Plasma Biomarkers of Brain Injury in Neonatal Hypoxic-Ischemic Encephalopathy

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Objectives To evaluate plasma brain specific proteins and cytokines as biomarkers of brain injury in newborns with hypoxic-ischemic encephalopathy (HIE) and, secondarily, to assess the effect of erythropoietin (Epo) treatment on the relationship between biomarkers and outcomes.

Study design A study of candidate brain injury biomarkers was conducted in the context of a phase II multicenter randomized trial evaluating Epo for neuroprotection in HIE. Plasma was collected at baseline (<24 hours) and on day 5. Brain injury was assessed by magnetic resonance imaging (MRI) and neurodevelopmental assessments at 1 year. The relationships between Epo, brain-specific proteins (S100B, ubiquitin carboxy-terminal hydrolase-L1 [UCH-L1], total Tau, neuron specific enolase), cytokines (interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-12P70, IL-13, interferon-gamma [IFN-γ], tumor necrosis factor alpha [TNF-α], brain-derived neurotrophic factor [BDNF], monocyte chemoattractant protein-1), and brain injury were assessed.

Results In 50 newborns with encephalopathy, elevated baseline S100B, Tau, UCH-L1, IL-1β, IL-6, IL-8, IL-10, IL-13, TNF-α, and IFN-γ levels were associated with increasing brain injury severity by MRI. Higher baseline Tau and lower day 5 BDNF were associated with worse 1 year outcomes. No statistically significant evidence of Epo treatment modification on biomarkers was detected in this small cohort.

Conclusions Elevated plasma brain-specific proteins and cytokine levels in the first 24 hours of life are associated with worse brain injury by MRI in newborns with HIE. Only Tau and BDNF levels were found to be related to neurodevelopmental outcomes. The effect of Epo treatment on the relationships between biomarkers and brain injury in HIE requires further study. (J Pediatr 2018;194:67-75).

Trial registration ClinicalTrials.gov: 01913340.

Perinatal hypoxic ischemic encephalopathy (HIE) remains an important cause of neonatal death and long-term disability, despite successful incorporation of therapeutic hypothermia into standard care.1-5 Adjunct therapies are needed and are under active investigation.

Erythropoietin (Epo) is a hematopoietic cytokine induced in settings of chronic fetal hypoxia6 and is known to have neurotrophic properties and neuroreparative effects on the brain after injury.7-10 In the Neonatal Erythropoietin and Therapeutic Hypothermia Outcomes Study (ClinicalTrials.gov: 01913340), infants with HIE randomized to Epo in conjunction with hypothermia had reduced brain injury on neonatal magnetic resonance imaging (MRI) and improved motor performance at 1 year compared with those who received hypothermia alone.11

To advance neurotherapeutics and improve outcomes in HIE, biomarkers of brain injury are needed to identify infants who are failing to respond to cooling alone and who might benefit from adjuvant therapy such as Epo. Biomarkers might help individualize care by enabling assessment of treatment efficacy acutely, offering prognostic information for families, and directing need for later rehabilitative care. Currently, bedside assessment of evolving brain injury in encephalopathic newborns relies on clinical examination12-14 and electroencephalogram (EEG).
background severity. However, these tools are limited by their subjective nature, required expertise for interpretation, and confounding by medical interventions such as the use of sedatives. Peripheral blood biomarkers that reflect end-organ injury might provide objective quantitative measures that are free from these limitations. However, no blood-based biomarker is in current clinical use for newborns with HIE.

Several candidate brain injury biomarkers have recently been investigated in small cohorts of babies with HIE undergoing therapeutic hypothermia. Brain-specific proteins released in the setting of cerebral injury have been related to brain injury by MRI and early neurodevelopmental outcomes. These include proteins from neurons (neuron specific enolase [NSE]) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and glial cells (S100B protein and glial fibrillary acidic protein [GFAP]), as well as Tau protein present in all cells of the central nervous system. Likewise, inflammatory cytokines and chemokines measured during cooling and rewarming have been linked to adverse outcomes in babies with HIE. Although not previously investigated in newborns with HIE, endogenous plasma Epo levels at birth have been related to poor outcomes in infants with intrauterine growth restriction and other high-risk newborns.

We investigated a panel of candidate plasma biomarkers including brain specific proteins, inflammatory cytokines, and endogenous Epo levels to evaluate their relationship with brain injury assessed by MRI at 4-7 days of life and neurodevelopmental outcomes at 1 year. We hypothesized that elevated brain specific protein, cytokine, and Epo levels would be associated with worse brain injury and, secondarily, that these relationships would be mediated by treatment with Epo.

**Methods**

We conducted an ancillary study in the context of a phase II multicenter randomized trial evaluating Epo for neuroprotection in HIE. Term or near-term (≥36 weeks gestational age) newborns with moderate or severe encephalopathy and evidence of perinatal depression (10-minute Apgar score ≤5, pH < 7.00 or base deficit ≥15, or ongoing resuscitation at 10 minutes) being treated with hypothermia were randomized to receive Epo 1000 U/kg IV or placebo on days 1, 2, 3, 5, and 7 of life. The study received institutional review board approval at all hospitals, and signed informed consent was obtained from the parent of each participant.

**Biomarker Determinations**

Whole blood specimens (0.5 mL) were collected prospectively in heparinized tubes at baseline (at enrollment, prior to study drug, and <24 hours of life) and on day of life 5. Specimens were refrigerated at 4°C as soon as possible after collection. Specimens were then centrifuged at 6000 rpm for 10 minutes; plasma collected and stored at -80°C ideally within 4 hours but allowable up to 48 hours from time of collection. Prior studies have demonstrated stability of cytokine measurements up to 8 hours from time of collection at room temperature (20°C) and up to 48 hours at 4°C. Brain-specific proteins Tau, UCH-L1, and NSE levels were quantified using a multiplexed bead-based enzyme-linked immunosorbent assay (ELISA) (Luminex Corp, Austin, TX) via a commercially available assay kit (Human Neurologic Disorders Magnetic Bead Panel 1; EMD Millipore, Darmstadt, Germany).

S100B protein was assayed separately using a commercially available ELISA (Human S100B ELISA kit; Millipore, Germany). GFAP, brain-derived neurotrophic factor (BDNF), and monocyte chemoattractant protein-1 (MCP-1) were measured using a custom multiplexed electrochemiluminescence-based assay (Meso Scale Discovery, Rockville, MD). Two additional commercially available electrochemiluminescence-based assays were used to measure inflammatory cytokines interfereron-gamma (IFN-γ), tumor-necrosis factor alpha (TNF-α), interleukin (IL)-1b, IL-6, IL-8, IL-10, IL-12p70, and IL-13 (V-PLEX Proinflammatory Panel 1; Meso Scale Discovery, Rockville, Maryland) and baseline (endogenous) Epo level (Human EPO Base Kit; Meso Scale Discovery). Samples were thawed once for the Epo and proinflammatory panel assays, with aliquots made (ie, 1 additional freeze-thaw cycle) for the remaining assays. All assays were performed in duplicate, and results averaged for analyses. Assays were performed by individuals blinded to study endpoints.

Brain MRI was performed as part of routine clinical care at 4-7 days of age. T1, T2, and diffusion weighted images were reviewed independently by 2 experienced readers, and discrepancies were resolved by consensus. Images were scored according to a previously described system that provides a global brain injury score (range 0-138, categorized as “none” = 0; “mild” = 1-11; “moderate” = 12-32; or “severe” = 33-138), as well as 5 regional scores (basal ganglia, white matter, cortex, brainstem, and cerebellum). Two infants who died before undergoing MRI were conservatively assigned a global injury score of 80, which is approximately the midpoint of the “severe” global brain injury category and 10 points higher than the maximum observed score.

**Neurodevelopmental Assessment**

Early neurodevelopmental outcomes were assessed at age 1 year with a standardized neuromotor examination (Alberta Infant Motor Scale [AIMS]) and the Warner Initial Developmental Evaluation (WIDEA). The AIMS evaluates gross motor abilities of infants from birth to independent walking, providing norm-referenced percentile ranks for motor performance. The WIDEA is a 43-item parental questionnaire that assesses 4 domains of infant development: self-care, mobility, communication, and social cognition. As all deaths occurred secondary to redirection of medical care in infants with poor neurologic or overall prognosis, infants who died were assigned 12-month AIMS and WIDEA scores corresponding to moderate to severe neurodevelopmental impairment (ie, AIMS less than 5th percentile for age [AIMS = 44], and WIDEA more than 2 SDs below the normative mean [WIDEA = 76.4]).

**Statistical Analyses**

Descriptive statistics included medians and ranges; boxplots were used to characterize the log-distribution of each biomarker
at baseline and day 5 by group. Spearman correlation coefficients were used to assess bivariate associations between biomarker levels and outcome groups both overall and separately for each treatment group. We used multivariate linear regression analyses to assess the association between imaging or neurodevelopmental outcomes and biomarkers, adjusting for important study design factors of treatment group (Epo vs placebo) and severity of encephalopathy at presentation (moderate or severe). Separately for each biomarker of interest, we assessed effect modification via treatment with Epo between the day 5 biomarker level and each outcome by evaluating the interaction between the day 5 biomarker level and an indicator of treatment group assignment, controlling for the baseline biomarker level. Linear mixed effects regression models were used to assess for differences in biomarker levels between baseline and day 5, adjusting for treatment and severity of encephalopathy at presentation. For all analyses, biomarker data were log-transformed to satisfy normality assumptions. All statistical analyses were performed using R statistical software (v 3.3.0; R Foundation for Statistical Computing, Vienna, Austria). All reported P values are 2-sided, and statistical significance was assessed at α = .05. No adjustment was made for multiple testing as the present study serves to generate hypotheses to be formally tested in a future larger randomized controlled trial. For the primary analyses examining the correlation between biomarkers and outcome measures, this study was statistically powered at >0.80 to detect correlation coefficients of ±0.4 or larger with a type 1 error rate (2-sided alpha = .05) uncorrected for multiple comparisons.

Results

Fifty singleton neonates with moderate or severe encephalopathy were randomized to receive Epo with therapeutic hypothermia (n = 24) or hypothermia alone (n = 26) at 7 centers between December 2013 to November 2014. Detailed characteristics of the study sample, as well as safety, efficacy, and outcome data from the parent trial have been previously reported.

In summary, mean ± SD birthweight was 3.3 ± 0.7 kg, gestational age 38.7 ± 1.7 weeks, 52% were male, and severe encephalopathy was present in 9 (18%) infants. Maternal chorioamnionitis (based on clinical diagnosis by the treating physician) was noted in 7 (14%) infants. Of the 35 infants for whom placental pathology was available, 13 (37%) had inflammatory abnormalities noted. Death occurred in 7 (14%) patients, brain MRI was performed in 48 (96%) infants at mean age of 5.2 days (SD 2.2), and 41 of 43 survivors (95%) were evaluated for developmental outcomes at mean age 12.7 months (SD 0.9). Global brain injury score on MRI ranged from 0 to 70 (IQR 1-11). Two infants died prior to MRI (1 in each treatment group), and injury was classified in the remaining infants as none (n = 11), mild (n = 22), moderate (n = 7), and severe (n = 6). Five infants were classified as having moderate to severe neurodevelopmental impairment evidenced by WIDEA scores greater than 2 SDs below the normative mean at 12 months, and 2 of these infants also had significant motor delay based on AIMS less than the fifth percentile for age. All 50 infants had baseline plasma samples drawn at median age of 16.2 hours (range 3.3-23.9 hours), prior to first dose of study drug. Three infants died prior to day 5; plasma was obtained in the remaining 47 infants at median age of 4.7 days (range 4.0-5.0 days).

Specimen processing times (from collection to freezing) were not different by treatment group (Table I; available at www.jpeds.com), and no observable patterns, outliers, or heterogeneity were noted in biomarker levels based on increasing processing time.

Brain Specific Proteins and Brain Injury by MRI

Biomarker levels generally decreased between baseline and day 5 (Figure 1). Higher plasma S100B, UCH-L1, and total Tau at baseline were significantly associated with higher MRI global injury score after adjusting for severity of HIE and treatment allocation (Table II).

Baseline NSE was not associated with MRI injury (P > .05). As GFAP levels were largely below the assay lower limits of detection (<0.2 ng/mL), these data are not presented nor further analyzed. Significant correlations were observed between baseline brain specific proteins, and regional MRI injury subscores in the basal ganglia and brainstem (Table II). On day 5, only Tau was significantly associated with MRI global injury score after adjusting for baseline level, encephalopathy grade at presentation, and treatment allocation (r, 0.32, P = .004).

Cytokine levels also decreased between baseline and day 5 (except for MCP-1 and BDNF), with many cytokines below limits of detection at the later time point (Figure 1). IL-12p70 levels were largely below the assay lower limits of detection (<.05 pg/mL) at both time points so these data are not presented nor further analyzed. Significant correlations between baseline proinflammatory (IL-1β, IL-6, IL-8, IFN-γ, and TNF-α) and anti-inflammatory (IL-10, IL-13) cytokines and MRI global brain injury scores were observed (Table II). Baseline BDNF and MCP-1 were not associated with brain injury by MRI (P > .05). Although baseline endogenous Epo level was not significantly associated with MRI global brain injury score, significant correlations were observed between baseline Epo level and regional MRI subscores in the basal ganglia (r, 0.16, P = .05) and brainstem (r, 0.26, P = .03). Likewise, other cytokines were significantly associated with injury in these regions only (Table II). Higher Epo levels on day 5 were associated with lower MRI global brain injury scores (r, -0.39, P = .008). Otherwise, relationships between day 5 cytokine levels and MRI global injury score were not observed (Table III).

Biomarkers and Neurodevelopmental Outcomes

Baseline brain specific proteins were negatively correlated with neurodevelopmental outcomes at 1 year (Table II), but these relationships were not statistically significant except for baseline Tau level, which was inversely related to WIDEA score (r, -0.24, P = .05). At day 5, Tau was also inversely related to AIMS and WIDEA scores, but these relationships were not statistically significant (Table III). Baseline cytokines across the entire study cohort were not significantly associated with neurodevelopmental outcomes (Table II). At day 5, higher
Figure 1. Biomarker levels at baseline and day 5 by treatment group. $P$ values denote differences by group and by time point, adjusted for specimen processing time. Boxplots depict median and IQRs with outliers denoted by open circles.
Table II. Spearman correlation of baseline biomarker levels with MRI injury scores and neurodevelopmental outcomes at 1 year

<table>
<thead>
<tr>
<th>Biomarker name</th>
<th>Baseline biomarkers</th>
<th>MRI injury score</th>
<th>Year 1 neurodevelopmental outcomes</th>
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<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>Global score</td>
<td>Basal ganglia</td>
</tr>
<tr>
<td>Brain specific proteins</td>
<td></td>
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<tr>
<td>S100B (pg/mL)</td>
<td>0.50 (.07-19)</td>
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<td>UCH-L1 (ng/mL)</td>
<td>0.44 (.05-191)</td>
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<td>0.29*</td>
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<tr>
<td>Tau (ng/mL)</td>
<td>0.50 (.01-12.8)</td>
<td>0.16*</td>
<td>0.24*</td>
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<td>NSE (ng/mL)</td>
<td>98.8 (43.8-758)</td>
<td>.04</td>
<td>0.21</td>
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<tr>
<td>Cytokines/Chemokines</td>
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<td></td>
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<tr>
<td>IL-1b (pg/mL)</td>
<td>0.39 (.06-990)</td>
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<td>.09</td>
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<tr>
<td>IL-6 (pg/mL)</td>
<td>24.1 (2.67-1534)</td>
<td>0.17*</td>
<td>0.17*</td>
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<tr>
<td>IL-8 (pg/mL)</td>
<td>105 (10.9-990)</td>
<td>0.31*</td>
<td>0.13</td>
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<tr>
<td>IL-10 (pg/mL)</td>
<td>4.6 (0.4-668)</td>
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<td>0.25*</td>
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<tr>
<td>IL-13 (pg/mL)</td>
<td>0.4 (.17-15.5)</td>
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<td>IFN-γ (pg/mL)</td>
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<td>0.29*</td>
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<td>TNF-α (pg/mL)</td>
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<td>0.13</td>
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<td>MCP-1 (pg/mL)</td>
<td>746 (63-5000)</td>
<td>0.10</td>
<td>.09</td>
</tr>
<tr>
<td>BDNF (pg/mL)</td>
<td>80 (0.3-1008)</td>
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<td>0.15</td>
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<td>Epo (mIU/mL)</td>
<td>23.4 (0.3-5047)</td>
<td>0.02</td>
<td>0.16*</td>
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</table>

*Treatment and HIE severity adjusted P value of < .05.

BDNF was associated with higher WIDEA score at 1 year (r = 0.37, P = .01).

The relationships between biomarkers and MRI injury did not significantly differ by Epo treatment allocation (biomarker*treatment group interaction P > .05). Day 5 Tau, BDNF, and Epo levels by treatment group are shown in Figure 2.

Discussion

We demonstrate the ability of several brain specific proteins and cytokines, when measured during the acute postnatal period in newborns with HIE, to serve as biomarkers of brain injury assessed by MRI. In particular, plasma Tau appears to have high promise as it remains correlated with brain injury when measured out to day 5 and is also related to functional outcomes at 1 year. BDNF may have utility as a marker of neuronal repair with higher levels at day 5 associated with reduced brain injury by MRI and improved motor performance at 1 year. These results support the potential importance of developing a specimen biorepository within the context of a phase III multicenter randomized therapeutic trial and will help direct optimized timing and selection of biomarkers for future larger validation studies.

Consistent with prior studies, we observed significant correlations between brain injury and brain specific proteins S100B protein, UCH-L1, and total Tau. S100B is a calcium-binding protein released in the setting of astroglial injury that is known to be neurotoxic at supraphysiologic concentrations. Several small studies have related S100B levels in the first few days of
life to clinical grade of encephalopathy, brain injury by MRI, and/or short-term neurodevelopmental outcomes in newborns with HIE in both the pre- and postcooling era. UCH-L1, also known as neuronal-specific protein gene product 9.5, is an abundant protein concentrated in neurons of the central nervous system. Recent studies have related UCH-L1 to brain injury in neonatal HIE. To our knowledge, no studies have evaluated S100B and UCH-L1 levels out to day 5 after perinatal asphyxia. Our findings are consistent with prior studies relating these biomarkers measured in the first 24 hours of life with brain injury by MRI, but suggest limited utility of measurements performed at day 5 of life. Tau protein is a microtubule-associated protein that is abundant in neuronal axons. In the setting of hypoxia-ischemia, phosphorylated Tau accumulates intracellularly leading to axonal injury and cell death. Two recent studies have reported correlation between outcomes and peripheral blood Tau measurements performed serially up to day 7 in newborns with HIE. In 1 study, hypothermia-treatment was associated with reduced serial Tau measures compared with normothermic infants managed conventionally, demonstrating the potential of Tau to reflect treatment modification after neurotherapeutic intervention. Our results suggest that Epo treatment may reduce Tau at day 5 (Figure 1), although we did not demonstrate a statistically significant difference in Epo treatment modification. We acknowledge that our study was underpowered to identify such treatment modification effects. Future studies will need to evaluate whether Epo treatment modifies the trajectory of these biomarkers and their relationships with neurologic outcomes.

That we did not observe a relationship between NSE and brain injury supports the limitation of this brain specific protein as a viable biomarker of neonatal brain injury. With known variability in the setting of hemolysis, prior studies have demonstrated conflicting predictive abilities for neurological injury in newborns with HIE. We cannot make conclusions about the utility of GFAP as a biomarker of brain injury given that most levels in our cohort were below the limits of detection by our assay (0.2 ng/mL). We used a customized multiplexed assay for GFAP determinations that was validated in the setting of mild traumatic brain injury. Other studies using high-affinity assays have demonstrated association between GFAP and brain injury in HIE, and have described increasing levels over the first week of life in babies with poor outcomes. Future studies will need to incorporate high-affinity assays for GFAP to evaluate the comparative predictive

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**Figure 2.** Relationships between day 5 A, Epo, B, Tau, and C-D, BDNF levels and brain injury, assessed by A-C, MRI Global Brain Injury Score and D, WIDEA score at 1 year, were not different by Epo treatment (red) compared with placebo (black). P values are adjusted for encephalopathy severity, treatment group, and specimen processing time.
ability of this brain specific protein with other biomarkers of brain injury in babies with HIE.

We describe modest associations between proinflammatory (IL-1β, IL-6, IL-8, IFN-γ, and TNF-α) and anti-inflammatory (IL-10, IL-13) cytokines and brain injury. Consistent with prior studies in neonatal HIE, these associations are mainly observed in the first 24 hours as the neuroinflammatory response to injury peaks during this time period. Although prior studies have suggested that IL-6 and IL-8 may continue to differentiate severity of brain injury out to 48 to 72 hours, our study suggests limited utility to delayed assessment of inflammatory cytokine response at 5 days after insult. BDNF is a cytokine secreted by neural cells to promote cell survival and regeneration in the setting of insults such as hypoxia-ischemia and inflammation. Two studies have related early BDNF levels to severity of HIE in newborns. Although not statistically significant, we likewise observed a positive correlation between BDNF measured in the first 24 hours of life and severity of brain injury by MRI. In contrast, higher BDNF at day 5 was associated with a trend toward lower MRI global brain injury score and a higher WIDEA score at 1 year. These data suggest that BDNF release may serve as an acute marker of injury when measured early, but observation of a sustained elevation of BDNF may reflect neuroprotective and reparative processes. This positive association between BDNF and outcomes has been reported in patients with traumatic brain injury.

Although baseline Epo level was not associated with global MRI injury score, it is of interest that a positive correlation was observed between baseline (endogenous) Epo level and injury by MRI in the basal ganglia and brainstem. This may signify the role of early postnatal Epo level as a marker of in utero hypoxia and associated risk for brain injury. Conversely, Epo level at day 5, largely indicating Epo treatment status, was negatively correlated with brain injury. This is consistent with the significant effect of Epo treatment in reducing brain injury by MRI demonstrated in the parent study.

Our selection of candidate biomarkers was driven both scientifically and pragmatically. Given limited plasma volumes because of neonatal blood sampling restrictions, we prioritized analytes that had literature-supported evidence of being correlated to neonatal brain injury and were quantifiable via multiplexed or high-sensitivity assays utilizing minimal sample volume. We recognize that this selected panel is not exhaustive of all promising biomarkers as other brain specific proteins (eg, Activin A, spectrin breakdown products, neurofilament heavy chain protein) and cytokines (eg, macrophage inflammatory protein 1α and vascular endothelial growth factor) have been described as potential biomarkers in HIE. However, this approach enabled us to screen a relatively large number of candidate biomarkers within a single well-characterized cohort of newborns with HIE.

Likewise, our sampling approach in this ancillary study was pragmatically coordinated with time points designed for Epo pharmacokinetic analyses. The early time point (<24 hours of life) allowed for evaluation of biomarkers in the acute period when therapeutic decisions are often being made and when peak of secondary injury is known to occur. The later time point was following rewarming and in close proximity to when brain MRI, one of our primary outcomes, was performed. Evaluating cytokine levels later in the course of illness is important as prior studies have suggested biphasic response for some cytokines such as IL-6 and IL-8. In our study, most cytokines decreased by day 5 suggesting that the neuroinflammatory response to hypoxia-ischemia peaks in the first few days after injury and later serial measurements have limited utility in monitoring ongoing secondary injury. Meanwhile, BDNF and brain specific proteins such as plasma Tau may have a role in the longitudinal monitoring of evolving brain injury. Future studies might require a more frequent sampling strategy, particularly during the first 48-72 hours of life to establish trajectories of these biomarkers with more granularity.

Our study has several limitations. Sample size for this ancillary study was determined by the parent randomized trial. Therefore, it was not surprising that we were unable to detect statistically significant effects of Epo treatment modification across biomarkers. Likewise, sample size limitations precluded our ability to control for additional demographic and clinical covariates in our analyses, to evaluate combinative predictive values, and to adjust for multiple comparisons. As previously reported, 2 infants with myotonic dystrophy and brainstem malformation were identified after enrollment in the parent trial. We analyzed all available data according to the parent study was determined by the parent randomized trial. Our sampling approach in this ancillary study was pragmatically coordinated with time points designed for Epo pharmacokinetic analyses. The early time point (<24 hours of life) allowed for evaluation of biomarkers in the acute period when therapeutic decisions are often being made and when peak of secondary injury is known to occur. The later time point was following rewarming and in close proximity to when brain MRI, one of our primary outcomes, was performed. Evaluating cytokine levels later in the course of illness is important as prior studies have suggested biphasic response for some cytokines such as IL-6 and IL-8. In our study, most cytokines decreased by day 5 suggesting that the neuroinflammatory response to hypoxia-ischemia peaks in the first few days after injury and later serial measurements have limited utility in monitoring ongoing secondary injury. Meanwhile, BDNF and brain specific proteins such as plasma Tau may have a role in the longitudinal monitoring of evolving brain injury. Future studies might require a more frequent sampling strategy, particularly during the first 48-72 hours of life to establish trajectories of these biomarkers with more granularity.

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<table>
<thead>
<tr>
<th>Sample</th>
<th>Epo (max)</th>
<th>Placebo (max)</th>
<th>P value*</th>
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<tbody>
<tr>
<td>PK-1</td>
<td>1.3 (0.5-12.6)</td>
<td>1.2 (0.5-1.6)</td>
<td>.79</td>
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<tr>
<td>PK-2</td>
<td>0.9 (0.5-11.6)</td>
<td>0.8 (0.5-1.0)</td>
<td>.46</td>
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<tr>
<td>PK-3</td>
<td>0.7 (0.5-1.1)</td>
<td>0.8 (0.5-2.6)</td>
<td>.42</td>
</tr>
</tbody>
</table>

max, maximum; PK, pharmacokinetics.
Data presented as median (IQR).
*P value derived using a Wilcoxon-rank sum test.