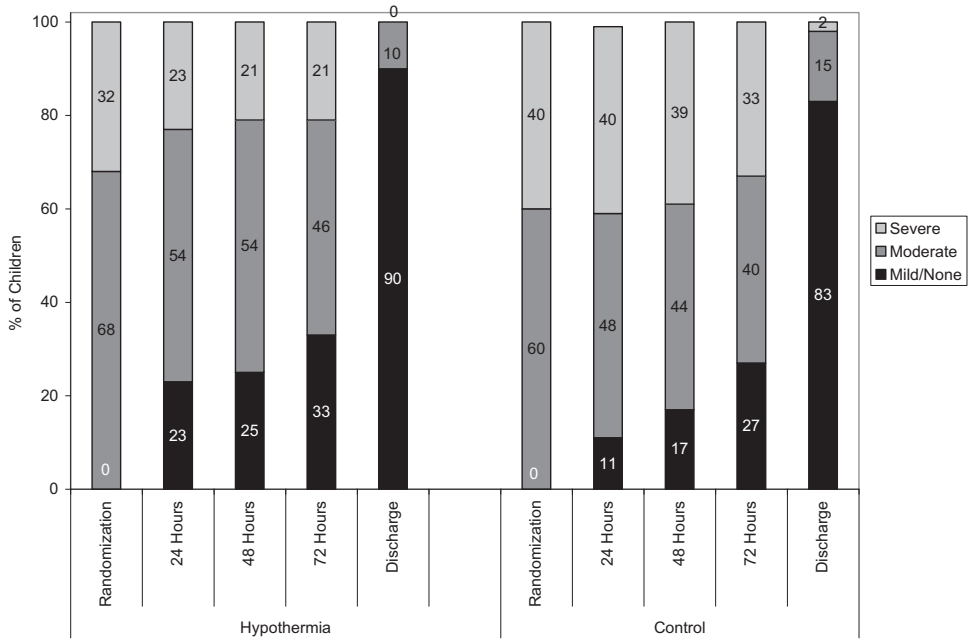

Figure 2. The stage of encephalopathy at <6 hours of age and at 24, 48, and 72 hours of study intervention and at discharge.

included: anticonvulsants, range 44%-58% of the infants; analgesics/sedatives, 16%-36%; and neuromuscular blocking agents, 4%-10% of infants. The frequency of exposure to each of these categories of medications, at each of these time points was comparable between the hypothermia and control group of infants (data not shown).

The stage of encephalopathy among infants who survived to the time points noted is shown in **Figure 2**. Although there were no significant differences in the stage of HIE among the 2 treatment groups at <6 hours of age (OR [95% CI = 0.70 (0.39, 1.25)]), $P = .23$, those in the hypothermia group had less severe encephalopathy at 24 hours [OR (95% CI) = 0.44 (0.25, 0.77)], $P = .004$, and at 48 hours [OR (95% CI) = 0.49 (0.28-0.87)], $P = .01$. The groups were comparable in stage of encephalopathy at 72 hours [OR (95% CI) = 0.65 (0.38-1.14)], $P = .13$, or at discharge [OR (95% CI) = 0.58 (0.22-1.54)], $P = .27$.

Improvement in stage of encephalopathy (from severe to moderate or moderate to mild/none) was achieved earlier among infants in the hypothermia group than those in the control group ($P = .04$). Among the infants in the hypothermia group, 34 (40%) experienced improvement in their stage of HIE within the first 24 hours of treatment, 14 (16%) between 48-72 hours, and 38 (44%) at ≥72 hours. Among the infants in the control group, 21 (24%) experienced improvement in 24 hours, 18 (20%) between 48-72 hours, and 50 (56%) at ≥72 hours. The groups did not differ significantly with respect to time before classification as none/mild HIE ($P = .07$). A deterioration (change in stage of encephalopathy from moderate to severe) occurred among 19 infants (8 hypothermia and 11 control) in the first 24 hours, 6 (2 hypothermia and 4 control) within 48 hours, and 3 hypothermia infants within 72 hours. Ten infants (7 hypothermia and 3 control) initially improved in stage of HIE to mild/none and then deteriorated to moderate encephalopathy within 72 hours.

The hypothermia group had significantly lower odds of the primary outcome of death or disability at 18 months (**Table I**). Based on the bivariate analyses (unadjusted), infants with more severe HIE at each time point had increased odds of death/disability. Similarly, increased risk of death/disability was associated with more than 72 hours (vs 24 hours) before improvement in the initial stage of HIE or before classification as none/mild HIE. At discharge, other than sustained clonus, the presence of any of the following was associated with significantly increased odds of death/disability: hypertonia, fisted hand, abnormal movements, absent gag reflex, or asymmetric tonic neck reflex.

The stage of HIE at <6 hours of age was a significant predictor of death/disability, after controlling for treatment group (**Table II**, Model 1). However, among survivors to 72 hours, after adding stage of HIE at 72 hours, the stage of HIE at <6 hours of age was no longer significant (**Table II**, Models 2 vs 3). The addition of stage of HIE at 72 hours significantly improved the model fit as measured by the area under the receiver operating characteristic curve (AUC) beyond that of stage of HIE at <6 hours of age and treatment group (0.87 vs 0.75, $P < .001$).

Among survivors to discharge, the stage of HIE at <6 hours of age and at discharge were significantly associated with death/disability while controlling for treatment in Model 5. Adding stage of HIE at discharge did not significantly improve the explanatory power of the model beyond that found with HIE stage at <6 hours of age and treatment group

Table I. Death/disability by stage of HIE and time in HIE stage

Variable	N	Death/disability			
		N (%)	Unadjusted OR	95% CI	*P*
Treatment group					
Hypothermia	101	45 (45)	0.49	0.28-0.86	.012
Control	103	64 (62)			
Stage of HIE at each time point					
<6 hours of age					
Severe	73	58 (79)	6.07	3.11-11.82	<.001
Moderate	131	51 (39)	REF		
24 hours					
Severe	61	54 (89)	55.93	15.11-207.02	<.001
Moderate	98	41 (42)	5.22	1.70-15.98	.004
Mild/none	33	4 (12)	REF		
48 hours					
Severe	54	48 (89)	66.00	17.27-252.19	<.001
Moderate	88	33 (38)	4.95	1.61-15.23	.005
Mild/none	37	4 (11)	REF		
72 hours					
Severe	47	41 (87)	81.99	21.64-310.65	<.001
Moderate	75	34 (45)	9.95	3.26-30.40	<.001
Mild/none	52	4 (8)	REF		
Discharge					
Severe/moderate	19	16 (84)	11.62	3.20-42.23	<.001
Mild/none	124	39 (31)	REF		
Time before improvement in stage of HIE					
Time before classification as none/mild HIE					
More than 72 hours	112	73 (65)	13.57	4.45-41.40	<.001
48-72 hours	29	2 (7)	0.54	0.09-3.17	.493
24 hours	33	4 (12)	REF		
Time before any improvement in HIE stage					
More than 72 hours	88	59 (67)	4.96	2.38-10.31	<.001
48-72 hours	32	5 (16)	0.45	0.15-1.38	.163
24 hours	55	16 (29)	REF		
Discharge findings					
Gavage/gastrostomy tube feedings					
Yes	32	27 (84)	16.97	5.97-48.24	<.001
No	116	28 (24)	REF		
Six additional findings					
Hypertonia					
Yes	35	24 (69)	5.87	2.56-13.48	<.001
No	107	29 (27)	REF		
Clonus					
Yes	12	6 (50)	1.76	0.54-5.78	.351
No	127	46 (36)	REF		
Fisted hand					
Yes	18	13 (72)	5.61	1.87-16.87	.002
No	120	38 (32)	REF		
Abnormal movements					
Yes	14	13 (93)	28.67	3.62-226.90	.002
No	125	39 (31)	REF		
Gag reflex absent					
Yes	33	25 (76)	9.26	3.74-22.95	<.001
No	107	27 (25)	REF		
Asymmetric tonic neck reflex					
Yes	15	9 (60)	3.12	1.04-9.37	.043
No	120	39 (33)	REF		

REF, reference category.
Moderate and severe HIE stage at discharge are combined because only one child had severe HIE at discharge.

(Table II, Models 4 vs 5; AUC 0.73 vs 0.68, $P = .07$). However, including the additional neurologic findings at discharge in Model 4 did significantly improve AUC over the base model (Table II, Models 6 vs 4; AUC 0.80 vs 0.68, $P = .003$). Those with mild/no HIE grade at discharge, but who had any of the additional findings, had significantly greater odds of death/disability than those with mild/no HIE who did not have the additional findings. Those with moderate/severe HIE at discharge had 8 times the odds of death/disability as those with mild/no HIE and no additional findings. The need for gastrostomy/gavage tube feeding at discharge was also associated with increased risk for death/disability.

Time before improvement in stage of HIE and time before classification as mild/no HIE were both significant predictors of death/disability (Table III) and both models (Model 1 and 2) had significantly higher AUC (0.84 and 0.86) than the model with treatment group and HIE grade at <6 hours of

Table II. Logistic regression models of death/disability by stage of HIE at <6 hours of age, 72 hours, and discharge

Variable	Adjusted OR	95% CI	P
All children (N = 204)			
Model 1: Stage of HIE at <6 hours of age			
Treatment group			
Hypothermia	0.51	0.28-0.94	.031
Control	REF		
HIE stage at <6 hours of age			
Severe	6.08	3.07-12.06	<.001
Moderate	REF		
Children assessed at 72 hours (N = 174)			
Model 2: Stage of HIE at <6 hours of age			
Treatment group			
Hypothermia	0.41	0.21-0.80	.009
Control	REF		
HIE stage at <6 hours of age			
Severe	5.54	2.66-11.53	<.001
Moderate	REF		
Model 3: HIE stage at <6 hours of age and 72 hours			
Treatment group			
Hypothermia	0.38	0.17-0.83	.016
Control	REF		
HIE stage at <6 hours of age			
Severe	2.25	0.94-5.37	.068
Moderate	REF		
HIE stage at 72 hours			
Severe	59.95	14.61-245.96	<.001
Moderate	9.15	2.85-29.39	<.001
Mild/none	REF		
Children assessed at discharge (N = 143)			
Model 4: Stage of HIE at <6 hours of age			
Treatment group			
Hypothermia	0.53	0.26-1.08	.082
Control	REF		
HIE stage at <6 hours of age			
Severe	3.01	1.35-6.72	.007
Moderate	REF		
Model 5: Stage of HIE at <6 hours of age and discharge			
Treatment group			
Hypothermia	0.57	0.27-1.20	.139
Control	REF		
HIE stage at <6 hours of age			
Severe	2.66	1.14-6.22	.025
Moderate	REF		
HIE stage at discharge			
Severe/moderate	9.85	2.61-37.16	<.001
Mild/none	REF		
Model 6: HIE stage at <6 hours of age and discharge findings			
Treatment group			
Hypothermia	0.58	0.25-1.37	.212
Control	REF		
HIE stage at <6 hours of age			
Severe	1.97	0.75-5.20	.168
Moderate	REF		
HIE stage/findings at discharge			
Severe/moderate	8.47	1.76-40.88	.008
Mild/none + additional findings	2.69	1.09-6.67	.033
Mild/none + no additional findings	REF		
Gavage/gastrostomy tube feeding at discharge	8.55	2.73-26.82	<.001

Models also include research center as a random effect.
AUC by model: 1 (AUC = 0.75), 2 (AUC = 0.75), 3 (AUC = 0.87), 4 (AUC = .68), 5 (AUC = 0.73), and 6 (AUC = 0.80).

Table III. Logistic regression models of death/disability by time before improvement in HIE stage

Variable	Adjusted OR	95% CI	P
Model 1: Time before classification as mild/none (N = 174)			
Treatment group			
Hypothermia	0.38	0.18-0.81	.013
Control	REF		
HIE stage at <6 hours of age			
Severe	3.44	1.52-7.77	.003
Moderate	REF		
Time before initial classification as none/mild HIE			
More than 72 hours	9.65	2.96-31.50	<.001
48-72 hours	0.43	0.07-2.75	.372
24 hours	REF		
Model 2: Time before any improvement in HIE stage (N = 175)			
Treatment group			
Hypothermia	0.43	0.20-0.95	.036
Control	REF		
HIE stage at <6 hours of age			
Severe	20.27	6.15-66.86	<.001
Moderate	REF		
Time before any improvement in HIE stage			
More than 72 hours	18.61	5.37-64.49	<.001
48-72 hours	0.74	0.17-3.21	.681
24 hours	REF		

Models also include research center as a random effect.
AUC: model 1 (AUC = 0.84) and model 2 (AUC = 0.87).

age (AUC 0.75, $P < .001$). Infants who did not improve the stage of HIE within the study intervention (72 hours) had significantly higher odds of death/disability than those who improved within the first 24 hours ($P < .001$).

Discussion

We have noted that infants who received whole body hypothermia for neonatal HIE had a higher likelihood of improving their stage of encephalopathy within 24 hours than infants in the control group. Persistence of the severe stage of HIE (compared with none/mild HIE) throughout the 72-hour study intervention period was associated with a greater chance of death or disability at 18 months of age. At the time of discharge, the presence of hypertonia, fisted hand, abnormal movements, absent gag reflex, an asymmetric tonic neck reflex, or need for gavage tube or gastrostomy feeds increased the risk for death or disability. In a secondary analysis of the Cool Cap Study, Gunn et al have noted that milder stage of encephalopathy at random assignment of cooling, greater improvement in encephalopathy to day 4, and the intervention of cooling were associated with favorable outcome at 18 months of age.[12]

The strength of this study is the information obtained from detailed neurologic examinations by physician examiners certified after rigorous training both during and after whole body hypothermia. A low rate of missing data and loss to follow-up provide another strength of the study. A neurologic

examination performed at multiple time points can be used as a clinical biomarker for prognostication. Using data from this study, it is possible to counsel parents regarding the possible risk of death or disability based on the stage of HIE during and at the end of the period of whole body hypothermia. The presence of continued severe encephalopathy at the end of 72 hours of cooling may reflect a more severe injury or ongoing injury distant from the time of the hypoxic-ischemic insult, as injury continues for days or weeks.[13,14] The information obtained from this study evaluating stage of HIE at the end of 72 hours of cooling raises the question whether cooling to 72 hours at 33.5°C is adequate or whether either the duration or depth of cooling should be increased.[15] This question is being addressed in an ongoing trial.

The limitations of this study are that the study did not distinguish between mild encephalopathy and no encephalopathy.

The neurologic examination at <6 hours of age has been shown to be a clinical biomarker of patient selection for neuroprotection. The results of our findings and those of the Cool Cap Study demonstrate that the neurologic examination at the end of hypothermia therapy continues to be a good predictor of outcome. The effects of cooling on clinical examinations and on the amplitude integrated electroencephalographic recovery[16] are highly consistent. The prevalence of abnormalities on the discharge examination is low; however, we have noted that when present, they are helpful in predicting death or disability. On the other hand, if abnormalities at discharge are not noted, some risk of disability cannot be ruled out.

There is increasing interest in identification of early biomarkers as a surrogate of childhood outcome following neonatal HIE.[1-6,17-20] It is not known whether an early/short-term clinical biomarker such as early neurologic examination can be used in lieu of long-term outcome. Biomarkers have limitations, the home environment influences outcome following hospital discharge, and the neonatal brain has the ability to recover from injury. Our research indicates that neurologic findings during and following hypothermia treatment are valuable for informing such predictions. ■

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Figure 1. Classification of infants according to the stage of encephalopathy.

Appendix 1

Members of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Neonatal Research Network

The Hypothermia Study Group

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Appendix 2

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