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Blood Biomarkers for Neonatal Hypoxic-Ischemic Encephalopathy in the Presence and Absence of Sentinel Events

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Abstract

OBJECTIVE—To determine if neonatal serum biomarkers representing different pathways of injury differ for cases of HIE of unknown cause to gain insight into timing and mechanism of injury.

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Disclosures

Under a license agreement between ImmunArray Ltd. and the Johns Hopkins University, the University and Dr. Everett are entitled to royalties on an invention described in this study and discussed in this publication. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policies.

The authors report no conflict of interest

STUDY DESIGN—In this cohort of all neonates with HIE admitted to our NICU, newborns with sentinel events were compared to those without during the 1st 3 days of life. Discard neonatal blood during the 1st 3 days of life was used for analysis.

RESULTS—Of 277 babies with HIE treated with whole-body hypothermia, 190 (68.6%) had blood available for biomarker analysis. 71 (37.4%) were born within our system, and 119 (62.6%) were transferred in from outside hospitals. Of these babies, 77 (40.5%) had a sentinel event and 113 (59.6%) had no sentinel event. Although the degree of metabolic acidosis was similar, repeated measures analysis showed that during the initial 3 days of life neonates born with HIE in the absence of sentinel events had 41.4% decreased VEGF (p=0.027) and 62.5% increased IL-10 serum concentrations (p=0.005).

CONCLUSION—These changes indicate that neonatal HIE in the absence of sentinel events is not related to an unrecognized acute intrapartum event and is possibly related to chronic hypoxia of lower severity or recovery from a remote event.

Keywords

Blood biomarkers; Hypoxic-ischemic encephalopathy; Neonate; Whole-body hypothermia; Vascular endothelial growth factor; Interleukin-10

Introduction

Hypoxic ischemic encephalopathy (HIE) is a major cause of neonatal morbidity and mortality.(1) It is a clinical syndrome that results from impaired cerebral perfusion and oxygen delivery to the fetus or newborn with resultant hypoxia, metabolic acidosis, and neurologic morbidity(1,2) characterized by depressed consciousness, abnormal muscle tone and reflexes, seizures, and respiratory distress.(3) HIE occurs in approximately 1.5 infants per 1000 live births and globally accounts for an estimated 491,900–724,000 neonatal deaths annually.(4,5) Significantly, 25% of HIE neonatal patients die in the postnatal period, and another 25% develop permanent neurological complications such as cerebral palsy, global developmental delays, intellectual disability, epilepsy, and autism spectrum disorders.(4)

HIE can be due to acute intrapartum sentinel events (known cause of injury) or can be unexplained in the absence of sentinel events.(3) Intrapartum sentinel events are those occurring immediately before or during delivery that could disrupt placental blood flow.(2) Such events include ruptured uterus, placental abruption, umbilical cord prolapse, shoulder dystocia, maternal cardiopulmonary arrest, massive fetomaternal hemorrhage, or amniotic fluid embolism. Sentinel events occur in approximately 5/1,000 births.(6) Only a minority (25–35%) of HIE cases are attributable to clear sentinel events in the intrapartum period.(7,8) For instance, only an estimated 10 to 20% of cases of spastic quadriplegia or dyskinetic cerebral palsy, which is a clinical manifestation of HIE, are attributed to intrapartum sentinel events.(2,9) However, potential mechanisms of injury for the development of HIE in the absence of sentinel events are poorly understood.

In our attempt to understand how moderate-severe neonatal brain injury occurs in the absence of an identifiable cause, this study examined cases with sentinel events in the intrapartum period and compared them to cases without sentinel events. We previously

found that nulliparity with histologic funisitis and longer duration of labor were associated with neonatal HIE in the absence of sentinel events,(3) which suggested the possibility of subclinical infection and hypoxia-ischemia of less severity but longer duration being related to the brain injury.

Circulating brain injury biomarkers have been used to gain insight into the mechanism and timing of brain injury.(10,11) In neonates with encephalopathy at birth of such severity that treatment is required within 6 hours of delivery, but where no sentinel event is identified, we examined biomarkers in neonatal serum during the first 3 days of life related to angiogenesis after hypoxia, neuronal survival, axonal transport, glial cytoskeletal support in the central nervous system (CNS), markers of inflammation/infection and protection of brain tissue. These include assaying for neuronal [neurogranin-NRGN, Tau] and glial [glial fibrillary acidic protein-GFAP] specific proteins that function as necrosis markers or molecules involved in the secondary injury process or recovery such as cytokines [interleukin (IL)-6, 8, 10, brain derived neurotrophic factor-BDNF and vascular endothelial growth factor-VEGF]. By measuring levels of this diverse array of blood biomarkers we sought to shed light on timing and mechanism of perinatal brain injury in the absence of an identifiable intrapartum cause.

Materials and Methods

We conducted a retrospective cohort study of all neonates with suspected HIE admitted to our university referral neonatal intensive care unit (NICU) for treatment with whole-body hypothermia from April, 2009 to November, 2019. This study was approved by the Johns Hopkins IRB #26068 on 3/26/2009, and informed consent was obtained from participants. To qualify for whole-body hypothermia, neonates had to be admitted and begin cooling within 6 hours of birth, be ≥ 35 weeks gestation and weigh > 1800 grams; however the neonatologists can begin cooling for a baby born at 34 weeks if they feel the benefits outweigh the risks. Exclusion criteria included major congenital anomalies, severe persistent pulmonary hypertension with anticipated need for extracorporeal membrane oxygenation, coagulopathy with active bleeding, and suspected sepsis with severe hemodynamic compromise requiring large doses of vasopressors. Those neonates transported from outside institutions were started on passive cooling upon recognition of need for therapeutic hypothermia with instructions for temperature goal. Whole-body hypothermia treatment consisted of being cooled to a rectal temperature of 33.5°C for 72 hours. All children enrolled in the hypothermia protocol were evaluated by a pediatric neurologist within 18 hours.

Infant and maternal medical records were reviewed to identify relevant clinical data. A sentinel event was defined as a ruptured uterus, abruption, umbilical cord prolapse, amniotic fluid embolism, maternal cardiopulmonary arrest, shoulder dystocia, ruptured vasa previa or massive fetomaternal hemorrhage.(3) The diagnosis of non-reassuring fetal heart rate tracing was made by the physician attending delivery prior to performing a cesarean delivery. Preeclampsia was defined as proteinuria, edema, and the presence of new onset hypertension. The clinical diagnosis of chorioamnionitis was made in the presence of maternal fever, with the presence of at least one other finding of fetal tachycardia, uterine

tenderness, or purulent vaginal discharge. Patients diagnosed with clinical chorioamnionitis were immediately started on intravenous antibiotics. Intrauterine growth restriction (IUGR) was defined as an estimated fetal weight less than the 10^{th} percentile.(12) Oligohydramnios was defined as an amniotic fluid index (AFI) < 5.0 cm with intact membranes at the time of the admission in which delivery occurred. Sepsis was considered present only for neonates with positive blood and/or cerebrospinal fluid cultures. A brain MRI with diffusion tensor images was performed between days 7 and 10 of life. These MRI images were reviewed by an experienced pediatric neuroradiologist at our institution and were used to assign a National Institute of Child Health and Development (NICHD) brain injury score based on the MRI findings with 0 indicating normal; 1A minimal cerebral lesions only; 1B more extensive cerebral lesions without other involvement; 2A basal ganglia, thalamic, internal capsule lesions only; 2B basal ganglia, thalamic, internal capsule and cerebral lesions and 3 cerebral hemispheric devastation.(13)

ELISA Assays

The immunoassays were run on discard neonatal serum after clinically indicated tests had been performed. Serum samples were held at 4°C for 48 hours before being aliquoted and stored at -80^0C until assayed. This study did not require additional blood draws for biomarker ascertainment. These 8 biomarkers were measured on admission to the NICU and on the subsequent 3 days of life. A custom multiplex enzyme-linked immunosorbent assay (ELISA) was developed to measure BDNF, IL-6, IL-8, IL-10, and VEGF simultaneously using robotically spotted capture antibodies on the 96-well plate format (Meso Scale Discovery [MSD], Rockville, MD). A custom duplex ELISA was developed to measure GFAP and NRGN simultaneously using robotically spotted capture antibodies on the 96-well plate format (MSD, Rockville, MD). Tau was measured using a commercial ELISA (MSD, Rockville, MD, Human Total Tau Kit, Cat # N451LAA-1). The use of these assays to measure plasma and cerebrospinal fluid biomarkers of neonatal encephalopathy severity have been confirmed as we have previously reported.(14)

Stata version 15.1 was used for statistical analysis (StataCorp LP, College Station, TX). Between-group differences in continuous variables were analyzed using Wilcoxon rank sum (Mann Whitney) test. Fisher's exact test was used to test differences in categorical variables. The median and interquartile ranges of non-normally distributed continuous variables, and absolute counts and proportions of categorical variables were reported. All biomarker measurements were logarithmically transformed before regression analysis, since they were skewed. Prior to logarithmic transformation, values less than the lower limit of detection (LLOD) were imputed as 0.5*LLOD, and values above the upper limit of detection were imputed to 2*ULOD. Repeated measures analysis of each of the biomarkers over all days of measurement adjusted for sampling epoch was performed using mixed model analysis nested within individual patients to account for within-person correlation. For those with sentinel events and separately those without, adjusted marginal geometric means and their 95% confidence were exponentiated for tabulation, thus to be interpreted in their original measurement units.

Results

Over a 10.6-year period, 277 neonates were treated for HIE at our facility with whole-body hypothermia within 6 hours of delivery. This includes neonates delivered at our facility and referrals from other facilities. Of these babies 190 (70.4%) had at least one serum sample drawn during the period from admission to the NICU through the first 3 days of life, with sentinel events occurring in 77 (40.5%) and no sentinel events in 113 (59.5%). Of the 190 neonates in this study 71 (37.4%) were born within our institution and 119 (62.6%) were transferred in from outside hospitals. The percentage of neonates transported in for whole-body hypothermia for HIE did not differ between the no sentinel event (51, 42.9%) and sentinel event groups (68, 57.1%) (p=0.45).

Maternal characteristics

Maternal demographics such as age and race (Table 1) were similar between mothers with sentinel events and those without. There was no significant difference in nulliparity (37.9% vs. 62.1%, p=0.22) or gestational age (38.7 vs. 39.1 weeks, p=0.07). There were no significant differences detected between the two groups with respect to maternal oxytocin administration, preeclampsia, oligohydramnios, IUGR, clinical chorioamnionitis and cesarean delivery. There was no difference in placental histopathologic abnormalities including histologic chorioamnionitis, funisitis or placental infarcts. (Table 1) The largest number of sentinel events were abruptions (32, 41.6%), and one of these abruptions was also complicated by an eclamptic seizure. Other sentinel events were isolated eclampsia (3), shoulder dystocia (17), uterine rupture (12), cord prolapse (5), fetal-maternal hemorrhage (1), ruptured velamentous cord insertion (1), tight nuchal cord x 3 which was difficult to reduce (1), abdominal trauma status post motor vehicle accident (1), head entrapment during a breech delivery (1), and 2 home deliveries, one complicated by the baby’s head hitting the floor with rupture of the cord and the other baby delivering into the toilet with a tight nuchal cord x 3.

Neonatal characteristics

Neonates born to mothers without recognized sentinel events had no difference in birth weight or level of metabolic acidosis.(Table 2) They did have a significantly lower initial glucose compared to those without maternal sentinel events (88 mg/dl vs 105 mg/dl, p=0.005); (Table 2) however, both these glucose levels were within the normal range. Initial neonatal arterial pH, base deficit, lactate, WBC count, hematocrit and nucleated red blood cell counts did not show any statistically significant differences between neonates with and without sentinel events. There were also no significant differences between the two groups with regards to respiratory distress, positive neonatal blood cultures, seizures, death or NICHD brain injury score based on an MRI performed between days 7 and 10 of life.(Table 2) There were 4 neonates cooled at a gestational age of 34 weeks during this 10.6 year period at the discretion of the neonatology attending based on the decision that benefits would outweigh risks.

Biomarker analysis

Analysis of daily biomarker levels of babies from NICU admission to day 3 of life showed significant decreases of VEGF on days 2 and 3 of life and increases for IL-6 on day 3 of life, IL-10 on days 1–3 of life and IL-8 on days 1 and 3 of life for neonates with HIE in the absence of sentinel events.(Table 3, Figure 1) GFAP, NRGN, BDNF and Tau were not different between the sentinel event versus no sentinel event groups. Repeated measures analysis adjusted for the day of sampling for the entire period from NICU admission to day 3 of life showed that neonates without sentinel events had significantly lower VEGF levels and significantly higher IL-10 levels.(Table 4) IL-6 showed a trend toward an increase in neonates without sentinel events ($p=0.06$) which mirrored the increase seen in IL-10. During the initial 3 days of life neonates born with HIE in the absence of sentinel events had 41.4% decreased VEGF ($p=0.027$) and 62.5% increased IL-10 serum concentrations ($p=0.005$).

Discussion

This study sought to determine if there are any differences in biomarker levels in neonates with moderate-severe HIE that could shed light on the timing and mechanism of injury in the absence of an identifiable intrapartum cause. The proportion of neonatal HIE in our study with documented sentinel events (40.5%) is slightly higher than proportions in some previous studies (25–35%).(7,8)

The main findings of this study are:

1. neonates born with unexplained HIE in the absence of sentinel events have significantly lower VEGF and higher IL-10 in the first 3 days of life compared to neonates with HIE related to an acute intrapartum sentinel event. IL-6 showed a trend towards increase that mirrored the increase seen in IL-10.
2. The other biomarkers we measured in this cohort of neonates with HIE; IL-8, GFAP, TAU, BDNF and NRGN were not significantly different in the first 3 days of life between the groups based on the presence of sentinel events. Elevations in these biomarkers are common in HIE in general, regardless of the etiology of the hypoxia-ischemia, and represent a more final common pathway of clinical injury.

We chose blood biomarkers based on their association with different possible pathways in the process leading to brain injury. VEGF is a polypeptide growth factor secreted by the placenta and other organs(15) which is activated by tissue hypoxia(15,16) and promotes the proliferation and angiogenesis of vascular endothelial cells.(17–19) There is increased VEGF expression in astrocytes and neurons during cerebral ischemia(20) which induces proliferation of astrocytes and is anti-apoptotic to dopaminergic neurons.(21) In addition to oxygen, VEGF is also useful in delivering glucose, lactate, and glycogen derived from the astroglia to the neurons for optimal neuronal activity.(22,23) The VEGF gene is regulated by the binding of hypoxia-inducible factor to elicit a response from target genes.(16) Acute hypoxia may lead to abrupt increases in VEGF increasing angiogenesis, while chronic hypoxia downregulates VEGF and inhibits angiogenesis leading to a lower intensity, longer duration of hypoxia.(24)

VEGF promotes hypoxia induced angiogenesis,(25) and mediates endothelial cell proliferation through stimulation of endothelial nitric oxide synthase.(26,27) Increased VEGF concentrations are detectable within 3 hours after ischemic stroke with peak levels between 12 to 48 hours.(28) The observed higher levels of VEGF in neonates with sentinel events in our study may be attributable to the intensity of the acute hypoxic–ischemic episode, which causes a rapid and sharp increase in serum VEGF levels compared to neonates without sentinel events where the hypoxic exposure may be longer and less severe. VEGF has both neuroprotective and neurotoxic properties since it can either be upregulated or downregulated in response to hypoxia.(29). Our finding of lower levels of VEGF in neonates with HIE without a sentinel event suggests that a longer duration, less severe hypoxic episode probably downregulated VEGF with resultant low levels during the 1st 3 days of life, which could indicate that these babies may suffer the consequences of inhibition of angiogenesis and ultimately worsened ischemic injury.(24)

Adenovirus VEGF gene therapy in sheep and guinea pig models of fetal growth restriction have been shown to increase fetal growth.(30,31) There is also an ongoing clinical trial to ascertain the utility of adenovirus VEGF gene therapy as treatment for IUGR,(32) one of the manifestations of chronic hypoxia in the fetus. VEGF improves recovery of sensorimotor and cognitive deficits secondary to focal cerebral ischemia.(33) Studies have demonstrated the association of higher VEGF levels in cord blood with improved long term neurodevelopmental outcomes among babies with HIE.(20,34). The indispensable role of VEGF in neurodevelopmental processes like neurogenesis, plasticity, neuronal migration, neuronal survival, and axon guidance has been recently well documented and has promise as the therapeutic molecule to improve cognition after a hypoxic insult.(22,35)

IL-10 plays a protective role in brain tissue by inhibiting the secretion of IL-1β, IL-8 and TNF-α, inhibiting the production of chemokines, decreasing leukocyte aggregation, and reducing inflammatory responses in the brain.(17) IL-10 levels are significantly elevated in the acute phase of injury in neonates with HIE.(36) IL-10 plays a crucial role in restoring brain vascular function that can occur after ischemia or trauma.(37) Low or absent IL-10 results in changes to the cerebral vasculature leading to harmful vascular remodeling and impaired vascular relaxation exacerbating secondary brain damage following acute injury. (37) Although in vitro and in vivo models of brain ischemia have shown IL-10 mediated neuroprotection, the role of IL-10 in predicting clinical outcomes is not clear. In a limited number of clinical studies higher IL-10 levels seen post-injury have been associated with worse outcomes, so while IL-10 is consistently elevated following brain injury, the effect of these high levels appears to be pathology dependent with preclinical and clinical studies often yielding conflicting results.(37) The higher IL-10 levels seen in the first 3 days of life in neonates with HIE without sentinel events, suggest that the hypoxic insult occurred earlier than in those neonates with intrapartum sentinel events. (37)

Neonatal blood biomarkers could provide information about cell types injured due to HIE which could be related to timing and mechanisms of injury. Future research should examine if there is correlation of blood biomarkers in the period just after birth with pediatric MRI imaging and whether they can provide prognostic information related to infant neurodevelopment.

Our analysis involved biomarker measurements of this cohort of neonates over the first 3 days of life. Although serum samples were drawn daily and cohorted into 24 hour epochs, the time during the day at which the samples were obtained was not accounted for in this study, which fails to account for daily fluctuations in biomarker levels.(38) Our study did not control for the timing of blood draws during each day since we used serum from clinically scheduled blood draws that would otherwise have been discarded and did not perform blood draws specifically for this study. The problem with this approach stems from how the rapidly changing extrauterine environment could affect each neonate differently with implications for differences in the levels of the biomarkers of interest.(20) Another major limitation is the small number of neonates in our cohort who had placental and umbilical cord histopathology results, though an earlier study by Novak et al showed that placentas from unexplained HIE cases more often exhibited histologic chorioamnionitis and funisitis.(3) The increased levels of interleukins found in our current study could be related to undiagnosed subclinical infection in neonates with HIE but without a recognized sentinel event. During this 10.6 year period there were 4 neonates born at 34 weeks who received cooling, a gestational age at which the benefit of this therapy has not been demonstrated, but these neonates were included in this study to determine if blood biomarkers correlated with degree, timing and mechanism of brain injury even if the cooling therapy was not efficacious. Most importantly our study is limited due to the lack of long-term follow-up for neurodevelopmental outcomes in this cohort of HIE neonates. There is an urgent need for long-term studies to correlate serum biomarker levels with neurodevelopmental outcome in HIE with or without identifiable sentinel events. The strength of this study is that we have collected a continuous stream of data including maternal and neonatal clinical information, blood biomarkers during the 1st week of life and neonatal MRI imaging with plans for neurodevelopmental follow-up for all neonates admitted to our NICU for HIE over a 10 year period.

In conclusion, our study finding of lower VEGF and higher IL-10 levels in the first 3 days of life in neonates with HIE without sentinel events sets the stage for further exploration of the role biomarkers play in elucidating the mechanism and timing of injury of HIE in neonates without an obvious intrapartum hypoxic-ischemic event and their relationship to long term neurodevelopmental outcome. The underlying causes of HIE remain complex and multifactorial, and biomarkers may help unravel the cause of HIE in this subgroup of neonates without an obvious cause. In neonates with HIE and a similar degree of metabolic acidemia at birth begun on whole-body hypothermia within 6 hours, a longer duration and less severe hypoxic exposure could have downregulated VEGF and increased IL-10 production during the 1st 3 days of life indicating that these cases are not related to an unrecognized sentinel event and put these neonates at increased risk of adverse neurodevelopmental outcomes.

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CONDENSATION

Neonatal hypoxic-ischemic encephalopathy in the absence of sentinel events is associated with lower levels of vascular endothelial growth factor and higher levels of interleukin-10.

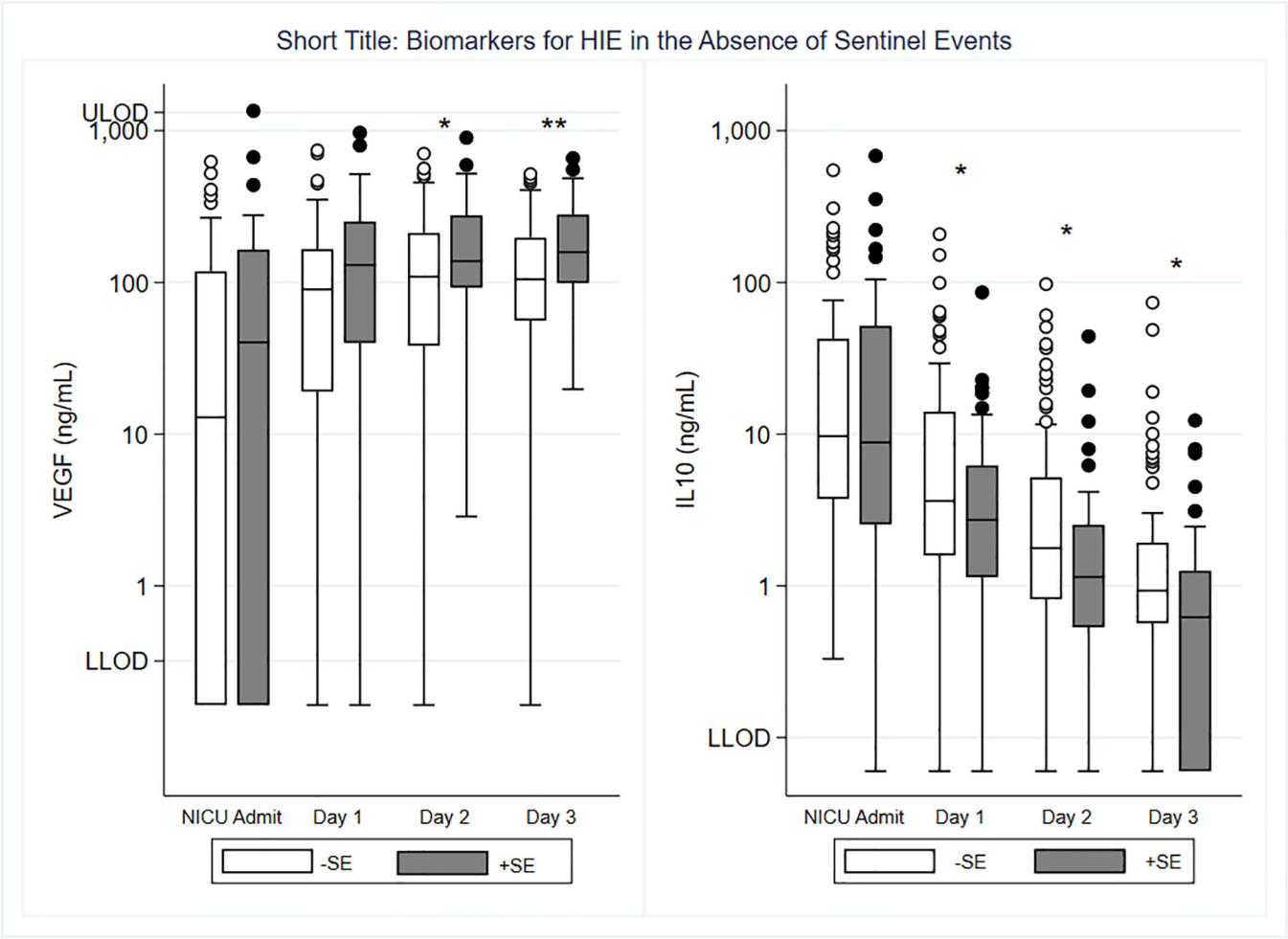

Figure 1.

Vascular Endothelial Growth Factor (VEGF) and Interleukin (IL)-10 levels measured at Neonatal Intensive Care Unit (NICU) admission and daily for the first 3 days of life for neonates with hypoxic-ischemic encephalopathy treated with whole-body hypothermia within 6 hours of birth without sentinel events (-SE) and with sentinel events (+SE). Box and central line represent median and interquartile range; ULOD=upper limit of detection, LLOD=lower limit of detection

Table 1.

Bivariate analysis of maternal variables for neonates with moderate-severe hypoxic-ischemic encephalopathy treated with whole-body hypothermia within 6 hours of birth comparing those with an intrapartum sentinel event to those without.

	HIE + SE N= 77	HIE − SE N= 113	P value
Maternal Age, years †	29 (26–33)	29 (24–33)	0.90
Nulliparity	55 (37.9%)	90 (62.1%)	0.22
Race			0.21
Caucasian	26 (33.8%)	46 (40.7%)	
African-American	31 (40.3%)	38 (33.6%)	
Hispanic	8 (10.4%)	8 (7.1%)	
Asian	2 (2.6%)	11 (9.7%)	
Other	10 (13.0%)	10 (8.8%)	
Gestational Age (weeks) †	38.7 (37.1–40.0)	39.1 (38.0–40.1)	0.07
Oxytocin	23 (29.9%)	42 (37.2%)	0.35
Preeclampsia	7 (9.1%)	14 (12.4%)	0.64
Intrauterine growth restriction	1 (1.3%)	5 (4.4%)	0.40
Oligohydramnios	1 (1.3%)	2 (1.8%)	0.49
Abruption	32 (41.6%)	0	<0.001 *
Nonreassuring fetal heart rate	49 (63.6%)	61 (54.0%)	0.23
Meconium	12 (15.6%)	47 (41.6%)	<0.001 *
Cesarean delivery	55 (71.4%)	72 (63.7%)	0.40
Clinical chorioamnionitis	6 (7.8%)	15 (13.3%)	0.35
Histologic chorioamnionitis	8/27 (29.6%)	19/44 (43.2%)	0.32
Histologic funisitis	3/27(11.1%)	10/44 (22.7%)	0.34
Histologic placental infarcts	4/27 (14.8%)	6/44 (13.6%)	1.0

HIE = hypoxic-ischemic encephalopathy

SE = sentinel event

* indicates $P < 0.05$

† (median, interquartile range)

Table 2.

Bivariate analysis of neonatal variables for neonates with moderate-severe hypoxic-ischemic encephalopathy treated with whole-body hypothermia within 6 hours of birth comparing those with an intrapartum sentinel event to those without. For continuous variables, normally distributed data are presented as mean ± standard deviation and skewed data as median, interquartile range.

	HIE + SE N = 77	**HIE − SE N = 113**	**P value**
Birth weight (grams) †	3120 (2785–3825)	3280 (3008–3670)	0.56
Male gender	45 (58.4%)	68 (60.2%)	0.88
1 min Apgar < 7	76 (98.7%)	104 (92.0%)	0.08
5 min Apgar < 7	65 (84.4%)	84 (74.3%)	0.19
Arterial cord pH †	6.92 (6.80–7.07)	6.95 (6.87–7.06)	0.27
Arterial cord base deficit (mM) †	14.3 (9.8 to 20.0)	14.0 (11.0 to 18.0)	0.79
pH<7.0 or base deficit>12 mM	42 (54.5%)	65 (57.5%)	0.29
Initial neonatal arterial pH †	7.16 (6.99–7.25)	7.15 (7.07–7.24)	0.55
Initial neonatal arterial BD (mM) †	16.0 (9.0 to 21.0)	15.7 (11.0 to 18.1)	0.68
Initial neonatal lactate (mmol/L) †	5.1 (3.1–9.5)	5.3 (2.7–9.5)	0.99
Initial neonatal glucose (mg/dL) †	105 (77–152)	88 (47–118)	0.005 *
Initial neonatal WBC count (K/mm^3) †	17.2 (12.5–24.2)	19.5 (13.9–24.6)	0.25
Initial neonatal hematocrit (%) †	45.7 (40.6–52.1)	45.6 (40.6–51.1)	0.73
Initial neonatal platelet count (K/mm^3) †	191 (150–230)	182 (137–221)	0.23
Respiratory distress	34 (44.2%)	43 (38.1%)	0.45
+ Blood cultures	2 (2.6%)	6 (5.3%)	0.48
Seizures	24 (31.2%)	37 (32.7%)	0.87
Length of stay (days) †	13 (9–20)	11 (9–24)	0.55
Death	3 (3.9%)	5 (4.4%)	1.0
NICHD Brain Injury Score			0.06
0	41 (53.2%)	63 (55.8%)	
1A	7 (9.1%)	14 (12.4%)	
1B	0	3 (2.7%)	
2A	5 (6.5%)	5 (4.4%)	
2B	1 (1.3%)	9 (8.0%)	
3	3 (3.9%)	0	

HIE = hypoxic-ischemic encephalopathy

SE = sentinel event

† (median, interquartile range)

* indicates P < 0.05

Table 3.

Daily biomarker levels from admission to the neonatal intensive care unit through day 3 of life.

Serum Biomarker	HIE + Sentinel Event N=77 neonates	HIE – Sentinel Event N=113 neonates	N biomarker readings	P value
NRGN NICU Admission (ng/mL)	0.014 (0.009–0.021)	0.009 (0.006–0.014)	98	0.22
NRGN day of life 1	0.014 (0.009–0.022)	0.014 (0.010–0.021)	143	0.91
NRGN day of life 2	0.017 (0.010–0.028)	0.022 (0.014–0.032)	150	0.42
NRGN day of life 3	0.016 (0.010–0.26)	0.023 (0.015–0.034)	148	0.26
GFAP NICU Admission (ng/mL)	0.48 (0.25–0.94)	0.24 (0.14–0.44)	98	0.13
GFAP day of life 1	0.52 (0.29–0.93)	0.28 (0.18–0.45)	143	0.11
GFAP day of life 2	0.41 (0.23–0.74)	0.39 (0.24–0.61)	149	0.87
GFAP day of life 3	0.50 (0.29–0.85)	0.40 (0.26–0.63)	148	0.54
BDNF NICU Admission (pg/mL)	1199 (858–1675)	1065 (790–1434)	97	0.60
BDNF day of life 1	1222 (960–1557)	1076 (881–1315)	147	0.43
BDNF day of life 2	945 (770–1161)	901 (766–1058)	154	0.72
BDNF day of life 3	782 (625–978)	813 (672–985)	147	0.79
TAU NICU Admission (pg/mL)	202.7 (117.0–351.2)	108.7 (66.7–177.2)	95	0.10
TAU day of life 1	183.3 (115.7–290.6)	110.3 (76.1–160.0)	144	0.09
TAU day of life 2	148.1 (90.4–242.7)	137.0 (93.2–201.3)	145	0.81
TAU day of life 3	171.6 (104.1–282.8)	160.9 (104.6–247.6)	138	0.85
VEGF NICU Admission (pg/mL)	12.8 (4.92–33.2)	7.20 (3.14–16.5)	95	0.37
VEGF day of life 1	72.0 (41.5–125.1)	37.5 (23.6–59.6)	145	0.08
VEGF day of life 2	129.9 (86.7–194.5)	68.9 (50.0–94.9)	152	0.017*
VEGF day of life 3	158.8 (115.7–217.3)	79.1 (60.3–103.8)	145	0.001*
IL-6 NICU Admission (pg/mL)	19.3 (11.3–32.8)	30.2 (18.8–48.5)	97	0.22
IL-6 day of life 1	24.2 (16.6–35.3)	36.0 (26.4–49.1)	148	0.11
IL-6 day of life 2	18.7 (13.6–25.9)	26.2 (20.3–33.8)	154	0.11
Il-6 day of life 3	9.18 (6.99–12.1)	13.2 (10.5–16.6)	148	0.049*
IL-8 NICU Admission (pg/mL)	86.8 (57.7–130.5)	134.7 (92.4–196.4)	89	0.12
IL-8 day of life 1	97.6 (74.9–127.1)	139.4 (110.5–175.8)	126	0.048*
IL-8 day of life 2	95.1 (73.2–123.7)	109.0 (87.4–136.0)	130	0.44
IL-8 day of life 3	60.7 (47.8–77.1)	86.0 (69.2–106.9)	121	0.037*
IL-10 NICU Admission (pg/mL)	9.03 (4.73–17.2)	13.7 (7.55–24.9)	89	0.35
IL-10 day of life 1	2.31 (1.50–3.55)	4.75 (3.26–6.94)	126	0.01*
IL-10 day of life 2	0.98 (0.63–1.53)	1.96 (1.36–2.85)	130	0.02*
IL-10 day of life 3	0.44 (0.29–0.68)	0.98 (0.67–1.44)	121	0.008*

HIE = hypoxic-ischemic encephalopathy; NRGN=neurogranin; GFAP=glial fibrillary acidic protein; BDNF=brain derived neurotrophic factor; VEGF=vascular endothelial growth factor; IL=interleukin

Table 4.

Biomarker levels adjusted for sampling time/day from admission to the neonatal intensive care unit through day 3 of life given as geometric mean (95% confidence interval).

Biomarker	HIE + SE N=77 neonates	HIE − SE N=113 neonates	Biomarker Readings	P value
NRGN (ng/mL)	0.016 (0.011–0.024)	0.017 (0.013–0.023)	539	0.86
GFAP (ng/mL)	0.473 (0.302–0.742)	0.333 (0.232–0.480)	538	0.24
BDNF (pg/mL)	995 (852–1162)	958 (842–1089)	545	0.71
TAU (pg/mL)	171.6 (120.0–245.5)	122.5 (91.6–163.7)	522	0.15
VEGF (pg/mL)	73.0 (50.6–105.2)	42.8 (31.6–57.9)	537	0.027 *
IL-6 (pg/mL)	17.2 (13.4–22.1)	23.5 (19.1–29.0)	547	0.06
IL-8 (pg/mL)	88.6 (72.6–108.3)	108.7 (91.4–129.3)	466	0.13
IL-10 (pg/mL)	1.60 (1.16–2.19)	2.90 (2.20–3.83)	466	0.005 *

HIE = hypoxic-ischemic encephalopathy; SE = sentinel event; NRGN=neurogranin; GFAP=glial fibrillary acidic protein; BDNF=brain derived neurotrophic factor; VEGF=vascular endothelial growth factor; IL=interleukin

* indicates $P < 0.05$