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Biomarkers for Severity of Neonatal Hypoxic-Ischemic Encephalopathy and Outcomes in Newborns Receiving Hypothermia Therapy

Lina F. Chalak, MD, MSCS[1], **Pablo J. Sánchez, MD**[1], **Beverley Adams-Huet, MS**[2], **Abbot R. Laptook, MD**[3], **Roy J. Heyne, MD**[1], and **Charles R. Rosenfeld, MD**[1]

[1]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX

[2]Department of Clinical Sciences and Statistics, University of Texas Southwestern Medical Center, Dallas, TX

[3]Department of Pediatrics, Brown University, Women & Infants Hospital of Rhode Island, Providence, RI

Abstract

Objective—To evaluate serum neuronal and inflammatory biomarkers to determine whether measurements of umbilical cords at birth can stratify severity of hypoxic-ischemic encephalopathy (HIE), whether serial measurements differ with hypothermia-rewarming, and whether biomarkers correlate with neurological outcomes.

Study design—This is a prospective cohort of inborn term newborns with varying degrees of HIE by neurological assessment. Neuronal glial fibrillary acidic protein (GFAP), ubiquitin carboxyl-terminal hydrolase L1, and inflammatory cytokines were measured in serum from umbilical artery at 6–24, 48, 72, and 78 hours of age. Neurodevelopmental outcomes (Bayley Scales of Infant and Toddler Development-III scales) were performed at 15–18 months.

Results—Twenty neonates had moderate (n = 17) or severe (n = 3) HIE and received hypothermia; 7 had mild HIE and were not cooled. At birth, serum GFAP and ubiquitin carboxyl-terminal hydrolase L1 increased with the severity of HIE ($P < .001$), and serial GFAP remained elevated in neonates with moderate to severe HIE. Interleukin (IL)-6, IL-8, and vascular endothelial growth factor were greater at 6–24 hours in moderate to severe vs mild HIE ($P < .05$). The serial values were unaffected by hypothermia-rewarming. Elevated GFAP, IL-1, IL-6, IL-8, tumor necrosis factor, interferon, and vascular endothelial growth factor at 6–24 hours were associated with abnormal neurological outcomes.

Conclusions—The severity of the hypoxic-ischemic injury can be stratified at birth because elevated neuronal biomarkers in cord serum correlated with severity of HIE and outcomes.

Reprint requests: Lina F. Chalak, MD, MSCS, Division of Neonatal-Perinatal Medicine, Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9063. lina.chalak@utsouthwestern.edu.

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Hypoxic-ischemic encephalopathy (HIE) is a complex disease process in which injury severity, duration, and timing of the antenatal injury are difficult to discern. This knowledge gap results in hypothermia being delivered to all newborns with moderate or severe encephalopathy in an identical fashion. Although whole body hypothermia therapy has improved outcomes,[1,2] 40% of neonates with HIE have neurological disability at 18–24 months of age despite therapy. This lack of apparent efficacy suggests that neonates treated with hypothermia therapy are a diverse group who need to be further stratified. At present, there are no specific, easily measured serum biomarkers that identify the extent of neurological injury at birth or thereafter.[2,3] The availability of markers of neuronal injury that correlate with disease severity and are predictive of neurodevelopmental disability in childhood would likely facilitate a more targeted therapeutic approach using adjunctive therapies.

The rewarming phase of whole body hypothermia in patients beyond the newborn period has been associated with a secondary reperfusion phenomenon in multiple organs and tissues, resulting in the release of circulating inflammatory mediators that may contribute to additional pathology.[4–8] The rewarming process and the potential surge of inflammatory biomarkers during reperfusion also could result in additional injury remote from the primary insult but have not been evaluated in the newborn.[9]

Due to the complex multifaceted nature of HIE, we selected a combination of serum biomarkers that have been shown to be mechanistically involved in the energy-depleting, free radical, excitotoxic, and inflammatory cascade that results in brain injury. Glial fibrillary acidic protein (GFAP) is a key cytoskeleton intermediate filament protein that is specific to astrocytes. Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is a neuron-specific cytoplasmic enzyme that is concentrated in dendrites.[10,11] Both GFAP and UCH-L1 have been used as markers of neuronal apoptosis. We also examined an array of circulating proinflammatory cytokines and growth factors that are mechanistically involved in the pathogenesis of brain injury.[12–14]

We addressed 3 objectives: (1) to measure circulating neuronal and inflammatory biomarkers in umbilical cord serum at birth in order to evaluate the severity of antenatal injury in infants with HIE; (2) to assess whether a surge in inflammatory mediators or neuronal biomarkers occurs during the rewarming phase; and (3) to determine if levels of these potential biomarkers soon after birth correlate with abnormal neurodevelopmental outcomes at 15–18 months of age.

Methods

This prospective cohort pilot study included all inborn infants ≥36 weeks of gestation and birth weight ≥1800 g who were admitted to the neonatal intensive care unit at Parkland Memorial Hospital, Dallas, TX, from June 2010 to June 2011 and had perinatal asphyxia with metabolic acidosis. Exclusion criteria included the presence of congenital anomalies or if comfort care was planned. The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center. Written informed consent was obtained from each mother after delivery.

Perinatal acidemia was determined by measuring blood gases in umbilical arterial cord plasma that is evaluated routinely on all deliveries from a double-clamped section of umbilical cord.[15] Criteria for an infant to qualify for a detailed neurological examination were as described in the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Neonatal Research Network study of whole body hypothermia[1]: (1) pH ≤7.00 or base deficit ≥16 mEq/L in umbilical arterial cord plasma or (2) history of an acute perinatal event and either no arterial blood gas available or a pH from cord arterial serum ranging from 7.01 to 7.15 or a base deficit from 10 to 15.9 mEq/L, in addition to a 10-minute Apgar score ≤5 or assisted ventilation initiated at birth. To establish the diagnosis and severity of encephalopathy, a neurological examination was performed by 1 of 2 certified examiners (L.C., P.S.) within 6 hours of birth according to the NICHD classification for modified Sarnat staging.[16] The hypothermia group was composed of newborns with a composite exam (at least 3 of 6 abnormal categories) consistent with a diagnosis of moderate or severe encephalopathy who received hypothermia therapy. The mild encephalopathy group was composed of newborns with 1–2 abnormal categories in the neurologic assessment but who did not meet criteria for hypothermia and received supportive care that included an indwelling umbilical catheter. This group of newborns was used in the time-series analyses for comparisons with the hypothermia group.

Whole body hypothermia was started within 6 hours after birth by placing the newborn on a cooling blanket (Blanketrol II; Cincinnati Sub-Zero, Cincinnati, Ohio) and maintaining the esophageal temperature at 33.5°C with the blanket servomechanism for 72 hours.[1]

Then, 1 mL of blood was collected from each newborn at birth (umbilical arterial serum), and serially. Time zero was representative of initiation of hypothermia: sample A (6–24 hours), sample B (48–72 hours), sample C (immediately at the end of rewarming and on achieving normothermia at 78 hours), and sample D (6–12 hours after completion of rewarming: 84–90 hours). Testing of umbilical cord arterial plasma that had been refrigerated and stored in the Transfusion Services Department was performed with informed consent. We used serial samples from the lower aorta via an umbilical arterial catheter to reflect changes during hypothermia (samples A and B) and rewarming (samples C and D). Neonates with mild HIE had serial sampling after 24 hours of age only while indwelling arterial catheters were in place. Samples were centrifuged at 2700 rpm for 10 minutes, aliquoted to subsamples, and stored at −80°C until time of immunoassay. Samples underwent enzyme-linked immunosorbent assay for interleukin (IL)-1, IL-6, IL-8, vascular endothelial growth factor (VEGF), tumor necrosis factor-α, interferon-γ, Regulated on activation, normal T cell expressed and secreted (Bio-Plex Pro Human Cytokine, Bio-Rad, Hercules, California), UCH-L1 (Banyan Biomarkers, Gainesville, Florida), and GFAP (Banyan Biomarkers). The lower level of detection for the GFAP assay was 0.03 ng/mL. All samples were assayed in duplicate with the average recorded. The coefficient of variation for intra- and inter-assay precision was <10%.

Magnetic resonance imaging (MRI) was performed from 7 to 14 days of age. Conventional T1 and T2 images were assessed using the NICHD MRI classification[17] by a pediatric neuroradiologist who had no knowledge of the outcomes.

All infants who received hypothermia were seen after hospital discharge at Children's Medical Center Dallas at 15–18 months of age for neurodevelopmental assessment using the Bayley Scales of Infant and Toddler Development-III (BSID-III). An abnormal neurological outcome was defined a priori by BSID-III developmental scores <85 on any of the 3 cognitive, language, or motor domains or the presence of deafness, blindness, or cerebral palsy.[18]

Statistical Analyses

Statistical analysis was performed using Sigma Plot 11.0 (Systat Software, San Jose, California) and SAS 9.2 (SAS Institute, Cary, North Carolina). The results are reported in a tabular format as medians, 25th–75th quartiles, mean±SD, or number and percentage. The Wilcoxon rank sum test was used to compare newborns with moderate to severe HIE who received hypothermia to newborns with mild HIE and no hypothermia treatment. Trends across mild, moderate, and severe HIE newborns were assessed with use of the Jonckheere-Terpstra test. After log transformation, serial serum biomarkers were compared using mixed-model repeated-measures analysis. Serial cytokine data were plotted and analyzed using geometric means ($\log_{10}$). Data for samples (C and D) collected 6–12 hours apart during rewarming were comparable so the average values are summarized in Table I (available at www.jpeds.com) to represent the rewarming interval. The predictive values for development of abnormal neurological outcome were calculated using sensitivity, specificity, and positive and negative predictive values. Receiver-operator characteristic (ROC) curves were constructed to determine area under the curve for each serum biomarker value obtained from the first sample after birth (6–24 hours) to identify at the earliest possible time neonates with abnormal outcomes. Sample size in this pilot study was determined by all consecutive patients who met entry criteria over the predefined study period.

Results

Of 14 000 inborn neonates, 100 (0.7%) had perinatal acidosis and were admitted to the neonatal intensive case unit for neurologic assessment of encephalopathy. Twenty-two infants, 1.6 per 1000 live births, had moderate to severe encephalopathy and were treated with hypothermia. One patient with severe HIE was excluded because he died within hours of birth, and there was no parental consent for another newborn. The hypothermia study group was composed of 20 newborns (17 with moderate and 3 with severe). The mild HIE group consisted of 7 of 13 newborns who had fetal acidosis, mild abnormalities on neurologic examination in the first 6 hours of age, and an indwelling arterial catheter. There was no significant difference in maternal variables; two-thirds of all neonates were delivered via cesarean and >85% of pregnancies had maternal complications (Table II). There was evidence of either abnormal fetal heart rate tracing, meconium-stained amniotic fluid, or another perinatal event in the 25% of neonates in the moderate to severe HIE group who were born via elective cesarean. There was no difference for results of umbilical arterial blood gases and Apgar scores between those with moderate to severe and those with mild HIE. However, the moderate to severe HIE group was more likely to have persistent metabolic acidosis at 1 hour of age, need for mechanical ventilation, and a longer duration of

hospitalization ($P = .01$). Neonates with mild HIE were discharged at 6 to 9 days of age on full oral feeds. Newborns with moderate to severe HIE received hypothermia therapy for 72 hours, and all had evidence of multiple organ involvement at 24 hours (Table II). Two had fat necrosis, and 3 had severe pulmonary hypertension requiring nitric oxide therapy; 2 of these received extracorporeal membrane oxygenation between 2 and 4 days of age. During the hospitalization, 1 neonate required renal dialysis and 2 had placement of a gastrostomy tube for inability to nipple feed.

MRI results were available for all 20 patients in the hypothermia group, and MRI was performed after hypothermia treatment at a median age of 7 days. Four (20%) neonates with moderate to severe HIE had abnormal MRI results that demonstrated diffuse white matter injury and watershed infarcts. None had basal ganglia injury and no infant died.

Blood samples were obtained from all newborns with HIE who received hypothermia at all time points specified. However, 4 of 7 neonates in the mild HIE group had missing samples after 24 hours of age due to loss of arterial access.

Analysis of umbilical arterial serum demonstrated significant differences in GFAP and UCH-L1 between neonates with mild vs those with moderate and severe encephalopathy; values increased progressively with worsening encephalopathy (Figure 1; GFAP, $P = .001$; UCH-L1, $P = .03$). Figure 1 shows umbilical cord measurements of biomarkers for each of mild, moderate, and severe encephalopathy groups.

GFAP was the only serum biomarker at birth that correlated significantly with other indicators of birth depression and multiple organ dysfunctions: 5-minute Apgar scores ($r = 0.86$, $P = .001$), alanine aminotransferase ($r = 0.75$, $P = .01$), aspartate aminotransferase ($r = 0.71$, $P = .02$), and creatinine ($r = 0.75$, $P = .01$).

The remaining systemic inflammatory biomarkers in umbilical arterial plasma were not significantly different between groups (Table III).

GFAP levels remained significantly higher in neonates with moderate to severe HIE compared with those with mild HIE at each time point after birth ($P < .02$; Table I). In contrast, UCH-L1 no longer differed between groups by 6–24 hours. When the postnatal levels of the other cytokines and biomarkers were compared between moderate to severe vs mild HIE, levels of IL-6 and IL-8 were greater in the moderate to severe group at 12 hours and IL-8 remained significantly higher at 48 hours, at which time VEGF also was elevated (Table I). When cytokine, GFAP, or UCH-L1 levels across time were examined in the newborns treated with hypothermia, there were no significant differences during hypothermia and rewarming.

BSID-III developmental scores were performed on cooled infants at 20 ± 5 months. Five (25%) of these cooled infants had abnormal outcomes. Of these 5 children with abnormal outcome, 1 had severe cerebral palsy and was not able to perform further testing, and the remaining had scores <85 in Bayley domains (cognitive, language, and motor) tested. The median (25%–75% quartiles) BSID-III scores in each of the domains tested were cognitive, 75 (67–89); language, 72 (65–78); and motor, 82 (79–83). Notably, at 6–24 hours of age, the

serum concentrations of all biomarkers, except for UCH-L1, were significantly greater in infants who had abnormal vs normal neurodevelopmental outcomes ($P < .05$; Table I).

Neurologic examination at hospital discharge was abnormal in 3 infants, all of whom had a sustained GFAP value >0.05 ng/mL beyond 72 hours of age and an abnormal BSID-III outcome at 24months of age. Abnormal outcomes in infants with persistent neurologic abnormalities were significantly more likely than in the remaining infants whose neurologic exam normalized before hospital discharge ($P = .0001$).

Seizures occurred during rewarming in 1 neonate who, at 24 months of age, had an abnormal BSID-III test (cognitive score 65, motor score 82). Five other infants had abnormal background electrical activity on ambulatory electroencephalography in the first 72 hours of life. Four (80%) infants with abnormal ambulatory electroencephalographic backgrounds had GFAP values >0.05 and abnormal BSID-III results at 18 months.

ROC curve analysis with cut-off for sensitivity and specificity is plotted in Figure 2. Significant area under the curve values >0.85 ($P < .05$) were selected with optimal tradeoff sensitivity and specificity obtained at 6–24 hours for GFAP, 0.06 ng/mL; IL-1, 722 pg/mL; IL-6, 650 pg/mL; IL-8, 149 pg/mL; and VEGF, 650 pg/mL. Specifically, GFAP measurements demonstrated discriminative ability with GFAP values >0.08 pg/mL (100% positive predictive value) in all infants with an abnormal outcome.

Discussion

This pilot cohort study examined an array of neuronal specific serum biomarkers and circulating inflammatory cytokines known to be involved in the excitatory-oxidative cascade of brain injury in infants with HIE. Key findings were: (1) a concentration-dependent relationship between serum GFAP at birth and the severity of encephalopathy; (2) the absence of a surge in any serum biomarkers after rewarming; and (3) evidence that a panel of inflammatory and neuronal biomarkers measured at 6–24 hours of age in newborns with moderate to severe HIE treated with hypothermia may predict abnormal 15- to 18-month neurodevelopmental outcomes.

Although whole body hypothermia in neonates with HIE is associated with a reduction in death and neurologic impairment,[2,19] there is no real-time means of determining the therapeutic efficacy of hypothermia and who ultimately may benefit from adjunctive therapy. There is a need to improve stratification of this heterogeneous group of neonates with HIE who are currently identified by a categorical neurologic examination. The neurologic evaluation changes over time, is influenced by medications, and is subject to examiner bias:

Although physiological challenges such as the timing, severity, pattern and duration of the fetal insult as well as the degree of recovery via fetal adaptive mechanisms determine the spectrum of disease, the magnitude of the insult, and the responses to therapy, each is unclear and difficult to estimate.[20] Thus, identification of biomarkers of neuronal injury at the time of birth could help in ascertaining the severity of the fetal insult. Moreover, they

might provide risk stratification to guide additional neuroprotective therapies and improve our early ability to predict outcomes.

GFAP and UCH-L1 were selected to reflect the extent of neuronal injury because they are expressed in neurons and astrocytes, then released into the circulation after breakdown of the blood-brain barrier that occurs with ischemic injury, and are easily measured.[21,22] GFAP is a key protein in astrocytes that is functionally involved during regeneration and gliosis and whose expression is induced by brain damage.[11] Cerebrospinal fluid GFAP levels are reported to be predictive of abnormal neurologic outcomes with traumatic brain injury[23] and extracorporeal membrane oxygenation[24] as well as death in asphyxiated newborns.[25] Moreover, levels in children with traumatic brain injury are unaltered by hypothermia therapy.[21] UCH-L1 also is detected in the cerebrospinal fluid of patients after traumatic brain injury and surgically induced circulatory arrest.[26,27] UCH-L1 values in circulating blood were >100 ng/mL in neonates with HIE who subsequently died.[28] Limited information is available regarding serum levels of these neuron-specific biomarkers in newborns with HIE who receive hypothermia therapy. In a recent report, 50% of newborns with HIE and evidence of brain injury on MRI had elevated serum GFAP after completing hypothermia.[29] We observed significantly higher serum GFAP levels in neonates with moderate to severe HIE who had treatment with whole body hypothermia vs neonates with mild HIE. More importantly, we observed a graded increase in serum neuronal specific biomarkers in the umbilical arterial plasma that paralleled the insult severity based on the neurological examination. Massaro et al[30] have recently reported optimal time of detection of UCH-L1 at time 0 vs GFAP at 24 and 72 hours of life, further confirming our observed trends in this study where UCH-L1 was elevated only in the umbilical arterial cord plasma compared with more sustained increases in GFAP at all time points studied. Our observation that levels of GFAP and UCH-L1 were greatest in the umbilical arterial plasma and unchanged during the hypothermia-rewarming period suggests the impact of injury is predominantly prenatal. These neuronal biomarkers, therefore, could potentially provide a means of determining the severity or degree of brain injury before initiating neuroprotective therapies because neonates with the highest values in the cord plasma also were more likely to have abnormal neuroimaging and neurodevelopmental outcomes. These findings, if replicated in larger studies, ultimately may be useful in facilitating the stratification of the severity of HIE at birth and response to treatment.

Because multiple organ dysfunction is known to be an integral part of HIE, we also examined an array of circulating proinflammatory cytokines as potential biomarkers of severity of injury that have been mechanistically involved in the pathogenesis of brain injury.[3,12–14,31–33] IL-1, IL-6, and IL-8 were selected because their expression has been reduced following hypothermia during in vivo experimental studies.[34,35] We and others[36–38] have reported that IL-6 and IL-8 are associated with a variety of inflammatory challenges and represent a final common pathway of brain injury. VEGF is a growth factor that may contribute to the inflammatory responses by causing edema and increased vascular permeability in animal models[39]; it has been detected in the cord serum of newborns with HIE.[40] Our current pilot data further suggest that inflammatory serum biomarkers are also predictive of abnormal neurological outcomes.

Currently, supportive data for the rewarming process in the newborn are limited, and there are concerns that too rapid an increase in temperature may be detrimental.[41,42] Brain injury following asphyxia may evolve over days to even weeks[43] and may be reactivated or exacerbated during rewarming via peripheral organ reperfusion resulting in the systemic release of inflammatory cytokines.[7,44,45] We did not observe a surge in circulating inflammatory or neuronal biomarkers during or after rewarming. The possibility exists that such a surge could have occurred but was masked by concomitant increased clearance of the serum biomarkers. The small sample size of neonates with HIE and, specifically, with severe HIE, limits the ability to make definitive associations of biomarkers with events during the rewarming phase and neurodevelopmental outcomes. In addition, because the first postnatal blood sample was at 6–24 hours, it is possible that the values could have been suppressed by the hypothermia therapy.[46]

The observations reported in this study are limited by the small sample size of encephalopathic infants who were all inborn. The HIE incidence in this study of 1.6:1000 was in accordance with the expected prevalence in such a population.

Strengths of this study include a prospective design, a mechanism-oriented choice of selective neuronal as well as general inflammatory biomarkers, and serial arterial measurements spanning birth, cooling, and postrewarming in all hypothermia patients. Other strengths include the performance of the neurologic examination and MRI readings by an examiner blinded to clinical outcomes and a priori definition of abnormal neurologic outcomes that was available for all cooled infants. The inclusion of the mild HIE group strengthens the comparisons because it widens the spectrum of clinical encephalopathy at birth. This group of neonates with mild HIE are poorly characterized, and recent evidence suggests that up to 20% can have abnormal short-term outcomes.[47] Unfortunately, data on multiple organ injury (eg, creatinine, liver enzymes) in these neonates with mild HIE were not evaluated. Findings in the neonates with encephalopathy support the search for biomarkers in addition to the neurological examination to better stratify subgroups that might benefit from neuroprotective therapies.

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Glossary

BSID-III	Bayley Scales of Infant and Toddler Development-III
GFAP	Glial fibrillary acidic protein
HIE	Hypoxic-ischemic encephalopathy
IL	Interleukin
MRI	Magnetic resonance imaging

NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
ROC	Receiver-operating characteristic
UCH-L1	Ubiquitin carboxyl-terminal hydrolase L1
VEGF	Vascular endothelial growth factor

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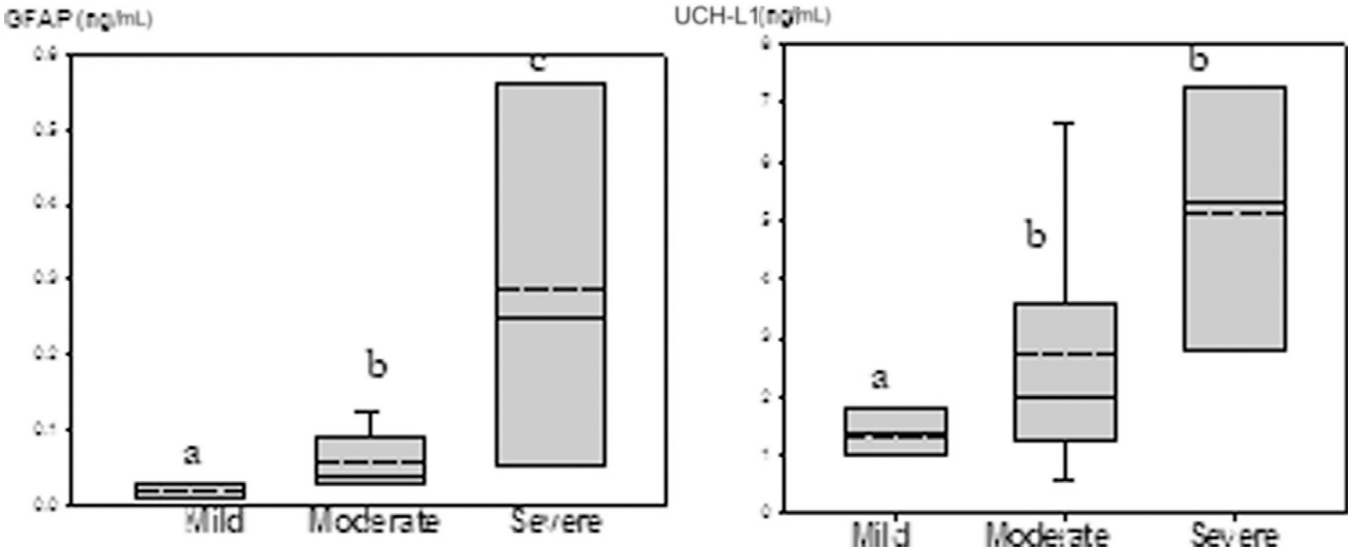

Figure 1.

Umbilical cord plasma GFAP and UCH-L1 and severity of encephalopathy at birth. A box and whisker plot is shown with median as *solid line*, mean as *dotted line*, and 25th and 75th quartiles as *lower* and *upper borders*. Analyses include neonates with mild (n = 7), moderate (n = 17), and severe (n = 3) HIE. *P* = .001 (GFAP) and *P* = .03 (UCH-L1) by Jonckheere-Terpstra test. **a, b**, and **c** denote significant differences among each group.

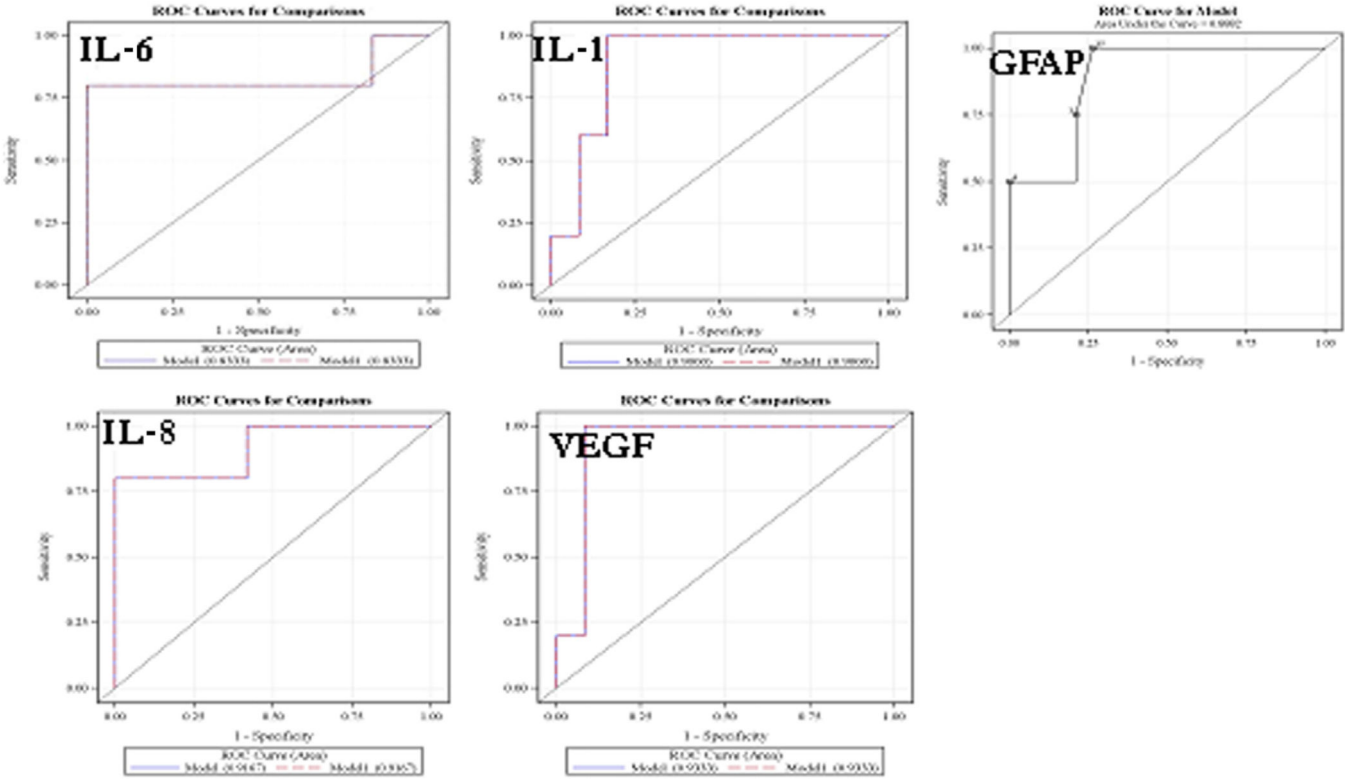

Figure 2.

ROC curves of serum biomarkers at 6–24 hours predicting abnormal BSID-III results (<85) at 18–24 months.

Table I

Serum biomarkers measured at 12 h and relationship to BSID III outcomes

Serum biomarkers	Normal outcome (N = 22)	Abnormal outcome (N = 5)	*P*-value
IL-1	452 (354–577)	1324 (533–3289)	.03
IL-6	62 (24–165)	5404 (89–326 766)	.04
IL-8	154 (98–240)	2535 (295–21 801)	.02
VEGF	243 (137–430)	1514 (456–5027)	.008
TNF	6.6 (4–11)	24.3 (13–44)	.001
IFN	2745 (1823–4133)	7869 (7143–8668)	.0002
GFAP	0.03 (0.03–0.05)	0.1 (0.08–0.6)	.002
UCH-L1	1.88 (1.13–3.24)	2.38 (1.90–3.00)	.45

Data presented as geometric mean and 95% CIs.

Table II

Characteristics of the mothers and infants

	Moderate to severe HIE (n = 20)	Mild HIE (n = 7)
Mothers		
Mode of delivery, n		
Cesarean (emergency)	12 (60%)	4 (57%)
Cesarean (elective)	5 (25%)	1 (14%)
Vaginal	3 (15%)	1 (14%)
Age, y (mean ± SD)	27 ± 8	25 ± 4
Gravida (mean ± SD)	2 ± 1	3 ± 1
Gestational hypertension, n	8 (40%)	1 (14%)
Diabetes mellitus, n	3 (15%)	3 (42%)
Clinical chorioamnionitis, n (≥38 °C)	2 (10%)	2 (28%)
Placental abruption, n	4 (20%)	1 (14%)
Infants		
Large for gestational age, n	2 (10%)	2 (28%)
Small for gestational age, n	3 (15%)	1 (14%)
Weight, g (mean ± SD)	3156 ± 624	3037 ± 752
Gestational age, wk (mean ± SD)	39 ± 2	38 ± 2
Race		
Hispanic	75%	57%
Black	14%	43%
Sex, n		
Male	14 (70%)	4 (57%)
Female	6 (30%)	3 (43%)
Apgar score 1 min	2 (2–3)	3 (2–3)
Apgar score 5 min	6 (5–7)	7 (5–8)
Cord arterial pH (mean ± SD)	6.98 ± 0.09	7.00 ± 0.11
O_2, mm Hg	16 (11–20)	36 (15–44)
CO_2, mm Hg	77 (66–99)	78 (65–101)
Base deficit, mm Hg (mean ± SD)	19 ± 6	19 ± 6
Base deficit at 1 h* (mean ± SD)	12 ± 6*	7 ± 3
Mechanical ventilation, n*	12 (60%)*	1 (14%)
CPAP, n	3 (15%)	3 (43%)
Room air, n	5 (25%)	3 (43%)
Days in hospital*	24 (10–50)*	7 (6–9)
Time to start cooling, h	5 (3–6)	Not cooled
Non-CNS organ involvement		
AST, U/L	208 (86–514)	NA
ALT, U/L	59 (19–329)	NA
Troponin, yg/L	0.17 (0.10–0.33)	NA

	Moderate to severe HIE (n = 20)	Mild HIE (n = 7)
Creatinine, mg/dL	1 (0.8–1.5)	NA

ALT, alanine aminotransferase (normative data <40); *AST*, aspartate aminotransferase (normative data <40); *CNS*, central nervous system; *CPAP*, continuous positive airway pressure; *NA*, no samples were obtained for testing in infants with mild HIE.

Data presented as mean ± SD or median and IQR. Creatinine (normative data <1).

* $P < .05$.

Table III

Serial serum biomarker measurements in newborns with moderate to severe HIE vs newborns with mild HIE

Moderate to severe HIE	**Cord (n = 20)**	**6–24 h (n = 20)**	**48–72 h (n = 20)**	**78–96 h (n = 20)**
GFAP, ng/mL	0.05 (0.03–0.1)*	0.06 (0.03–0.15)*	0.05 (0.03–0.14)*	0.08 (0.04–0.2)*
UCH-L1, ng/mL	2.6 (1.5–4.6)†	0.6 (0.3–2.2)	0.5 (0.3–0.6)	0.5 (0.3–0.6)
IL-1, pg/mL	497 (347–1003)	401 (342–436)	385 (337–440)	365 (320–541)
IL-6, pg/mL	301 (9–715)	106 (24–4482)*	56 (17–144)	38 (11–103)
IL-8, pg/mL	566 (154–997)	161 (134–1677)*	307 (70–817)*	94 (64–473)
VEGF, pg/mL	1344 (390–1683)	456 (164–943)	307 (230–833)*	475 (325–901)
IFN-γ, pg/mL	4834 (3491–6652)	5715 (3032–7599)	3273 (2209–4542)	2193 (1921–4313)
TNF, pg/mL	11 (6–13)	10 (5–24)	7 (4–11)	6 (5–10)
RANTES, pg	15 121 (12 238–77 513)	11 464 (8691–14 284)	8485 (4237–10 926)	9533 (6824–12 955)
Mild HIE	**Cord (n = 7)**	**6–24 h (n = 7)**	**48–72 h (n = 4)**	**84–96 h (n = 4)**
GFAP, ng/mL	0.008 (0.008–0.03)	0.01 (0.01–0.0.03)	0.01 (0.01–0.05)	0.008 (0.008–0.04)
UCH-L1, ng/mL	1.3 (1–1.7)	0.7 (0.4–1.1)	1 (0.4–1.8)	0.9 (0.3–1.9)
IL-1, pg/mL	499 (468–664)	423 (361–529)	433 (401–559)	492 (467–517)
IL-6, pg/mL	19 (7–135)	18 (10–25)	10 (9–20)	12 (12–15)
IL-8, pg/mL	159 (70–315)	103 (63–261)	51 (41–70)	50 (40–70)
VEGF(pg/mL	654 (299–1057)	237 (162–1038)	143 (46–1297)	70 (32–135)
IFN-γ, pg/mL	4271 (2737–5429)	3393 (1446–4556)	3000 (1729–4158)	1000 (700–2500)
TNF, pg/mL	14 (10–17)	4 (0–9)	7 (0–10)	5 (0–10)
RANTES, pg	15 367 (12 910–21 894)	14 284 (12 773–19 318)	14 252 (11 072–18 593)	9512 (8137–11 250)

IFN, interferon; *RANTES*, Regulated on Activation, Normal T cell Expressed and Secreted; *TNF*, tumor necrosis factor.

Data given as median and IQR. Last column (84–96 h) represents the pooled rewarming interval.

* $P < .05$, comparisons between groups of moderate to severe and mild HIE groups.

† $P < .05$, serial time points (cord, 6–24 h, 48–72 h, 84–96 h) in each group separately.