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# Plasma and CSF Candidate Biomarkers of Neonatal Encephalopathy Severity and Neurodevelopmental Outcomes

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## Abstract

**Objectives—**To identify candidate biomarkers in both plasma and cerebrospinal fluid (CSF) that are associated with neonatal encephalopathy severity measured by encephalopathy grade, seizures, brain injury by magnetic resonance imaging (MRI), and neurodevelopmental outcomes at 15–30 months.

**Study design—**A retrospective cohort study of plasma (N=155, day of life 0–1) and CSF (n=30, day of life 0–7) from neonates with NE and healthy term neonates (N=30, ≥36 weeks' gestation) was conducted. We measured CNS necrosis (glial fibrillary acidic protein [GFAP], neurogranin [NRGN], Tau), inflammatory (IL-6, IL-8, IL-10), and trophic (brain-derived neurotrophic factor [BDNF], vascular endothelial growth factor [VEGF]) proteins. Clinical outcomes were Sarnat scores of encephalopathy, seizures, MRI scores, and Bayley Scales of Infant and Toddler Development III (Bayley-III) at 15–30 months.

**Results—**Plasma NRGN, Tau, IL-6, IL-8, and IL-10 were higher, whereas BDNF and VEGF were lower in NE versus controls. In plasma, Tau, GFAP, and NRGN were directly and BDNF inversely associated with encephalopathy grade. IL-6 was inversely related to seizures. Tau was

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directly related to MRI abnormalities. Tau was inversely associated with Bayley-III cognitive and motor outcomes. In CSF, NRGN was inversely associated with cognitive, motor, and language measures. GFAP, IL-6, and IL-10 were inversely related to cognitive and motor outcomes. IL-8 was inversely related to motor outcomes. CSF candidate biomarkers showed no significant relationships with encephalopathy grade, seizures, or MRI abnormalities.

**Conclusions—**Plasma candidate biomarkers predicted encephalopathy severity, seizures, MRI abnormalities, and neurodevelopmental outcomes at 15–30 months.

## Keywords

Neurology; development; neonate; marker; brain injury

Neonatal encephalopathy is a syndrome defined by clinical features of neurological dysfunction during the first few days of life, including difficulty initiating or maintaining respiration, altered consciousness, and seizures.[1,2] NE occurs in as many as 3 per 1000 livebirths.[3] Common causes include hypoxic-ischemic insult leading to hypoxic-ischemic encephalopathy (HIE), perinatal infections, maternal conditions including placental abnormalities, and neonatal conditions including metabolic disorders, coagulopathies, and neonatal stroke.[1,4] The only validated treatment for this syndrome remains therapeutic hypothermia with intensive supportive care.[5] Despite the successful implementation of TH, approximately 29% of neonates with NE still have unfavorable outcomes of neurological disability and death.[6] The modest efficacy of TH may depend on accurate assessment of injury severity for targeted intervention.[7,8] Presently, current clinical methods are insufficient to identify risk, stratify severity, and monitor therapeutic efficacy which warrants further research into multidimensional approaches to assessing and monitoring evolving brain injury in neonates with NE.[9,10]

As a potential component of a multidimensional diagnostic/prognostic approach, biological fluid markers can provide objective, serial, and non-invasive information to identify pathological processes reflected by distinct peripheral concentrations. Biomarker assays have been extensively studied in adults to predict neurological pathologies, including traumatic brain injury and neurodegenerative diseases.[11–13] However, there is limited research and clinical application of pediatric biomarkers due to the need for large sample validation studies, especially in neonatal brain injury.[14]

Several candidate biomarkers, including CNS-necrosis, inflammatory, and trophic proteins, were identified in adult studies on brain injury and some have been investigated in neonatal populations. Glial fibrillary acidic protein (GFAP), a CNS-specific astrocyte cytoskeletal intermediate filament protein, was associated with abnormal magnetic resonance imaging (MRI) and neurodevelopmental outcomes.[15–18] Neurogranin (NRGN), a brain-specific protein kinase C substrate, has not yet been studied in neonatal populations, but has associations with adult traumatic brain injury (TBI) and neurodegeneration.[11,19–22] Tau, another CNS-necrosis factor, was associated with asphyxia and NE.[23–25] Inflammation plays an important role in brain injury, and increased cytokines, especially interleukins 6, 8, and 10 (IL-6, IL-8, IL-10), have been associated with infants with brain injury.[17,24,26–29] Brain-derived neurotrophic factor (BDNF), a trophic protein, was associated with severity

of injury and worse neurodevelopmental outcomes.[24,30,31] Another trophic factor, vascular endothelial growth factor (VEGF), was also associated with worse NE severity, abnormal imaging, and mortality.[17,32,33] Research in brain injury biomarkers suggests that a combination of biomarkers is the most effective for providing evidence of injury.[9,34,35] However, review of the current literature reports a need for validation studies before these biomarkers can be introduced for routine clinical care.[9,10]

We investigated a candidate multi-biomarker panel to identify molecules in both plasma and CSF that are associated with NE severity measured by encephalopathy grade, seizures, brain injury by MRI, and neurodevelopmental outcomes at 15–30 months.

## Methods:

In this multicenter retrospective cohort study of NE, we identified and analyzed the concentrations of CNS necrosis markers (GFAP, NRGN, Tau), inflammation markers (IL-6, IL-8, IL-10) and trophic-factor markers (BDNF, VEGF) from a cohort of neonates with NE and a cohort of healthy neonatal controls. The study received institutional review board approval at all hospitals, and signed informed consent was obtained from the parent of each participant. Johns Hopkins University Institutional Review Board approved the use of all cohorts in this study.

### Control Patient Cohort:

Healthy term neonates (≥36 weeks' gestation) had plasma samples collected from National Maternity Hospital Dublin and Coombe Women and Infants University Hospital (Trinity College) from 2016–2018 from day of life (DOL) 0–7 with a median collection of DOL 2. The samples were stored in the Trinity Translational Medicine Institute Biobank in Dublin, Ireland. De-identified plasma samples (n=30) from healthy term neonates were analyzed in collaboration with the Neonatal Inflammation and Multiorgan Dysfunction and Brain Injury Research group (NIMBUS) at Trinity College Dublin, Ireland (JHU MTA A33285).

### Neonatal Encephalopathy Patient Cohort:

NE was defined as requiring resuscitation at birth and having an abnormal neurological examination. Inclusion criteria were as follows: all infants with NE Sarnat score 2 or 3[36] requiring TH, NE in the first 48 hours of life without TH, or postnatally diagnosed with brain injury on cranial ultrasound.[33,37] Exclusion criteria consisted of maternal substance abuse and major congenital abnormalities. The NE cohort was drawn from National Maternity Hospital Dublin and Children's National Health System, Washington, D.C. The National Maternity Hospital (Trinity College) NE neonates had plasma and CSF samples collected as previously described. At Children's National neonates with NE had plasma samples collected from 2012–2016 as part of a prospective study evaluating candidate biomarkers of brain injury in NE. From both studies, a single de-identified plasma sample at enrollment from DOL 0–1 and clinical data (n=155) were analyzed. From the Trinity College NE cohort only, de-identified CSF samples from DOL 0–7, with a median of DOL 3, and clinical data (n=30) were analyzed.

Clinical outcomes of neurologic injury severity were evaluated for significant relationships with candidate biomarker concentrations. To measure clinical severity, degree of encephalopathy was determined by the Sarnat classification and stratified as mild (Sarnat score 0–1) or moderate-to-severe (Sarnat score 2–3).[36] Clinical evidence of brain injury was defined as the presence of seizures or severity of injury by MRI on DOL 5–15 according to the Barkovich scale, evaluating the basal ganglia area, watershed area, and the combined basal ganglia/watershed area.[38,39] The Barkovich scale was categorized into two groups: score of 0 and scores 1–5.

Neurodevelopmental outcomes were evaluated using cognitive, motor, and language scores of the Bayley Scales of Infant and Toddler Development (Bayley-III) at 15–30 months.[40] We evaluated the Bayley-III as both a continuous and binary variable. For our binary analysis, we categorized the scores into two groups normal, scores ≥85 and abnormal, scores <85 or death.[41] The patients who died prior to neurodevelopmental follow-up were assigned a score of 39 for the continuous Bayley-III analysis.[42]

## Laboratory Methods:

All primary plasma samples were stored up to 24 hours at 4°C until aliquoted and stored at −80°C. All sample aliquots were exposed to 1–2 freeze/thaws prior to assaying. All assays were performed from 2018–2019 in the same laboratory (Everett Laboratory) at the Johns Hopkins University School of Medicine in Baltimore, MD.

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). Capture antibody-spotted plates were washed with 1xPBS supplemented with 0.05% TWEEN (PBS-T). Calibrators for BDNF, VEGF, and IL-6, IL-8, and IL-10 (MSD) were produced using commercially provided diluent (product number R50AG-2, MSD). The detection antibody cocktail was prepared in commercial diluent (product number R51BA-5, MSD). Plasma and CSF samples were diluted 5-fold. The lower limits of quantification for the BDNF, IL-6, IL-8, IL-10, and VEGF assays were 48.47 pg/mL, 0.47 pg/mL, 0.56 pg/mL, 1.26 pg/mL, and 1.59 pg/mL, respectively, with interassay coefficients of variation of 9.7%, 4.7%, 1.8%, 1.7%, and 2.7%, respectively.

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). The development of the capture antibodies, detection antibodies, and calibrators of NRGN and GFAP have been previously described.[21,43] Plasma and CSF samples were diluted 2-fold. The lower limits of quantification for the GFAP and NRGN assays were 0.014 and 0.016 ng/mL, respectively, with interassay coefficients of variation of 2.6% and 2.8%, respectively.

Tau was measured using a commercial ELISA (product number N451LAA-1, MSD). Plasma and CSF samples were diluted 4-fold and assayed according to manufacturer instructions. The lower limits of quantification for Tau was 82.03 pg/mL, with an interassay coefficient of variation of 5.1%.

### Statistical Analyses:

Demographic and functional data are presented as median and interquartile range (IQR) or percentages, as appropriate. As demographic, candidate biomarker, and clinical outcome data were not normally distributed for the NE cohort, the Mann Whitney U test was used for categorical variables and Spearman correlation was used for continuous variables. For the binary demographic data, the Fisher exact test was used for comparison. Adjusted analysis was performed by logistic and linear regression using log transformed candidate biomarker concentrations and adjusted for gestational age and sex. For all statistical analyses, a *P* value of ≤0.05 was considered significant. Statistical analysis was performed using GraphPad Prism (Version 8.0.0 (131); 2018; GraphPad Software, San Diego, CA) and Stata (Version 15, StataCorp LLC, College Station, TX).

## Results:

### Subject Demographics:

There were 30 healthy term control neonates and 155 neonates with NE available for analysis. From the NE cohort, 155 plasma samples and 30 CSF samples were available for analysis (Table I).

Clinical outcomes of the NE cohort are summarized in Table II. The NE cohort were predominately (92%) moderate-to-severe NE (Sarnat score of 2–3). 54% of the NE cohort had seizures. For MRI-derived brain injury, the majority had low Barkovich scores of 0 for basal ganglia (76%), watershed (74%), and combined basal ganglia/watershed area (67%). At 15–30 months, the majority of the NE cohort had normal (Bayley-III score ≥85) Bayley-III cognitive (75%), motor (72%), and language (70%) composite scores. Clinical outcomes were not available for the control cohort.

### Plasma candidate biomarkers in NE and healthy term neonates:

Plasma candidate biomarker concentrations significantly differed in patients with NE compared with controls (Table III; available at www.jpeds.com). CNS necrosis markers NRGN (p=0.03) and Tau (p<0.001) and inflammation markers IL-6 (p<0.001), IL-8 (p<0.001), and IL-10 (p<0.001) were higher, whereas trophic-factors BDNF (p<0.001) and VEGF (p<0.001) were lower in NE compared with controls.

### Candidate biomarkers and clinical outcomes of brain injury:

In univariate analysis of plasma candidate biomarkers, higher Tau (p<0.001), GFAP (p=0.05), and NRGN (p=0.03) and lower BDNF (p=0.002) and VEGF (p=0.05) were associated with moderate-to-severe encephalopathy (Table IV; available at www.jpeds.com). IL-6, IL-8, and IL-10 were all higher but not significantly. When adjusted for gestational age and sex, Tau (p=0.002), GFAP (p=0.03), and NRGN (p=0.05) were directly related and BDNF (p=0.04) was inversely related to severity of encephalopathy (Table V). There were no significant associations of CSF candidate biomarkers with moderate-to-severe encephalopathy in univariate or adjusted analysis (Table VI; available at www.jpeds.com).

In univariate analysis of plasma candidate biomarkers and seizure occurrence, lower IL-6 (p=0.02) was associated with seizures (Table VII; available at www.jpeds.com). In adjusted analysis, IL-6 showed an inverse relationship (p=0.02) with seizure occurrence (Table V). There were no significant associations of CSF candidate biomarkers with seizures in univariate or adjusted analysis (Table VIII; available at www.jpeds.com).

### Candidate biomarkers and brain injury by MRI:

In adjusted analysis, only plasma Tau was directly related to Barkovich basal ganglia (p=0.002), watershed (p=0.03), and basal ganglia/watershed (p=0.006) scores (Table V). CSF candidate biomarkers did not have any significant relationships with MRI abnormalities (Table IX; available at www.jpeds.com).

### Candidate biomarkers and neurodevelopmental outcomes:

In univariate analysis of plasma candidate biomarkers, Tau and IL-6 negatively correlated with Bayley-III cognitive (p=0.02, p=0.05) and motor (p=0.02, p=0.03) composite scores (Table X; available at www.jpeds.com). IL-8 negatively correlated with cognitive (p=0.005), motor (p<0.001), and language (p=0.02) composite scores. IL-10 negatively correlated with motor composite scores only (p=0.03). VEGF positively correlated with motor composite scores (p=0.03). In univariate analysis of CSF candidate biomarkers, NRGN and IL-6 negatively correlated with Bayley-III cognitive (p=0.04, p=0.05) and motor (p=0.03, p=0.02) scores, respectively (Table XI; available at www.jpeds.com). IL-8 negatively correlated with motor scores only (p=0.03).

In adjusted analysis of plasma candidate biomarkers, only Tau was associated with decreased cognitive (p=0.03) and motor (p=0.03) outcomes (Table XII). In adjusted analysis of CSF candidate biomarkers, NRGN was inversely related to all three outcomes of cognitive (p=0.001), motor (p<0.001), and language (p=0.007) measures (Table XII). GFAP, IL-6, and IL-10 were inversely related to cognitive (p=0.02, p=0.02, p=0.02) and motor (p=0.04, p=0.009, p=0.008) outcomes, respectively. IL-8 was inversely related to motor outcomes only (p=0.03).

In univariate analysis of plasma candidate biomarkers and binary neurodevelopmental outcomes (abnormal [Bayley-III <85 or death] or normal [≥85]), higher Tau and IL-8 were associated with abnormal cognitive (p=0.03, p=0.05), motor (p=0.01, p=0.009), and language (p=0.04, p=0.005) outcomes, respectively (Table XIII; available at www.jpeds.com). In adjusted analysis, greater than 1 standard deviation of increased Tau between individuals presented a 1.76 times increased likelihood for abnormal cognitive outcomes (p=0.04), 1.82 times increased likelihood for abnormal motor outcomes (p=0.03), and 1.71 times increased likelihood for abnormal language outcomes (p=0.05).

## Discussion:

In this multicenter retrospective cohort study of NE, we demonstrated a candidate multi-biomarker panel of CNS necrosis, inflammatory, and trophic-factor proteins differentiated neonates with NE from healthy term neonates within the first 24 hours of life. Moreover, this candidate biomarker panel, with the best performance from Tau, differentiated

severity of NE measured by clinical encephalopathy, seizures, brain injury by MRI, and neurodevelopmental outcomes at 15–30 months. Although previous research focused on candidate biomarkers such as cytokines, Tau, and GFAP, our study contributes novel investigation of less studied candidate biomarkers, NRGN, VEGF, and BDNF, providing important performance comparisons.

We found that CNS necrosis markers (NRGN and Tau) and inflammation markers (IL-6, IL-8, and IL-10) were higher, whereas trophic factors (BDNF and VEGF) were lower in NE compared with controls. Higher IL-6[27–29] and Tau,[23] and lower VEGF[44] in neonates with NE compared with controls is consistent with previous smaller, single center studies. The findings of higher NRGN[21,22] and lower BDNF[45–47] in patients with brain injury compared with healthy controls has also been reported in adults with traumatic brain injury and delirium. Conversely, others showed higher BDNF in neonates with asphyxia compared with controls using cord blood samples.[30,31] Olin et al found that cord blood is not representative of neonatal blood, and therefore our use of plasma samples from the first 24 hours of life is a strength of our study.[48] Additionally, timing variation of sample collection and TH may have also contributed to discrepancies, as cooling may affect candidate biomarker concentrations.[32,44] However, because the standard of care is to begin TH within 6 hours of birth, and the plasma samples in our study span DOL 0–1, TH was considered a mediator, not a confounder, and was not included in the adjusted analysis. The median plasma concentrations of GFAP and Tau are higher in the control cohort than the infants with mild encephalopathy in the NE cohort. This difference was driven by three control outliers not excluded from analysis that each had elevated GFAP, Tau and neurogranin. In addition, although the control cohort had no overt clinical brain injury at birth, they were neonates admitted to the NICU and could have had subclinical brain injury to account for the elevated GFAP and Tau. Finally, there are multiple factors as previously discussed including the influence of TH and timing variation of sample collection that may contribute to this observation.

The identification of early candidate biomarkers to discriminate NE severity is becoming increasingly important with potential adjuvant therapies to TH, such as xenon, erythropoietin, and stem cells.[24,49–52] In our study, using adjusted analysis, plasma Tau, GFAP, NRGN, and BDNF differentiated between mild and moderate-to-severe encephalopathy. The higher Tau, GFAP, and NRGN concentrations in moderate-to-severe encephalopathy supported the hypothesis that these CNS necrosis markers are indicators of acute and possibly ongoing neuronal injury.[11,17,23,35] Meanwhile, BDNF was lower in moderate-to-severe encephalopathy. BDNF is a neuronal survival factor, suggesting an inadequate concentration to protect the brain in more severe injuries.[53–55] CSF candidate biomarkers did not differentiate encephalopathy severity in our study, although others reported significant results for CSF GFAP,[56,57] CSF IL-6,[29] and CSF VEGF.[32] This could possibly be due to availability of CSF samples at a single time point compared with 0–7 days of life.

Seizures are a common sequelae of NE and infants with seizures are associated with worse neurodevelopmental outcomes or death.[7,8,36,58] Seizures were common in our cohort, and neonates with seizures had predominately moderate-to-severe NE (99% Sarnat score 2–3).

We found lower plasma IL-6 concentrations could differentiate and were predictive of seizures in neonates with NE. Conversely, Numis et al showed higher IL-6 was associated with epilepsy in newborns with NE.[59] This discrepancy could reflect variation in the dynamic inflammatory process and possible dysregulation in severely injured neonates. Furthermore, our study did not control for infectious etiologies, due to limited sample size and availability of clinical data. Further evaluation of inflammatory cytokines considering infectious etiologies is warranted in larger cohorts. Candidate biomarkers in CSF were not able to differentiate seizure occurrence.

Evidence of structural injury, especially the basal ganglia, in NE is well described.[38] We found with adjusted analysis that plasma Tau directly related to Barkovich score for basal ganglia, watershed, and combined basal ganglia/watershed area injury. Previous research supports our findings that plasma Tau is associated with worse brain injury severity on MRI.[24] Tau is an axonal protein and particularly enriched in the brain white matter.[60,61] The presence of elevated Tau suggests additional white matter injury in NE, which has been suspected based on clinical outcomes and MRI.[62] However, MRI is not diagnostic until 24 hours after the injury and for neonates treated with TH, TH devices are not MRI-compatible delaying MRI use.[10,63,64] Our data suggest that plasma Tau, collected on DOL 0–1, could be an effective predictor of brain injury and help to fill this crucial time gap for intervention. CSF candidate biomarkers were not associated with brain injury measured on MRI.

When evaluating neurodevelopmental outcomes related to brain injury severity, we found with adjusted analysis, that plasma Tau and CSF GFAP, NRGN, IL-6, IL-8, and IL-10 were negatively associated with Bayley-III scores at 15–30 months. Specifically, higher plasma Tau collected on DOL 0–1 predicted abnormal cognitive and motor outcomes at 15–30 months. Adding multiple candidate biomarkers to the regression model did not significantly improve the association of outcomes beyond that seen with Tau. Other studies using plasma and CSF also demonstrated higher levels of GFAP, IL-6, IL-8, and Tau were associated with abnormal neurodevelopmental outcomes at 15–30 months.[14,15,17,24,25,29,65,66] Previous research also supports that lower VEGF is associated with abnormal neurodevelopmental outcomes.[32] Our study suggests that higher levels of CNS necrosis, especially Tau, and inflammatory markers could predict poor neurodevelopmental performance in the future.

Furthermore, we analyzed the baseline characteristics of infants without reported neurodevelopmental assessments, to evaluate the impact of candidate biomarker association with abnormal outcomes because the data from neonates without MRI Barkovich scores (n=38) and without Bayley-III scores at 15–30 months (n=56) were not included in analysis. Those without reported MRI Barkovich scores appeared to be healthier infants with greater gestational age, and neonates with higher chance of vaginal delivery, lower rates of TH, and lower proportion of moderate-to-severe Sarnat Scores. Those without reported Bayley-III scores importantly did not differ in degree of encephalopathy, and therefore should not affect our conclusions about abnormal neurodevelopmental outcomes. Future studies should include analyses of factors that can impact follow-up rates and neurodevelopmental outcomes at follow-up, including socioeconomic status.

Limitations of this study include small sample size, heterogeneity of NE cases, a single time point for some samples, sample variation over 24 hours, and unavailability of clinical data, which limited the ability to evaluate some outcomes. The NE criteria for this study included infants with NE identified <6 hours with TH, <48 hours without TH, and postnatally with brain injury on cranial ultrasound. This reflects a heterogeneity of cases; however, all infants had perinatal asphyxia. Due to the limited availability of samples and clinical data, the absence of CSF samples from healthy term neonates precluded any comparison of CSF candidate biomarkers in the NE cohort compared with controls. Additionally, CSF candidate biomarker analysis of neurodevelopmental outcomes was limited by a lack of CSF samples from neonates with abnormal (<85) Bayley-III scores (n=1). In addition for the CSF analysis, there was a small number of neonates with mild encephalopathy (n=4) compared with moderate-to-severe encephalopathy (n=26). Lastly, the inclusion of CSF samples spanning DOL 0–7 (median DOL 3) may influence the associations with clinical outcomes.

In conclusion, the ideal biomarker for identifying, stratifying, and monitoring NE would need to be stable, measurable at a high sensitivity in an easy-to-access biofluid, have peak concentrations early in life, and have the ability to discriminate NE severity and predict neurodevelopmental outcomes. Our study provided novel insight into a selection of candidate biomarkers that fit these optimal criteria for NE, with Tau as the best performer in multiple measures of brain injury and outcomes. Furthermore, plasma candidate biomarkers were able to identify neonates with NE, discriminate clinical severity, and predict seizures, brain injury by MRI, and neurodevelopmental outcomes at 15–30 months. Our study also identified potential adjunctive therapies for NE. Larger validation studies are needed to further investigate this candidate biomarker panel and its implementations into clinical settings.

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## Abbreviations

| | |
|---|---|
| **BDNF** | brain-derived neurotrophic factor |
| **Bayley-III** | Bayley Scales of Infant and Toddler Development III |
| **CNS** | central nervous system |
| **CSF** | cerebrospinal fluid |

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|---|---|
| **DOL** | day of life |
| **ELISA** | enzyme-linked immunosorbent assay |
| **GFAP** | glial fibrillary acidic protein |
| **HIE** | hypoxic-ischemic encephalopathy |
| **IL-6** | interleukin-6 |
| **IL-8** | interleukin-8 |
| **IL-10** | interleukin-10 |
| **IQR** | interquartile range |
| **MRI** | magnetic resonance imaging |
| **NRGN** | neurogranin |
| **VEGF** | vascular endothelial growth factor |

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**Table I:**

Demographic data of healthy term neonates and neonates with neonatal encephalopathy (NE)

| | Control Cohort (n=30) | Total NE Cohort (n=155) | Trinity College Cohort (n=57) | Children's National Cohort (n=98) | p-value |
|---|---|---|---|---|---|
| **Median (IQR)** | | | | | |
| Gestational age (weeks) | 39.1 (37.9, 40.0) | 39.6 (38, 40.7) | 40.7 (40.0, 41.6) | 39.0 (38.0, 40.0) | 0.22[a] |
| Birthweight (kg) | 3.3 (3.1, 3.6) | 3.3 (2.9, 3.8) | 3.6 (3.2, 4.0) | 3.1 (2.8, 3.6) | 0.73[a] |
| First blood gas pH | 7.3 (7.2, 7.4) | 7.0 (6.9, 7.1) | 7.0 (6.9, 7.2) | 7.0 (6.8, 7.1) | <0.001[a] |
| 5-minute Apgar score | 10 (10, 10) | 4 (2, 6) | 5 (3, 7) | 3.5 (2, 5) | <0.001[a] |
| **Sex, n (%)** | | | | | |
| Male | 12 (40) | 88 (57) | 38 (68) | 50 (51) | 0.11[b] |
| Female | 18 (60) | 66 (43) | 18 (32) | 48 (49) | |
| **Mode of Delivery, n (%)** | | | | | |
| Cesarean | 9 (30) | 66 (52) | 20 (36) | 46 (64) | 0.04[b] |
| Vaginal | 21 (70) | 61 (48) | 35 (64) | 26 (36) | |
| **Therapeutic Hypothermia, n (%)** | | | | | |
| Yes | 0 (0) | 135 (88) | 37 (66) | 98 (100) | <0.001[b] |
| No | **30 (100)** | **19 (12)** | 19 (34) | 0 (0) | |

NE = neonatal encephalopathy.

[a]Mann Whitney U test was used for comparison of the control cohort and the Total NE cohort.

[b]Fisher's exact test was used for comparison of control cohort and the Total NE cohort.

**Table II:**

Clinical data of neonates with neonatal encephalopathy (NE)

| | Total NE Cohort (n=155) | Trinity College Cohort (n=57) | Children's National Cohort (n=98) |
|---|---|---|---|
| **Degree of Encephalopathy, n (%)** | | | |
| Sarnat 0 | 3 (2) | 3 (5) | 0 (0) |
| Sarnat 1 | 9 (6) | 9 (16) | 0 (0) |
| Sarnat 2 | 116 (75) | 36 (63) | 80 (82) |
| Sarnat 3 | 27 (17) | 9 (16) | 18 (18) |
| **Seizures, n (%)** | | | |
| Yes | 74 (54) | 33 (67) | 41 (47) |
| No | 63 (46) | 16 (33) | 47 (53) |
| **Brain Injury by MRI: Barkovich Score, n (%)** | | | |
| Barkovich Basal Ganglia (n=117) | | | |
| Barkovich 0 | 89 (76) | 26 (84) | 63 (73) |
| Barkovich 1 | 5 (4) | 0 (0) | 5 (6) |
| Barkovich 2 | 6 (5) | 1 (3) | 5 (6) |
| Barkovich 3 | 9 (8) | 0 (0) | 9 (10) |
| Barkovich 4 | 8 (7) | 4 (13) | 4 (5) |
| Barkovich Watershed (n=117) | | | |
| Barkovich 0 | 86 (74) | 22 (71) | 64 (74) |
| Barkovich 1 | 7 (6) | 3 (10) | 4 (5) |
| Barkovich 2 | 7 (6) | 2 (6) | 5 (6) |
| Barkovich 3 | 1 (1) | 0 (0) | 1 (1) |
| Barkovich 4 | 11 (9) | 2 (6) | 9 (10) |
| Barkovich 5 | 5 (4) | 2 (6) | 3 (4) |
| Barkovich Basal Ganglia: Watershed (n=117) | | | |
| Barkovich 0 | 78 (67) | 21 (68) | 57 (66) |
| Barkovich 1 | 7 (6) | 1 (3) | 6 (7) |
| Barkovich 2 | 12 (10) | 5 (16) | 7 (8) |
| Barkovich 3 | 16 (14) | 3 (10) | 13 (15) |
| Barkovich 4 | 4 (3) | 1 (3) | 3 (4) |
| **Neurodevelopmental Outcomes: Bayley-III, Median (IQR)** | | | |
| Cognitive Score (n=99) | 99.5 (85, 105) | 105 (95, 110) | 95 (39, 100) |
| Motor Score (n=99) | 97 (76, 107) | 103 (97, 118) | 91.5 (39, 100) |
| Language Score (n=96) | 94 (74, 106) | 100 (86, 112) | 91 (39, 106) |
| **Neurodevelopmental Outcomes: Bayley-III, n (%)** | | | |
| Cognitive Score (n=99) | | | |
| Normal (≥85) | 74 (75) | 34 (87) | 40 (67) |
| Abnormal (<85) | 5 (5) | 1 (3) | 4 (7) |
| Dead | 20 (20) | 4 (10) | 16 (27) |

| | Total NE Cohort (n=155) | Trinity College Cohort (n=57) | Children's National Cohort (n=98) |
|---|---|---|---|
| Motor Score (n=99) | | | |
| Normal (≥85) | 71 (72) | 34 (87) | 37 (62) |
| Abnormal (<85) | 8 (8) | 1 (3) | 7 (12) |
| Dead | 20 (20) | 4 (10) | 16 (27) |
| Language Score (n=96) | | | |
| Normal (≥85) | 67 (70) | 32 (82) | 35 (61) |
| Abnormal (<85) | 9 (9) | 3 (8) | 6 (11) |
| Dead | 20 (21) | 4 (10) | 16 (28) |

NE = neonatal encephalopathy, Bayley-III = Bayley Scales of Infant and Toddler Development III at 15–30 months. Patients with missing data: Sarnat n=0, Seizures n=18, Barkovich Scores n=38, Bayley-III Cognitive n=56, Motor n=56, Language n=59

**Table III:**

Comparison of plasma candidate biomarker concentrations in healthy term neonates and neonates with NE

| | Control Cohort Median (IQR) | NE Cohort Median (IQR) | p |
|---|---|---|---|
| GFAP | 80 (31, 553) | 221 (9.0, 1008) | 0.75 |
| NRGN | **8 (8, 8)** | **34 (7, 374)** | **0.03** |
| BDNF | **1376.9 (867.8, 2629.0)** | **407.3 (152.5, 1161.0)** | **<0.001** |
| IL-6 | **4.9 (2.6, 14.3)** | **28.4 (9.8, 119.9)** | **<0.001** |
| IL-8 | **32.2 (24.4, 47.0)** | **113.5 (52.3, 394.9)** | **<0.001** |
| IL-10 | **0.7 (0.3, 2.2)** | **8.5 (2.2, 48.8)** | **<0.001** |
| VEGF | **276.1 (142.3, 319.9)** | **12.9 (0.9, 60.7)** | **<0.001** |
| Tau | **111.4 (32.8, 182.3)** | **243.1 (115.1, 541.0)** | **<0.001** |

NE = neonatal encephalopathy. All analyses used the Mann-Whitney U test for comparisons of non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): Control n=24, NE n=141; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): Control n=29, NE n=141; Tau: Control n=27, NE n=123

**Table IV:**

Univariate analysis of plasma candidate biomarker concentrations of neonates with NE and clinical encephalopathy

| | **Mild (Sarnat 0–1) Median (IQR)** | **Moderate-to-Severe (Sarnat 2–3) Median (IQR)** | **p** |
|---|---|---|---|
| GFAP | **9 (9, 45)** | **251 (9, 1154)** | **0.05** |
| NRGN | **7 (7, 7)** | **40 (7, 403)** | **0.03** |
| BDNF | **1596.5 (1081.2, 2324.1)** | **387.2 (136.1, 1006.5)** | **0.002** |
| IL-6 | 21.4 (7.2, 39.1) | 29.7 (9.8, 148.5) | 0.57 |
| IL-8 | 64.7 (46.1, 104.4) | 119.6 (52.6, 404.4) | 0.11 |
| IL-10 | 4.6 (1.3, 16.7) | 9.2 (2.3, 59.5) | 0.23 |
| VEGF | **87.0 (0.9, 330.2)** | **11.7 (0.9, 55.3)** | **0.05** |
| Tau | **45.8 (45.8, 103.6)** | **276.7 (135.5, 554.4)** | **<0.001** |

NE = neonatal encephalopathy. Univariate analyses used the Mann-Whitney U test for comparisons of non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): Mild n=11, Moderate-to-Severe n=130; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): Mild n=10, Moderate-to-Severe n=131; Tau: Mild n=11, Moderate-to-Severe n=112.

**Table V:**

Association of plasma candidate biomarker concentrations of neonates with NE and clinical encephalopathy, seizure occurrence, and brain injury by MRI *

| | Clinical Encephalopathy | | Seizure Occurrence | | MRI Barkovich Score | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Basal Ganglia | | Watershed | | BG:WS Score | |
| | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** |
| GFAP | **0.38 (0.04–0.71)** | **0.03** | −0.007 (−0.15–0.14) | 0.93 | 0.11 (−0.07–0.30) | 0.22 | 0.08 (−0.10–0.26) | 0.39 | 0.003 (−0.16–0.17) | 0.98 |
| NRGN | **0.48 (−0.001–0.95)** | **0.05** | −0.06 (−0.22–0.11) | 0.50 | 0.14 (−0.06–0.34) | 0.16 | 0.06 (−0.13–0.25) | 0.54 | 0.04 (−0.14–0.22) | 0.70 |
| BDNF | **−0.61 (−1.18–−0.03)** | **0.04** | −0.09 (−0.29–0.11) | 0.37 | −0.23 (−0.47–0.01) | 0.06 | −0.20 (−0.45–0.04) | 0.10 | −0.17 (−0.40–0.06) | 0.14 |
| IL-6 | 0.04 (−0.24–0.31) | 0.80 | **−0.19 (−0.36–0.03)** | **0.02** | 0.03 (−0.18–0.23) | 0.81 | 0.03 (−0.18–0.24) | 0.76 | −0.004 (−0.19–0.18) | 0.97 |
| IL-8 | 0.12 (−0.18–0.43) | 0.43 | −0.002 (−0.19–0.19) | 0.98 | 0.09 (−0.18–0.35) | 0.51 | 0.27 (−0.04–0.58) | 0.09 | 0.11 (−0.13–0.36) | 0.37 |
| IL-10 | 0.16 (−0.16–0.49) | 0.32 | −0.008 (−0.18–0.16) | 0.93 | −0.05 (−0.27–0.17) | 0.69 | 0.11 (−0.12–0.33) | 0.36 | −0.06 (−0.26–0.14) | 0.56 |
| VEGF | −0.23 (−0.53–0.07) | 0.14 | −0.02 (−0.16–0.13) | 0.84 | −0.06 (−0.23–0.11) | 0.50 | −0.09 (−0.26–0.08) | 0.30 | −0.04 (−0.20–0.12) | 0.65 |
| Tau | **1.46 (0.53–2.38)** | **0.002** | 0.08 (−0.23–0.39) | 0.62 | **0.74 (0.26–1.22)** | **0.002** | **0.47 (0.05–0.88)** | **0.03** | **0.60 (0.18–1.03)** | **0.006** |

NE = neonatal encephalopathy.

* Adjusted for gestational age and sex, the natural log of candidate biomarker concentrations were used in adjusted analyses. Clinical Encephalopathy: Duplex (GFAP and NRGN): n=141; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): n=141; Tau: n=123. Seizure Occurrence: Duplex (GFAP and NRGN): n=125; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): n=125; Tau: n=109. MRI: Duplex (GFAP and NRGN): n=107; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): n=107; Tau: n=93

**Table VI:**

Association of CSF candidate biomarker concentrations of neonates with NE and clinical encephalopathy

| | Univariate Analysis | | | Adjusted Logistic Regression* | |
|---|---|---|---|---|---|
| | **Mild (Sarnat 0–1) Median (IQR)** | **Moderate-to-Severe (Sarnat 2–3) Median (IQR)** | **p** | **Coefficient (95% CI)** | **p** |
| GFAP | 322 (220, 395) | 247 (156, 415) | 0.54 | −0.118 (−0.953–0.716) | 0.78 |
| NRGN | 7 (7, 24) | 7 (7, 7) | 0.50 | −0.783 (−2.439–0.873) | 0.35 |
| BDNF | 30.3 (30.3, 30.3) | 30.3 (30.3, 30.3) | 0.57 | - | |
| IL-6 | 4.0 (3.1, 142.6) | 3.8 (0.2, 36.2) | 0.46 | −0.135 (−0.580–0.311) | 0.55 |
| IL-8 | 369.9 (160.4, 501.2) | 203.5 (78.4, 606.1) | 0.76 | −0.122 (−0.777–0.532) | 0.71 |
| IL-10 | 1.0 (1.0, 1.0) | 1.0 (1.0, 1.0) | 0.70 | - | |
| VEGF | 4.5 (2.0, 6.6) | 4.9 (3.3, 9.6) | 0.46 | 0.603 (−0.784–1.990) | 0.39 |
| Tau | 3223.9 (56.6, 2.8e+04) | 2626.1 (56.6, 6721.5) | 0.80 | −0.016 (−0.446–0.415) | 0.94 |

NE = neonatal encephalopathy, CSF = cerebrospinal fluid. Univariate analyses used the Mann-Whitney U test for comparisons of non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): Mild n=4, Moderate-to-Severe n=26; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): Mild n=4, Moderate-to-Severe n=26; Tau: Mild n=4, Moderate-to-Severe n=23.

*Adjusted for gestational age and sex, the natural log of candidate biomarker concentrations were used in adjusted analyses.

**Table VII:**

Univariate Analysis of plasma candidate biomarker concentrations of neonates with NE and seizure occurrence

| | **No Median (IQR)** | **Yes Median (IQR)** | **p** |
|---|---|---|---|
| GFAP | 224 (9, 878) | 153 (9, 1008) | 0.96 |
| NRGN | 57 (7, 549) | 17 (7, 313) | 0.64 |
| BDNF | 373.2 (149.6, 1112.5) | 480.5 (183.2, 1525.5) | 0.52 |
| IL-6 | **43.7 (13.4, 295.5)** | **22.7 (6.5, 66.8)** | **0.02** |
| IL-8 | 100.8 (53.6, 341.6) | 130.7 (47.5, 439.3) | 0.75 |
| IL-10 | 11.4 (2.9, 36.4) | 7.6 (1.3, 59.5) | 0.39 |
| VEGF | 10.0 (0.2, 76.5) | 14.3 (0.9, 52.6) | 0.79 |
| Tau | 251.9 (123.8, 528.2) | 239.8 (119.2, 554.5) | 0.90 |

NE = neonatal encephalopathy. Univariate analyses used the Mann-Whitney U test for comparisons of non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): No n=58, Yes n=67; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): No n=60, Yes n=65; Tau: No n=51, Yes n=58.

**Table VIII:**

Association of CSF candidate biomarker concentrations of neonates with NE and seizure occurrence

| | Univariate Analysis | | | Adjusted Logistic Regression* | |
|---|---|---|---|---|---|
| | **No Median (IQR)** | **Yes Median (IQR)** | **p-value** | **Coefficient (95% CI)** | **p-value** |
| GFAP | 258 (251, 345) | 202 (156, 636) | 0.57 | −0.048 (−0.682–0.587) | 0.88 |
| NRGN | 7 (7, 7) | 7 (7, 7) | 0.68 | 0.273 (−1.157–1.702) | 0.71 |
| BDNF | 30.3 (30.3, 30.3) | 30.3 (30.3, 30.3) | 0.48 | - | |
| IL-6 | 3.1 (0.7, 196.2) | 4.5 (0.2, 36.2) | 0.68 | −0.059 (−0.387–0.270) | 0.73 |
| IL-8 | 300.5 (81.3, 503.9) | 203.5 (74.2, 606.1) | 0.78 | 0.036 (−0.362–0.433) | 0.86 |
| IL-10 | 1.0 (1.0, 1.0) | 1.0 (1.0, 1.0) | 0.48 | - | |
| VEGF | 5.5 (2.8, 7.3) | 3.8 (2.6, 6.9) | 0.76 | −0.008 (−0.770–0.754) | 0.98 |
| Tau | 2337.5 (56.6, 6391.4) | 5290.6 (56.6, 7552.7) | 0.46 | 0.127 (−0.217–0.471) | 0.47 |

NE = neonatal encephalopathy, CSF = cerebrospinal fluid. Univariate analyses used the Mann-Whitney U test for comparisons of non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): No n=9, Yes n=18; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): No n=9, Yes n=18; Tau: No n=9, Yes n=15.

*Adjusted for gestational age and sex, the natural log of candidate biomarker concentrations were used in adjusted analyses.

**Table IX:**

Association of CSF candidate biomarker concentrations of neonates with NE and brain injury by MRI

| | Basal Ganglia | | Watershed | | BG:WS Score | |
|---|---|---|---|---|---|---|
| | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** |
| GFAP | 0.53 (−0.58–1.63) | 0.35 | 0.10 (−0.75–0.96) | 0.82 | 1.46 (−0.43–3.346) | 0.13 |
| NRGN | - | | 0.37 (−1.15–1.90) | 0.63 | - | |
| BDNF | - | | - | | - | |
| IL6 | 0.63 (−0.10–1.35) | 0.09 | 0.04 (−0.34–0.41) | 0.84 | 0.10 (−0.30–0.49) | 0.63 |
| IL8 | 0.53 (−0.28–1.35) | 0.20 | −0.02 (−0.45–0.42) | 0.95 | 0.07 (−0.43–0.57) | 0.78 |
| IL10 | - | | - | | - | |
| VEGF | −0.07 (−1.07–0.93) | 0.89 | 0.00 (−0.79–0.79) | 0.99 | −0.03 (−0.82–0.76) | 0.95 |
| Tau | 0.77 (−0.53–2.07) | 0.25 | 0.11 (−0.34–0.55) | 0.64 | 0.14 (−0.33–0.61) | 0.55 |

NE = neonatal encephalopathy. All analyses used adjusted logistic regression, adjusted for gestational age and sex, the natural log of biomarker concentrations were used in adjusted analyses. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): n=20; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): n=20; Tau: n=18

**Table X:**

Univariate analysis of plasma candidate biomarker concentrations of neonates with NE and Bayley Scales of Infant and Toddler Development III (Bayley-III) scores at 15–30 months

| | Cognitive | | Motor | | Language | |
|---|---|---|---|---|---|---|
| | **rho** | **p** | **rho** | **p** | **rho** | **p** |
| GFAP | −0.04 | 0.72 | −0.09 | 0.38 | 0.01 | 0.95 |
| NRGN | −0.10 | 0.36 | −0.16 | 0.12 | −0.05 | 0.67 |
| BDNF | 0.16 | 0.14 | 0.13 | 0.23 | 0.03 | 0.76 |
| IL-6 | **−0.21** | **0.05** | **−0.23** | **0.03** | −0.15 | 0.17 |
| IL-8 | **−0.29** | **0.005** | **−0.40** | **<0.001** | **−0.25** | **0.02** |
| IL-10 | −0.20 | 0.06 | **−0.23** | **0.03** | −0.17 | 0.11 |
| VEGF | 0.16 | 0.13 | **0.23** | **0.03** | 0.14 | 0.20 |
| Tau | **−0.27** | **0.02** | **−0.26** | **0.02** | −0.16 | 0.17 |

NE = neonatal encephalopathy. Univariate analyses used Spearman correlation for non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): Cognitive and Motor n=93, Language n=90; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): Cognitive and Motor n=92, Language n=89; Tau: Cognitive and Motor n=81, Language n=80.

**Table XI:**

Univariate Analysis of CSF candidate biomarker concentrations of neonates with NE and Bayley Scales of Infant and Toddler Development III (Bayley-III) scores at 15–30 months

| | Cognitive | | Motor | | Language | |
|---|---|---|---|---|---|---|
| | **rho** | **p** | **rho** | **p** | **rho** | **p** |
| GFAP | −0.31 | 0.19 | −0.27 | 0.25 | −0.12 | 0.62 |
| NRGN | **−0.46** | **0.04** | **−0.48** | **0.03** | −0.39 | 0.09 |
| BDNF | 0.12 | 0.61 | 0.10 | 0.69 | 0.03 | 0.90 |
| IL-6 | **−0.44** | **0.05** | **−0.51** | **0.02** | −0.26 | 0.26 |
| IL-8 | −0.40 | 0.08 | **−0.50** | **0.03** | −0.25 | 0.28 |
| IL-10 | −0.34 | 0.14 | −0.34 | 0.14 | −0.34 | 0.14 |
| VEGF | −0.06 | 0.80 | −0.06 | 0.79 | −0.07 | 0.78 |
| Tau | −0.23 | 0.38 | −0.25 | 0.33 | −0.07 | 0.80 |

NE = neonatal encephalopathy. Univariate analyses used Spearman correlation for non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): Cognitive and Motor n=93, Language n=90; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): Cognitive and Motor n=92, Language n=89; Tau: Cognitive and Motor n=81, Language n=80.

**Table XII:**

Association of plasma and CSF candidate biomarker concentrations of neonates with NE and Bayley Scales of Infant and Toddler Development III (Bayley-III) scores at 15–30 months*

| | Plasma | | | | | | CSF | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cognitive | | Motor | | Language | | Cognitive | | Motor | | Language | |
| | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** | **Coefficient (95% CI)** | **p** |
| GFAP | −0.03 (−2.8–2.7) | 0.98 | −0.4 (−3.2–2.5) | 0.80 | 0.3 (−2.8–3.3) | 0.87 | **−9.2 (−17−-1.9)** | **0.02** | **−8.7 (−17−-0.5)** | **0.04** | −7.0 (−15–1.1) | 0.09 |
| NRGN | −0.07 (−3.4–3.3) | 0.97 | −0.6 (−4.1–2.9) | 0.73 | 0.2 (−3.5–3.9) | 0.90 | **−22.4 (−34−-11.3)** | **0.001** | **−25.3 (−37−-13.8)** | **<0.001** | **−19.1 (−32−-6.0)** | **0.007** |
| BDNF | 1.4 (−2.3–5.2) | 0.45 | 1.6 (−2.3–5.4) | 0.43 | 0.9 (−3.3–5.0) | 0.68 | 4.2 (−9.5–18.0) | 0.52 | 5.0 (−9.8–19.6) | 0.49 | 1.9 (−12.4–16.2) | 0.78 |
| IL-6 | −2.2 (−5.2–0.8) | 0.16 | −2.2 (−5.3–0.9) | 0.17 | −2.5 (−5.8–0.8) | 0.14 | **−4.4 (−8.0−-1.0)** | **0.02** | **−5.1 (−8.7-−1.5)** | **0.009** | −3.6 (−7.5–0.3) | 0.07 |
| IL-8 | −2.6 (−6.2–1.0) | 0.16 | −3.0 (−6.7–0.9) | 0.13 | −3.2 (−7.2–0.7) | 0.11 | −2.9 (−7.1–1.3) | 0.16 | **−4.8 (−8.9-−0.6)** | **0.03** | −2.5 (−7.0–1.9) | 0.25 |
| IL-10 | −0.3 (−4.0–3.5) | 0.89 | −0.6 (−4.5–3.3) | 0.76 | −1.6 (−5.6–2.5) | 0.45 | **−34.6 (−63−-5.9)** | **0.02** | **−42.0 (−71−-12.7)** | **0.008** | −20.2 (−54–13.0) | 0.22 |
| VEGF | 0.5 (−2.5–3.4) | 0.75 | 0.9 (−2.2–3.9) | 0.57 | 1.0 (−2.3–4.2) | 0.56 | −0.4 (−9.6–8.8) | 0.93 | 0.08 (−9.8–10.0) | 0.99 | −1.5 (−11.0–8.1) | 0.75 |
| Tau | **−5.6 (−10.6−-0.5)** | **0.03** | **−6.1 (−11.5−-0.7)** | **0.03** | −5.4 (−10.9–0.2) | 0.06 | −2.1 (−6.8–2.7) | 0.37 | −2.6 (−7.5–2.3) | 0.27 | −0.8 (−5.8–4.1) | 0.73 |

NE = neonatal encephalopathy.

*Adjusted for gestational age and sex, the natural log of candidate biomarker concentrations were used in adjusted analyses. Plasma: Duplex (GFAP and NRGN): n=93; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): n=92; Tau: n=81. CSF: Duplex (GFAP and NRGN): n=20; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): n=20; Tau: n=17.

**Table XIII:**

Association of plasma candidate biomarker concentrations of neonates with NE and binary outcomes of normal and abnormal Bayley Scales of Infant and Toddler Development III (Bayley-III) scores at 15–30 months

| Bayley-III Score | Univariate Analysis | | | Adjusted Logistic Regression* | |
|---|---|---|---|---|---|
| | Normal (≥85) Median (IQR) | Abnormal (<85 or death) Median (IQR) | p-value | Odds Ratio of Standard Deviation (95% CI) | p-value |
| **Cognitive** | | | | | |
| GFAP | 300.4 (8.7, 1154.2) | 284.6 (8.7, 821.8) | 0.68 | 0.88 (0.55–1.42) | 0.59 |
| NRGN | 35.9 (7.1, 498.7) | 49.9 (16.5, 243.2) | 0.68 | 0.94 (0.58–1.53 ) | 0.81 |
| BDNF | 444.6 (134.6, 1802.3) | 317.3 (77.0, 692.5) | 0.18 | 0.75 (0.46–1.22) | 0.25 |
| IL-6 | 28.8 (11.5, 116.8) | 35.1 (9.5, 2425.8) | 0.31 | 1.32 (0.80–2.16) | 0.28 |
| IL-8 | 115.3 (59.6, 462.6) | 324.8 (94.5, 2458.4) | 0.05 | 1.26 (0.73–2.15) | 0.41 |
| IL-10 | 11.6 (1.7, 60.5) | 13.6 (1.9, 111.8) | 0.76 | 0.92 (0.55–1.54) | 0.76 |
| VEGF | 11.7 (0.9, 82.2) | 9.3 (0.5, 43.8) | 0.35 | 0.89 (0.54–1.49) | 0.66 |
| Tau | 218.0 (102.4, 541.6) | 454.1 (210.8, 1410.2) | 0.03 | 1.76 (1.02–3.06) | 0.04 |
| **Motor** | | | | | |
| GFAP | 281.9 (8.7, 1154.2) | 429.1 (8.7, 1039.1) | 0.87 | 0.91 (0.57–1.45) | 0.69 |
| NRGN | 35.1 (7.1, 498.7) | 59.4 (16.5, 314.2) | 0.66 | 0.95 (0.59–1.52) | 0.82 |
| BDNF | 434.6 (134.6, 1720.7) | 414.6 (77.0, 868.7) | 0.37 | 0.80 (0.50–1.28) | 0.35 |
| IL-6 | 28.8 (10.9, 116.8) | 33.4 (10.9, 2365.0) | 0.26 | 1.34 (0.83–2.14) | 0.23 |
| IL-8 | 108.9 (53.9, 398.7) | 360.6 (106.8, 2458.4) | 0.009 | 1.43 (0.83–2.48) | 0.20 |
| IL-10 | 10.9 (1.4, 37.0) | 18.8 (2.8, 134.4) | 0.34 | 1.08 (0.66–1.75) | 0.77 |
| VEGF | 12.6 (0.9, 83.6) | 10.5 (0.5, 43.8) | 0.32 | 0.91 (0.56–1.48) | 0.70 |
| Tau | 215.0 (95.4, 541.0) | 412.8 (210.8, 1410.2) | 0.01 | 1.82 (1.06–3.12) | 0.03 |
| **Language** | | | | | |
| GFAP | 300.4 (8.7, 1154.2) | 99.1 (8.7, 902.6) | 0.46 | 0.79 (0.49–1.26) | 0.32 |
| NRGN | 34.3 (7.11, 498.7) | 38.0 (16.5, 208.2) | 0.98 | 0.83 (0.51–1.35) | 0.45 |
| BDNF | 446.6 (136.1, 1808.5) | 372.5 (77.0, 724.9) | 0.19 | 0.76 (0.47–1.22) | 0.25 |
| IL-6 | 27.4 (10.8, 113.7) | 35.1 (10.9, 2365.0) | 0.21 | 1.39 (0.86–2.26) | 0.18 |
| IL-8 | 101.3 (49.9, 390.7) | 360.6 (114.4, 1836.6) | 0.005 | 1.51 (0.87–2.63) | 0.15 |
| IL-10 | 9.2 (1.3, 31.6) | 22.1 (2.8 134.4) | 0.19 | 1.23 (0.75–2.01) | 0.42 |
| VEGF | 14.8 (0.9, 83.6) | 7.2 (0.2, 43.8) | 0.18 | 0.83 (0.51–1.36) | 0.46 |
| Tau | 218.0 (102.4, 541.0) | 412.8 (209.1, 1410.2) | 0.04 | 1.71 (1.00–2.93) | 0.05 |

NE = neonatal encephalopathy. Univariate analyses used the Mann-Whitney U test for comparisons of non-parametric data. Candidate biomarker units are pg/mL. Duplex (GFAP and NRGN): Cognitive: Normal n=69, Abnormal n=24, Motor: Normal n=66, Abnormal n=27, Language: Normal n=63, Abnormal n=27; Multiplex (BDNF, IL-6, IL-8, IL-10, VEGF): Cognitive: Normal n=68, Abnormal n=24, Motor: Normal n=64, Abnormal n=28, Language: Normal n=61, Abnormal n=28; Tau: Cognitive: Normal n=57, Abnormal n=19, Motor: Normal n=59, Abnormal n=22, Language: Normal n=55, Abnormal n=22

*Adjusted for gestational age and sex, the natural log of candidate biomarker concentrations were used in adjusted analyses.