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Serum Biomarkers of MRI Brain Injury in Neonatal Hypoxic Ischemic Encephalopathy Treated With Whole-Body Hypothermia: A Pilot Study

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Abstract

Objectives—To determine if candidate biomarkers, ubiquitin carboxyl-terminal esterase L1 and glial fibrillary acidic protein, are elevated in neonates with hypoxic ischemic encephalopathy who die or have severe MRI injury compared with surviving infants with minimal or no injury on brain MRI.

Design—Prospective observational study.

Setting—Level IIIC outborn neonatal ICU in a free-standing children's hospital.

Patients—Term newborns with moderate-to-severe hypoxic ischemic encephalopathy referred for therapeutic hypothermia

Interventions—Serum specimens were collected at 0, 12, 24, and 72 hours of cooling. MRI was performed in surviving infants at target 7–10 days of life and was scored by a pediatric neuroradiologist masked to biomarker and clinical data.

Measurements and Main Results—Serial biomarker levels were determined in 20 hypoxic ischemic encephalopathy patients. Ubiquitin carboxyl-terminal esterase L1 was higher at initiation and 72 hours of cooling, while glial fibrillary acidic protein was higher at 24 and 72 hours in babies with adverse outcome compared with those with favorable outcome.

Conclusions—This preliminary data support further studies to evaluate ubiquitin carboxyl-terminal esterase L1 and glial fibrillary acidic protein as immediate biomarkers of cerebral injury severity in newborns with hypoxic ischemic encephalopathy.

Keywords

asphyxia; biomarker; hypoxic ischemic encephalopathy; neonate; therapeutic hypothermia

Neonatal hypoxic ischemic encephalopathy (HIE) is a potentially devastating disorder associated with significant mortality and long-term morbidity in survivors (1, 2). Therapeutic hypothermia has recently been demonstrated to improve outcome in babies presenting with HIE. Further work is needed, however, as death and disability continue to occur in 30% to 70% of infants with moderate-to-severe encephalopathy despite treatment with cooling (3–5). Optimal depth and duration of cooling requires further refinement and adjuvant neurotherapies. Early serum biomarkers that reflect brain injury severity can be used to identify candidates for therapeutic interventions, gauge response to treatment, and serve as surrogate outcomes for future neurotherapeutic trials. Although several small studies have evaluated a variety of candidate neurologic biomarkers, no serum biomarker has been demonstrated to have sufficient reliability to advance into clinical use in patients with HIE (6). Further investigation to establish novel biomarkers of cerebral injury is needed.

Ubiquitin carboxyl-terminal esterase L1 (UCHL1) is a cytoplasmic enzyme selectively expressed in neurons (7). Cerebrospinal fluid (CSF) and/or serum UCHL1 levels have been demonstrated to be elevated in other neurologic diseases, such as subarachnoid hemorrhage (8), traumatic brain injury (9, 10), and animal/human studies of circulatory arrest during cardiovascular surgery (11, 12). Glial fibrillary acidic protein (GFAP) is a cytoskeletal protein specific to astrocytes in the central nervous system (13, 14). Increased serum levels have been reported in adult patients with acute traumatic brain injury (15, 16), cardiac arrest (17), and in infants and children undergoing extracorporeal membrane oxygenation life support (18). Few studies have evaluated either of these brain-specific proteins as potential serum biomarkers of brain injury in newborns with encephalopathy (19, 20).

The aim of this pilot study was to evaluate the utility of UCHL1 and GFAP as potential biomarkers of brain injury in babies with HIE. We hypothesized that UCHL1 and GFAP would be higher in infants who died or had severe MRI brain injury compared with surviving infants with minimal or no MRI injury.

Materials and Methods

Subjects

Subjects were participants in a prospective observational study evaluating predictors of outcome in hypothermia-treated neonatal encephalopathy. All infants referred to a level III out-born neonatal ICU (NICU) for treatment with whole-body hypothermia were approached for enrollment. Established National Institute of Child Health and Human Development (NICHD) inclusion and exclusion criteria were used to identify infants:

gestational age greater than 36 weeks, birth weight greater than 1,800 g, demonstrated metabolic acidosis and/or low Apgar scores/prolonged delivery room resuscitation, and exhibited signs of moderate-to-severe clinical encephalopathy. Infants with major congenital anomalies, suspected chromosomal abnormalities, or who presented in extremis were excluded (4). Parents of eligible participants provided initial verbal agreement with this minimal risk study either in person (at time of transfer) or via telephone (if parent not available at bedside on admission). This was followed by written informed consent for all patients continuing in the secondary longitudinal aspect of the study. The study was approved by the Institutional Review Board, and a Waiver of Documentation of Informed Consent was obtained to allow for the initial verbal consent used at enrollment. All data were collected in compliance with Health Information Portability and Accountability Act regulations. Participants with available stored serum were selected from the overall cohort for this study.

Clinical Management During Hypothermia

Infants were cooled to 33.5°C for 72 hours, followed by rewarming by 0.5°C/hour over 6 hours according to the NICHD protocol (3). Infants treated with hypothermia underwent routine continuous cerebral saturation monitoring with near-infrared spectroscopy (NIRS) and video electroencephalography monitoring with surface electrode placement according to the international 10–20 system modified for neonates. NIRS/electroencephalography monitoring was initiated as soon as possible after admission and continued through at least 12 hours after completion of rewarming. Medical management protocols include maintenance of euglycemia, fluid restriction to maintain high normal serum sodium levels (135–145 mEq/L), ventilatory support to maintain normocarbica (PaCO₂: 40–50), vasopressor support to maintain high normal systemic blood pressure (mean arterial blood pressure: 45–55 mm Hg) to promote cerebral perfusion, and timely treatment of coagulopathy and seizures. Postcooling imaging with MRI is performed in all surviving infants as described below.

Measurements

Data Collection—Demographic information and pertinent maternal and perinatal history were collected from the referral hospital maternal and newborn records. Clinical data from the infants' NICU course and status at final disposition were also recorded.

UCHL1 and GFAP Determinations—Blood specimens were collected at 0 (initiation), 12, 24, and 72 hours of cooling from indwelling umbilical arterial or venous lines. These time points coincided with routine clinical laboratory monitoring in patients undergoing hypothermia. Samples were centrifuged at 500 rpms and supernatants were stored at –70°C to allow for later assay in bulk. Stored serum from specimens obtained for clinical purposes at the time points of interest were used in the few cases where parents could not be initially reached for consent. Salvaged clinical specimens were likewise frozen at –70°C as soon as possible after processing and within 24 hours of collection. UCHL1 and GFAP levels were determined by sandwich enzyme-linked immunosorbent assay (ELISA) developed by Banyan Biomarkers (21) from 25 µL of serum for each assay. The inter- and intra-assay precision, as measured by the coefficient of variance (% CV), is lesser than 10% to 12% for

both measurements and no known cross-reactivity with other endogenous compounds has been reported.

Magnetic Resonance Imaging—MRI was performed in surviving infants at target age 7–10 days of life on a 1.5-Tesla scanner (Signa, General Electric, Milwaukee). Standard sequences included sagittal and axial spin-echo T1, double-acquisition axial fast spin-echo (FSE) T2 proton density images, coronal FSE T2, and axial diffusion-weighted images. Images were reviewed by two neuroradiologists (Drs. Kadom and Vezina) masked to clinical data and biomarker levels. Images were scored according to Barkovich with deep nuclear gray injury assigned a basal ganglia (BG) score ranging 0–4 and cortical/ white matter injury assigned a watershed score (WS) ranging 0–5 (22). Discrepancies in scoring were resolved by consensus. Patients were classified into two groups: group 1 = normal-to-mild injury (any BG score < 3 and WS < 4; Fig. 1A); group 2 = severe injury (BG ≥ 3 and WS ≥ 4; Fig. 1B) or died prior to MRI.

Data Analysis

Descriptive statistics are presented as medians (ranges) and frequencies for continuous and categorical variables, respectively. Means and *SD* are presented for parametric data. Group comparisons made with Mann-Whitney *U* tests, independent samples *t* test, and Fisher's exact tests were appropriate. Receiver operating curve (ROC) analyses were performed to evaluate the sensitivity and specificity of alternative cutoff values for each biomarker. ROC area under the curve (AUC) is presented as the variable estimate where values range from 0.5 (no predictive ability) to 1 (perfect discrimination of outcome). ROC analyses were also performed for clinical variables that differed significantly by outcome group in order to compare the relative magnitude of predictive ability (i.e., AUC) between biomarkers and clinical variables often used for prognostication. Statistical analyses were performed with SPSS 18.0 (SPSS, Chicago, IL).

Results

Serial biomarker levels were analyzed for 20 term newborns with moderate-to-severe encephalopathy. Demographic and clinical characteristics of the study population are presented in Table 1. Four patients died following withdrawal of care owing to poor neurologic prognosis based on clinical and electroencephalographic information. One of these patients died prior to 72 hours of cooling and thus had measurements at three of the four time points of interest. MRI was performed in all surviving infants at a median age of 9 (range: 5–14) days of life. Severe MRI injury was present in five infants. Significant deep nuclear gray injury was observed in three infants, while two infants had global MRI injury. Two of the infants with severe MRI injury required gastrostomy tube placement, while all infants with normal-to-mild injury were discharged home feeding independently. Infants in the poor outcome group were more likely to have severe encephalopathy, lower Apgar score at 10 minutes of life, and higher base deficit on initial blood gas. Otherwise, there were no significant differences in demographic or clinical data between outcome groups.

UCHL1 was significantly higher at initiation ($p = 0.005$) and at 72 hours ($p = 0.039$) of cooling, while GFAP was significantly higher at 24 ($p = 0.003$) and 72 hours ($p = 0.002$) in

babies who died or had severe MRI injury compared with survivors with mild or no injury on MRI (Fig. 2). Corresponding ROC curves are presented in Figure 3. Level at initiation of cooling (T0) was the best discriminator of outcome for UCHL1 (AUC = 0.852, $p = 0.010$). Conversely, GFAP at completion of cooling (T72) had the highest AUC (0.920, $p = 0.002$). These values were higher than other variables that were associated with adverse outcome (encephalopathy grade ROC AUC = 0.788, severe electroencephalography background at 24 hours AUC = 0.717, Apgar at 10 minutes AUC = 0.784, and presenting base-deficit AUC = 0.810).

Cutoff values were selected from the coordinates of the ROC curve for optimal predictive ability (i.e., highest combined sensitivity and specificity). At initiation of cooling, a UCHL1 cut-point value of 13.8 ng/mL had a sensitivity of 75%, specificity of 100%, positive predictive value (PPV) of 78%, and negative predictive value (NPV) of 90% for predicting adverse outcome. For GFAP at 72 hours of cooling, a cutpoint of 0.2 had a sensitivity of 87.5%, specificity of 82%, PPV of 78%, and NPV of 90%.

Discussion

Data from this pilot study suggest that both UCHL1 and GFAP are associated with death or severe MRI brain injury in newborns with HIE and have potential value as biomarkers of cerebral injury. Identifying valid biomarkers of brain injury is important for improving outcome after perinatal brain injury because they can be used to identify and risk stratify patients for neurotherapeutic interventions, gage response to therapy, and provide early prognostic information for families. In particular, serum markers that can be rapidly measured and objectively interpreted would offer advantages over current tools used to assess degree of brain injury in this population, such as clinical examination (23), brain MRI (22, 24), and electroencephalographic data (25, 26), which are subjective and/or require equipment and technical/interpretive expertise not available at all centers. Development of an early reliable laboratory test that reflects severity of brain injury is key to the treatment of these high-risk newborns in whom a narrow therapeutic window exists. Whether UCHL1 and/or GFAP will serve this role remains to be determined, but this study supports further investigation.

Previously evaluated candidate biomarkers in babies with HIE include a variety of inflammatory cytokines (27–29), physiologic markers of hypoxia-ischemia or oxidative stress (30–32), and brain-specific proteins (33–36). A recent meta-analysis concluded that while various blood, urine, and CSF markers have demonstrated promise, none have been studied extensively enough to warrant routine clinical use (6). Brain-specific proteins offer advantages as biomarkers over proteins involved with systemic processes as the latter may be elevated in severe illness while not being specifically representative of cerebral injury. We previously evaluated S100B and NSE in our cohort of HIE babies treated with cooling and demonstrated a significant association with death or severe MRI injury (37). However, limited sensitivity and specificity and variable predictive value reported in other studies (33, 36) tempers enthusiasm for continued study of these candidate biomarkers. Identification and validation of novel biomarkers are needed.

UCHL1 has several characteristics that argue for its desirability as a neurologic biomarker: it is brain specific, relatively abundant (1% to 5% total soluble brain protein), and of low-molecular weight (24 kDa [21]). It has also been demonstrated to be resistant to endogenous brain and serum proteases (8). Also known as neuronal-specific protein gene product (PGP) 9.5, UCHL1 is concentrated in the perikarya and dendrites of neurons in the central nervous system (7). While UCHL1 has been evaluated as a potential biomarker of brain injury in other neurological diseases (8–12); to our knowledge, only one prior study has been performed in newborns with encephalopathy. Douglas -Escobar and colleagues (19) reported that UCHL1 levels were elevated in 14 encephalopathic newborns (five who were treated with hypothermia) compared with a control population of healthy neonates. While they reported that UCHL1 levels had limited ability to discriminate babies with abnormal MRIs from those with normal studies, they demonstrated higher UCHL1 in a subgroup of infants who died or had cortical injury on MRI. These investigators analyzed blood specimens collected at variable times with pooling of results over 6- to 48-hour periods for comparison between outcome groups. As demonstrated by this study, UCHL1 levels change dramatically over the first 24–72 hours after birth, making comparisons at early specified time points diagnostically relevant.

GFAP is a 50 kDa intermediate filament protein that serves as a major constituent of the astroglial cytoskeleton and is involved in maintenance of the blood-brain barrier (13, 14). Animal data indicate that cerebral ischemia results in a widely distributed up regulation of GFAP expression and that this up regulation can be seen even at 30 days after reperfusion (38, 39). Its utility as a biomarker in neonates with encephalopathy has been explored by two prior studies. Blennow and colleagues (34) demonstrated that GFAP measured in CSF was elevated in asphyxiated newborns who died compared with surviving infants and healthy controls. The utility of a CSF biomarker is limited as CSF is not routinely sampled in infants presenting with encephalopathy and is often precluded by medical instability in the most critically ill patients. A more recent study evaluated serum levels of GFAP in 23 encephalopathic newborns treated with hypothermia (20). These investigators reported that daily GFAP levels during the first week of life were elevated in babies who had abnormal MRI (assessed by qualitative interpretation) compared with those with normal MRI. These differences were significant at 24 and 48 hours, and for days 4–7 of life (following rewarming). Our results were consistent with the findings of this study, demonstrating that infants with rising GFAP levels after 24 hours of life are at risk for adverse outcome despite treatment with cooling. This delayed rise in GFAP may be secondary to 1) its temporal profile and accumulation after postischemic up regulation, 2) the relatively larger molecular weight that impacts its ability to cross the blood-brain barrier compared with UCHL1 that rises acutely, and/or 3) its continued up regulation because of ongoing secondary injury in some infants. These patients may benefit from alterations in hypothermia protocols (i.e., deeper, longer cooling) or adjuvant therapies. Future investigations will need to include postrewarming time points to establish the time course of rising GFAP in infants with ongoing injury.

That UCHL1 offered greatest discriminatory ability at initiation of cooling, while GFAP levels best differentiated outcome at the completion of cooling supports the concept that a panel of biomarkers may offer more information and thus higher predictive value than any

single biomarker taken alone. High UCHL1 protein levels soon after birth in the adverse outcome group likely represents significant acute neuronal loss, while rising GFAP signifies ongoing secondary injury and associated gliosis. Individualized profiles of markers may be useful in tailoring neurotherapeutic regimens, particularly needed considering encephalopathy in newborns may be associated with different etiologies, timing of initial insult, and mechanisms of evolving injury. Once validated biomarkers are selected to move forward from research and development investigations, such as this study, issues surrounding translation to clinical care, such as availability of the assay, turn-around time (ideally development into a point-of-care bedside determination), and cost will need to be assessed.

Limitations of this study include small sample size, missing data (two subjects did not have initial blood gas performed by 1 hr of life and another two subjects did not have 10-minute Apgar assigned), and lack of follow-up information. The missing data and sample size limitations precluded controlling for these significant covariables via interpretable logistic regression analyses. Clinical predictors, such as grade of encephalopathy, initial base deficit, and Apgar score at 10 minutes have been shown to have prognostic value in encephalopathic newborns treated with hypothermia (40–42). Likewise, these covariables differed between outcome groups in our study population. Therefore, ROC analyses were performed in order to offer some measure of the predictive abilities of the proposed biomarkers in comparison with these clinical bedside variables. These ROC analyses demonstrated that UCHL1 at 0 hours and GFAP at 72 hours of cooling had higher AUC than any of these clinical factors. Sample size further limited our ability to evaluate death and severe MRI injury as individual outcomes or if biomarkers were associated with specific patterns of injury. Further investigation is needed (and underway) to confirm these preliminary findings and evaluate if these biomarkers, alone or in conjunction as a panel with other candidate brain-specific proteins (36), offer predictive value over and above clinically assessable variables in current use. Additional covariables associated with secondary injury, such as seizures, fever, hypotension, and hypoxia will also need to be considered as these will also have potential to affect biomarker trajectory. It is possible that the wide range of biomarker levels, noted in this pilot data, may not allow for adequate discrimination once covariables are considered.

Because determination of these biomarker levels was not the primary aim of this observational study, selection of patients may be subject to bias. Criteria for selection were based solely on having adequate remaining serum at each time point for biomarker assay. Thus, outcome groups were not evenly matched for comparison. Additionally, serum from healthy controls without encephalopathy was not available for comparison. However, as the goal of this study was to determine if these potential biomarkers could discriminate infants with severe brain injury among at-risk infants, incorporation of a healthy control group was not necessary to study aims. It has been demonstrated by prior studies that infants without neurological compromise have low values of these proteins in circulation—median healthy newborn, UCHL1 = 1.7 ng/mL (19) and daily-GFAP levels in nonencephalopathic NICU controls less than 0.1 ng/mL (20). It is acknowledged that technical variables, such as time to processing, effect of hemolysis, and effects of temperature may affect reproducibility and reliability results, particularly in salvaged clinical specimens used in some patients. Clinical specimens, however, were collected and handled in a comparable manner to specimens

collected specifically for research purposes (i.e., collected by bedside NICU nurses, processed in central laboratory within 1 hour of collection, and remaining serum frozen within 24 hours). Finally, as this study included only infants treated with whole-body hypothermia, findings may not be generalizable to normothermic HIE infants or infants treated with selective-head cooling. Planned future studies should include healthy newborn controls (and if possible infants treated with selective head cooling or normothermic HIE infants who missed cooling) for comparison.

Severe MRI brain injury was used as a short-term outcome measure for this study, as there is clear evidence that severe MRI abnormality is highly predictive of later deficits (22, 24). Although the well-described scoring system utilized in this study has not been reevaluated or validated for use in the postcooling era, other studies have demonstrated that the predictive value of qualitative MRI interpretation does not appear to be affected by treatment with hypothermia (43). Dependence on MRI as a leading qualified surrogate outcome marker in this population has been supported by the recent executive summary from the National Institutes of Health (44). However, reliability of brain MRI as a surrogate for later disability may be subject to differences in imaging protocols (i.e., timing of acquisition, image variables, and sequences used) as well as interrater reliability among neuroradiologists. It is recognized that some infants with less severe MRI injury will go on to have significant impairments and prediction of functional impairment with varying degrees of injury on conventional MRI is imprecise. Therefore, a dichotomized outcome was used to characterize severe injury where later significant neurologic disability would be unambiguous (22, 24). Predictive values from this study should be interpreted with caution given that patients classified as normal-to-mild injury may go on to exhibit some degree of later neurodevelopmental compromise. Future studies will need to assess whether these biomarkers are able to distinguish moderately affected infants from those with severe injury as defined in this pilot study. Further study evaluating the association of these promising biomarkers with long-term neurodevelopmental outcome (planned in this and future cohorts) are needed before application to therapeutic response monitoring or prognostication can be considered.

Conclusions

High UCHL1 at cooling initiation and GFAP after 24 hours of cooling appear to be predictive of death or severe MRI brain injury in term newborns treated with hypothermia for signs of hypoxic-ischemic encephalopathy. These brain-specific proteins may be useful biomarkers of neurologic injury in this population, making it possible to stratify infants according to the severity of the initial insult as well as response to hypothermic therapy. Further study is needed to confirm these promising findings and to correlate UCHL1 and GFAP levels with lasting neurological impairment after perinatal brain injury.

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References

1. Shankaran S, Woldt E, Koepke T, et al. Acute neonatal morbidity and long-term central nervous system sequelae of perinatal asphyxia in term infants. *Early Hum Dev.* 1991; 25:135–148. [PubMed: 1713544]
2. Dilenge ME, Majnemer A, Shevell MI. Long-term developmental outcome of asphyxiated term neonates. *J Child Neurol.* 2001; 16:781–792. [PubMed: 11732762]
3. Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med.* 2009; 361:1349–1358. [PubMed: 19797281]
4. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med.* 2005; 353:1574–1584. [PubMed: 16221780]
5. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: Multi-centre randomised trial. *Lancet.* 2005; 365:663–670. [PubMed: 15721471]
6. Ramaswamy V, Horton J, Vandermeer B, et al. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. *Pediatr Neurol.* 2009; 40:215–226. [PubMed: 19218035]
7. Wilkinson KD, Lee KM, Deshpande S, et al. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science.* 1989; 246:670–673. [PubMed: 2530630]
8. Lewis SB, Wolper R, Chi YY, et al. Identification and preliminary characterization of ubiquitin C terminal hydrolase 1 (UCHL1) as a biomarker of neuronal loss in aneurysmal subarachnoid hemorrhage. *J Neurosci Res.* 2010; 88:1475–1484. [PubMed: 20077430]
9. Papa L, Akinyi L, Liu MC, et al. Ubiquitin C-terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. *Crit Care Med.* 2010; 38:138–144. [PubMed: 19726976]
10. Siman R, Toraskar N, Dang A, et al. A panel of neuron-enriched proteins as markers for traumatic brain injury in humans. *J Neurotrauma.* 2009; 26:1867–1877. [PubMed: 19811094]
11. Siman R, Roberts VL, McNeil E, et al. Biomarker evidence for mild central nervous system injury after surgically-induced circulation arrest. *Brain Res.* 2008; 1213:1–11. [PubMed: 18456245]
12. Arnaoutakis GJ, George TJ, Wang KK, et al. Serum levels of neuron-specific ubiquitin carboxyl-terminal esterase-L1 predict brain injury in a canine model of hypothermic circulatory arrest. *J Thorac Cardiovasc Surg.* 2011; 142:902–910.e1. [PubMed: 21924148]
13. Eng LF, Vanderhaeghen JJ, Bignami A, et al. An acidic protein isolated from fibrous astrocytes. *Brain Res.* 1971; 28:351–354. [PubMed: 5113526]
14. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res.* 2000; 25:1439–1451. [PubMed: 11059815]
15. Lumpkins KM, Bochicchio GV, Keledjian K, et al. Glial fibrillary acidic protein is highly correlated with brain injury. *J Trauma.* 2008; 65:778–782. [PubMed: 18849790]
16. Honda M, Tsuruta R, Kaneko T, et al. Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S-100B and neuron-specific enolase. *J Trauma.* 2010; 69:104–109. [PubMed: 20093985]
17. Kaneko T, Kasaoka S, Miyauchi T, et al. Serum glial fibrillary acidic protein as a predictive biomarker of neurological outcome after cardiac arrest. *Resuscitation.* 2009; 80:790–794. [PubMed: 19411130]
18. Bembea MM, Savage W, Strouse JJ, et al. Glial fibrillary acidic protein as a brain injury biomarker in children undergoing extracorporeal membrane oxygenation. *Pediatr Crit Care Med.* 2011; 12:572–579. [PubMed: 21057367]
19. Douglas-Escobar M, Yang C, Bennett J, et al. A pilot study of novel biomarkers in neonates with hypoxic-ischemic encephalopathy. *Pediatr Res.* 2010; 68:531–536. [PubMed: 20736881]
20. Ennen CS, Huisman TA, Savage WJ, et al. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling. *Am J Obstet Gynecol.* 2011; 205:251.e1–251.e7. [PubMed: 21784396]

21. Liu MC, Akinyi L, Scharf D, et al. Ubiquitin C-terminal hydrolase-L1 as a biomarker for ischemic and traumatic brain injury in rats. *Eur J Neurosci.* 2010; 31:722–732. [PubMed: 20384815]
22. Barkovich AJ, Hajnal BL, Vigneron D, et al. Prediction of neuromotor outcome in perinatal asphyxia: Evaluation of MR scoring systems. *AJNR Am J Neuroradiol.* 1998; 19:143–149. [PubMed: 9432172]
23. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol.* 1976; 33:696–705. [PubMed: 987769]
24. Miller SP, Ramaswamy V, Michelson D, et al. Patterns of brain injury in term neonatal encephalopathy. *J Pediatr.* 2005; 146:453–460. [PubMed: 15812446]
25. Holmes G, Rowe J, Hafford J, et al. Prognostic value of the electroencephalogram in neonatal asphyxia. *Electroencephalogr Clin Neurophysiol.* 1982; 53:60–72. [PubMed: 6173201]
26. Obrecht R, Pollock MA, Evans S, et al. Prediction of outcome in neonates using EEG. *Clin Electroencephalogr.* 1982; 13:46–49. [PubMed: 7067153]
27. Aly H, Khashaba MT, El-Ayouty M, et al. IL-1beta, IL-6 and TNF-alpha and outcomes of neonatal hypoxic ischemic encephalopathy. *Brain Dev.* 2006; 28:178–182. [PubMed: 16181755]
28. Bartha AI, Foster-Barber A, Miller SP, et al. Neonatal encephalopathy: Association of cytokines with MR spectroscopy and outcome. *Pediatr Res.* 2004; 56:960–966. [PubMed: 15496611]
29. Sävman K, Blennow M, Gustafson K, et al. Cytokine response in cerebrospinal fluid after birth asphyxia. *Pediatr Res.* 1998; 43:746–751. [PubMed: 9621983]
30. Huang CC, Wang ST, Chang YC, et al. Measurement of the urinary lactate:creatinine ratio for the early identification of newborn infants at risk for hypoxic-ischemic encephalopathy. *N Engl J Med.* 1999; 341:328–335. [PubMed: 10423467]
31. Dellenbach P, Haberey P. Lactate as indicator for fetal and neonatal asphyxia. *Lancet.* 1982; 1:907. [PubMed: 6122121]
32. Dorrepaal CA, Berger HM, Benders MJ, et al. Nonprotein-bound iron in postasphyxial reperfusion injury of the newborn. *Pediatrics.* 1996; 98:883–889. [PubMed: 8909481]
33. Nagdyman N, Grimmer I, Scholz T, et al. Predictive value of brain-specific proteins in serum for neurodevelopmental outcome after birth asphyxia. *Pediatr Res.* 2003; 54:270–275. [PubMed: 12736385]
34. Blennow M, Sävman K, Ilves P, et al. Brain-specific proteins in the cerebrospinal fluid of severely asphyxiated newborn infants. *Acta Paediatr.* 2001; 90:1171–1175. [PubMed: 11697430]
35. Tekgul H, Yalaz M, Kutukculer N, et al. Value of biochemical markers for outcome in term infants with asphyxia. *Pediatr Neurol.* 2004; 31:326–332. [PubMed: 15519113]
36. Thorngren-Jerneck K, Alling C, Herbst A, et al. S100 protein in serum as a prognostic marker for cerebral injury in term newborn infants with hypoxic ischemic encephalopathy. *Pediatr Res.* 2004; 55:406–412. [PubMed: 14630979]
37. Massaro ANCT, Kadom N, Tsuchida T, et al. Biomarkers of brain injury in neonatal encephalopathy treated with hypothermia. *J Pediatr.* 2012; 161:434–440. [PubMed: 22494878]
38. Valentim LM, Michalowski CB, Gottardo SP, et al. Effects of transient cerebral ischemia on glial fibrillary acidic protein phosphorylation and immunocontent in rat hippocampus. *Neuroscience.* 1999; 91:1291–1297. [PubMed: 10391436]
39. Bidmon HJ, Jancsik V, Schleicher A, et al. Structural alterations and changes in cytoskeletal proteins and proteoglycans after focal cortical ischemia. *Neuroscience.* 1998; 82:397–420. [PubMed: 9466450]
40. Ambalavanan N, Carlo WA, Shankaran S, et al. Predicting outcomes of neonates diagnosed with hypoxemic-ischemic encephalopathy. *Pediatrics.* 2006; 118:2084–2093. [PubMed: 17079582]
41. Wyatt JS, Gluckman PD, Liu PY, et al. Determinants of outcomes after head cooling for neonatal encephalopathy. *Pediatrics.* 2007; 119:912–921. [PubMed: 17473091]
42. Gunn AJ, Wyatt JS, Whitelaw A, et al. Therapeutic hypothermia changes the prognostic value of clinical evaluation of neonatal encephalopathy. *J Pediatr.* 2008; 152:55–8. 58.e1. [PubMed: 18154900]

43. Rutherford M, Ramenghi LA, Edwards AD, et al. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: A nested substudy of a randomised controlled trial. *Lancet Neurol.* 2010; 9:39–45. [PubMed: 19896902]
44. Higgins RD, Raju T, Edwards AD, et al. Hypothermia and other treatment options for neonatal encephalopathy: An executive summary of the Eunice Kennedy Shriver NICHD workshop. *J Pediatr.* 2011; 159:851–858.e1. [PubMed: 21875719]

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Figure 1. MRI classification of severe brain injury. Representative T1- and T2-weighted images from infants with normal MRI (A) and severe injury (B). Note the diffuse cortical and basal ganglia/thalamic signal abnormality for the patient with severe (global) MRI injury.

Figure 2.

Levels of ubiquitin carboxyl-terminal esterase L1 (UCHL1) (A) and glial fibrillary acidic protein (GFAP) by outcome group over time (B). Box and whisker plots show median, interquartile range (IQR), and values within ± 1.5 of IQR. Outliers are depicted by open circles. Mann-Whitney U p values are shown for comparison between outcome groups.

Figure 3.

Receiver operating curves (ROC) for ubiquitin carboxylterminal esterase L1 (UCHL1) at 0 and 72 hours of cooling (**A**) and glial fibrillary acidic protein (GFAP) at 24 and 72 hours of cooling (**B**). AUC = area under the curve.

Table 1
Demographic and Clinical Characteristics of Study Population by Outcome Category

	Total (<i>n</i> = 20)	Normal-to-Mild MRI injury (<i>n</i> = 11)	Death or Severe MRI injury (<i>n</i> = 9)	<i>p</i>
Birthweight (kg) ^a	3.3 ± 0.6	3.2 ± 0.7	3.4 ± 0.5	0.514
Gestational age (wk) ^a	39 ± 1.8	39 ± 2.2	40 ± 1.0	0.203
Gender (<i>n</i> , % male)	12 (60)	5 (46)	7 (78)	0.157
Sentinel event (<i>n</i> , %) ^b	6 (30)	3 (27)	3 (33)	0.574
Cesarean delivery (<i>n</i> , %)	11 (55)	5 (46)	6 (67)	0.311
Apgar at 1 min	1 (0–6)	2 (0–6)	1 (0–3)	0.273
5 min	3 (0–7)	4 (0–7)	3 (0–6)	0.264
10 min ^c	5 (0–7)	6 (2–7)	3 (0–6)	0.039
Presenting pH ^c	6.9 (6.6–7.3)	7 (6.6–7.3)	6.8 (6.7–7.1)	0.230
Base deficit ^c	18 (12–36)	15 (12–36)	20 (17–30)	0.038
Severe encephalopathy (<i>n</i> , %)	7 (35)	1 (9)	6 (67)	0.012
Initiation of cooling (hr)	4.4 (2.6–6)	4.3 (2.9–6)	4.6 (2.6–5.6)	0.470
Electroencephalography seizure (<i>n</i> , %)	8 (40)	4 (36)	4 (44)	0.714
Severe electroencephalography at 24 hr ^d (<i>n</i> , %)	13 (65)	5 (45)	8 (89)	0.070
Ventilator days	1 (0–15)	1 (0–15)	3 (0–6) ^e	0.661
Vasopressor support (<i>n</i> , %)	9 (45)	4 (55)	5 (56)	0.653
Length of stay (d)	16 (7–37)	14 (7–37)	19 (11–23) ^e	0.180

Data presented as median (range) except where indicated.

^a Mean ± SD.

^b defined as placental abruption, uterine rupture, umbilical cord accident, traumatic delivery (e.g., dystocia), and maternal collapse (e.g., trauma, hemorrhage, cardiopulmonary resuscitation, demise).

^c Documented in 18 of 20 subjects.

^d Severe electroencephalography background abnormality (low voltage, burst suppression, or isoelectric) in first 24 hr of life.

^e Data for surviving infants.