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Perinatal Blood Biomarkers for the Identification of Brain Injury in Very Low Birth Weight Growth Restricted Infants

Shanna L. YUE, B.A.[1], **Ahizechukwu C. EKE, MD, MPH**[2], **Dhananjay VAIDYA, PhD**[3], **Frances J. NORTHINGTON, MD**[4,5], **Allen D. EVERETT, MD**[6], **Ernest M. GRAHAM, MD**[2,5]

[1.] School of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD

[2.] Department of Gynecology and Obstetrics, Division of Maternal-Fetal Medicine, Johns Hopkins University School of Medicine, Baltimore, MD

[3.] Biostatistics, Epidemiology and Data Management Core, Johns Hopkins University School of Medicine, Baltimore, MD

[4.] Department of Pediatrics, Division of Neonatology, Johns Hopkins University School of Medicine, Baltimore, MD

[5.] Neuroscience Intensive Care Nursery Program, Johns Hopkins University School of Medicine, Baltimore, MD

[6.] Department of Pediatrics, Division of Pediatric Cardiology, Johns Hopkins University School of Medicine, Baltimore, MD

Abstract

OBJECTIVE: To determine if blood biomarkers measured at delivery and shortly after birth can identify growth restricted infants at risk for developing severe brain injury.

STUDY DESIGN: In a cohort of very low birth weight neonates, fetal growth restricted (FGR) (birth weight < 10%) were compared to non-FGR neonates, and within the FGR group those with brain injury were compared to those without. Biomarkers were measured in cord blood at delivery, and daily for the 1st 5 days of life.

RESULT: FGR was associated with significantly higher levels of interleukin (IL)-6, IL-8, IL-10 and lower levels of vascular endothelial growth factor (VEGF). FGR and brain injury were associated with significantly higher levels of IL-6, IL-8, IL-10 and glial fibrillary acidic protein (GFAP).

Corresponding author: Ernest M. Graham, M.D., Johns Hopkins School of Medicine; Dept. of Gyn/Ob, Phipps 228; 600 N. Wolfe St., Baltimore, MD 21287-1228; Phone: 410-955-3187; Fax: 410-614-8305, egraham5@jhmi.edu.

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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.

CONCLUSION: Interleukins may be involved in a common pathway contributing to both the development of growth restriction and brain injury, and GFAP may help identify brain injury within this growth restricted group.

PRECIS:

Biomarkers measured in cord blood at delivery and neonatal serum after birth may identify growth restricted infants at risk for developing severe brain injury.

Keywords

Blood biomarkers; Fetal Growth Restriction; Neonate; Brain Injury

Introduction

One of the great challenges in perinatal medicine has been distinguishing the fetus or neonate that is small due to a pathological process from the normal, constitutionally small baby within the group with a birth weight less than the 10th percentile. Very low birth weight (VLBW, <1500g) neonates represent 1.5% of all live births and have an increased risk of brain injury thought to consist primarily of periventricular white matter injury (PWMI).[1] Periventricular white matter injury is frequently accompanied by neuronal/axonal disease affecting multiple parts of the brain, including the cerebral white matter, thalamus, basal ganglia, cerebral cortex, brain stem, and cerebellum, and the constellation of these damages is called “encephalopathy of prematurity”.[1] Encephalopathy of prematurity is thought to be caused by two main mechanisms: cerebral ischemia and inflammation, leading to activation of excitotoxicity and free radical attack by reactive oxygen species.[2] Placental insufficiency is the principal cause of fetal growth restriction (FGR), resulting in chronic fetal hypoxia that induces an adaptive response of blood redistribution that favors blood flow to the brain, termed “brain sparing”, which can be accompanied by cerebrovascular remodelling.[3] VLBW infants are also at risk for intraventricular hemorrhage (IVH), which occurs in around 20% of VLBW births.[4] Brain injury in VLBW infants leads to cognitive and motor delays that may last a lifetime, with a prevalence of 16.9% and 20.6% respectively, making this an important public health issue that warrants further research.[5]

The diagnosis of PWMI and IVH may not be apparent at birth, and are diagnosed with ultrasound in the first week of life to rule out IVH, and at 6 weeks to identify PWMI.[6] Other currently available tools to identify risk of brain injury in the early neonatal period include Apgar scores, fetal heart tracings, umbilical cord gases, and physical exam of the newborn, all of which lack precision. The discovery of novel biomarkers may be a way to quickly identify brain injury, assess brain injury severity, and give prognostic information. These biomarkers may also be able to shed light on a common pathway that causes both growth restriction and brain injury in VLBW neonates. Biomarker measurements are noninvasive, objective, and can be measured serially, making them an ideal technique for identifying brain injury. Previous research in this area has mostly focused on biomarkers of hypoxic-ischemic encephalopathy (HIE) in term and near-term infants, and more research is needed into biomarkers of brain injury in preterm infants. Our aim in this study is to determine if a

candidate multi-biomarker panel can provide information about common pathways through which VLBW neonates become growth restricted and later develop brain injury.

Materials and Methods

In this retrospective cohort study of all VLBW neonates admitted to our neonatal intensive care unit (NICU) from April 2009 to November 2019, we analyzed the concentrations of selected biomarkers in cord blood at delivery and in discard neonatal serum on days 1–5 after birth. Neonates with genetic abnormalities and major congenital malformations were excluded. This study was approved by the Johns Hopkins IRB #26068 on 3/26/2009, and informed consent was obtained from participants. We selected biomarkers for measurement related to multiple injury pathways including central nervous system necrosis (glial fibrillary acidic protein-GFAP, neurogranin-NRGN, Tau), trophic-factor markers (brain derived neurotrophic factor-BDNF, vascular endothelial growth factor-VEGF) and inflammation markers (interleukin (IL)-6, IL-8, IL-10).

Fetal growth restriction was defined as weight at birth less than the 10^{th} percentile for gestational age. Brain injury was defined as neonates who had either PWMI, grades 3 or 4 IVH, seizures or death. Within this cohort of VLBW neonates we compared those with FGR to non-FGR neonates. Within the group of FGR neonates we compared those with brain injury to those without.

Maternal and neonatal records were reviewed. Preeclampsia was defined as proteinuria, edema, and the presence of new onset hypertension. The diagnosis of nonreassuring fetal heart tracing was made by the physician attending the delivery prior to performing a cesarean delivery. 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. Oligohydramnios was defined as an amniotic fluid index <5.0 cm with intact membranes at the time of admission in which delivery occurred. During the 10.5 year period of this study we did not have a policy requiring paired cord gases be performed at all deliveries, but when they were they were checked to makes sure they were physiologic in that the arterial sample was more acidotic with a lower pH and pO_2 and a higher pCO_2.

ELISA Assays

Serum samples were held at 4°C for 48 hours before being aliquoted and stored at −80°C until assayed. A custom multiplex enzyme-linked immunosorbent assay (ELISA) was developed to measure BDNF, VEGF, IL-6, IL-8, and IL-10 simultaneously using robotically spotted capture antibodies on the 96-well plate format (Meso Scale Discover [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 formation (MSD, Rockville, MD). Tau was measured using a commercial ELISA (MSD, Rockville, MD, Human Total Tau Kit, Cat #N451LAA-1). Samples were run a single time with out of range samples (high or low) repeated. The use of these assays to measure biomarkers have been confirmed as we have previously reported.[7]

Statistical Analysis

Statistical analysis was done using STATA 15.1 (StataCorp LP, College Station, TX). Between group differences in continuous variables were analyzed using Wilcoxon rank-sum tests, and differences in categorical variables were analyzed using Chi squared tests. The median and interquartile range of non-normally distributed continuous variables and absolute counts and proportions of categorical variables were reported. All biomarker measurements were log-transformed prior to analysis. Prior to log transformation, values less than the lower limit of detection (LLOD) of the biomarker assays were imputed as 0.500. Repeated measures analysis of each of the biomarkers over all days of measurement was performed using a mixed model analysis to investigate how day of sampling affects biomarker changes between groups. Interaction p-values were used to investigate whether biomarker level changes differed by day of sampling in either direction or magnitude. Regression analysis was then performed for each of the biomarkers stratified by day of sampling to look at how changes varied in directionality or magnitude based on day. All analyses done on VLBW FGR vs non-FGR neonates were adjusted for gestational age. Geometric means and their 95% confidence interval were exponentiated for tabulation, thus to be interpreted in their original measurement units. When comparing clinical variables between groups a P value of ≤ 0.05 was considered significant, and for the biomarker comparisons we used a per-table correction for the first p-value of a sequence (8 biomarkers), and a conservative Bonferroni threshold for significance of $0.05/8 = 0.00625$.

Results

VLBW: FGR vs non-FGR neonates

Over this 10.5 year period, 486 VLBW neonates were admitted to our NICU and had at least one sample drawn on cord blood at delivery through the first 5 days of life. Of this group 107 (21.9%) had FGR and were compared to the 379 non-FGR neonates.

Maternal and Neonatal characteristics—Maternal age, gravidity, parity, and race were not significantly different between mothers who gave birth to VLBW neonates with FGR and compared to mothers of non-FGR neonates.(Table 1) Mothers of neonates with FGR gave birth at a significantly higher gestational age, were more likely to have a cesarean delivery, and had higher incidences of preeclampsia, oligohydramnios and histologic placental infarcts. They were significantly less likely to show evidence of preterm premature rupture of membranes, placental abruption, clinical chorioamnionitis, histologic chorioamnionitis and histologic funisitis.(Table 1) The decrease in birth weight in the FGR group did not reach statistical significance. Neonates born with FGR were significantly more likely to have a nonreassuring fetal heart rate requiring cesarean delivery and less likely to have an Apgar score <7 at both 1 min and 5 min.(Table 1) The FGR neonates had a significantly higher initial neonatal hematocrit and nucleated red blood cell count. They also had a lower arterial cord pH and higher arterial cord base deficit. FGR neonates had a significantly decreased incidence of respiratory distress, seizures, PWMI, and grades 3 or 4 IVH compared to non-FGR neonates. There was no significant difference in occurrence of culture positive blood infection, cerebrospinal fluid infection or death.(Table 1)

Biomarker analysis—Repeated measures analysis of biomarkers showed that the changes in IL-6, IL-8, IL-10, Tau, and VEGF levels between neonates with FGR and those without differ by day of life. (Table 2) Analysis of biomarkers stratified by day of life showed that IL-6 levels were higher in non-FGR neonates in cord blood, but the direction of change in biomarker levels was reversed for days 4–5, with FGR neonates having higher levels of IL-6 on those days. Additionally, neonates with FGR had significant increases in IL-8 and IL-10 on days 2–4 and Tau in cord blood compared to non-FGR neonates. Neonates with FGR also had significant decreases in VEGF in cord blood and across days 1–5. (Table 2)

VLBW FGR: Brain Injury vs No Brain Injury

Maternal and Neonatal characteristics—Within the group of neonates with VLBW and FGR the mothers of those with brain injury were significantly older than the mothers whose neonates were without brain injury.(Table 3) The brain injury group also had an increased incidence of placental abruption. There were no significant differences in gestational age, mode of delivery or other intrapartum complications. The group of 11 VLBW neonates with FGR and brain injury included 5 with PWMI, 4 with grades 3 or 4 IVH, and 4 that died. There was no significant difference in birth weight. Those with brain injury were more likely to have an Apgar score < 7 at 1 min and at 5 min, a higher arterial cord base deficit and a lower initial nucleated RBC count. They were significantly more likely to have culture positive blood infection.(Table 3)

Biomarker analysis—Repeated measures analysis of each biomarker from cord blood through day 5 of life in VLBW FGR neonates with brain injury showed significant increases in IL-6 (287.1%), IL-8 (128.7%), IL-10 (240.0%) and GFAP (296.8%).(Table 4) None of the interaction p-values were significant at the p= 0.05 level, indicating no significant interactions between brain injury and day of sampling. The supplementary table shows biomarker levels stratified by day of life for both the FGR with brain injury and FGR without brain injury neonates.

Discussion

This study sought to identify differences in serum biomarker levels in VLBW neonates with FGR, and determine if biomarkers might predict which VLBW FGR infants will go on to develop brain injury.

Our main findings:

1. VLBW neonates with FGR have significantly increased IL-6, IL-8, and IL-10 levels, and decreased VEGF levels during the period from birth to day 5 of life compared to their normally grown VLBW counterparts.
2. VLBW FGR neonates with brain injury have increased IL-6, IL-8, IL-10 and GFAP levels, compared to VLBW FGR neonates without brain injury for the period from birth to day 5 of life.

Our results show that the early increase in inflammatory markers IL-6, IL-8, and IL-10, found in both groups, may be an indicator of a pathological process that contributes to

both the development of FGR in VLBW neonates and then their later development of brain injury. FGR can have many causes, but the majority of cases that are not due to genetic anomalies, congenital malformation, or infections are thought to arise from deficient remodeling of the uterine spiral arteries supplying the placenta during early pregnancy, causing placental insufficiency.[8] As a result, malperfusion and hypoxia of organs, supported by the increased hematocrit and nucleated RBC counts seen in our FGR neonates in response to hypoxia, may activate proinflammatory pathways, leading to higher levels of inflammatory markers seen in neonates soon after birth. The decreased IL-6 in cord blood found in our VLBW neonates with FGR runs contrary to previous studies showing an increase in IL-6 in the cord blood of >35 week small for gestational age (SGA) infants compared to AGA infants.[9,10] It should be noted that these studies were conducted in term and near term infants, so it is possible that our VLBW neonates born at earlier gestational ages have a different mechanism of inflammation. However, on days 4–5, our study found that IL-6 levels were increased in FGR neonates. IL-8 is a chemo-attractant and activator that recruits neutrophils and T-lymphocytes to injured and inflamed tissue. Our findings of increased IL-8 levels in FGR neonates is supported by previous studies showing higher levels of IL-8 in cord blood of FGR infants compared to non-FGR infants in both pregnancies that were complicated by preeclampsia and those that were not.[11,12] IL-10 is an anti-inflammatory cytokine that plays a protective role in brain tissue by attenuating the synthesis of proinflammatory cytokines, reducing cytokine receptor expression and inhibiting receptor activation.[13] The increase in IL-10 seen in neonates with FGR may be a reaction to the inflammatory cascade being activated due to placental insufficiency and tissue hypoxia. Notably, the increased inflammatory markers associated with FGR seem unlikely to be due to infection, as the presence of clinical and histologic chorioamnionitis as well as histologic funisitis were found to be less likely in neonates with FGR.

IL-6, IL-8, and IL-10 may be implicated in a pathway that causes growth restriction first, and then later brain injury, as these inflammatory markers are also elevated in VLBW FGR neonates with brain injury compared to non-brain injured peers. This finding is consistent with past studies in preterm and term infants showing an association between elevated IL-6, IL-8, and IL-10 levels in cord blood at delivery and early plasma samples with the development of IVH and HIE.[7,12–15] High levels of IL-6 can induce inflammation and increase vascular permeability, leading to cerebral edema, providing a plausible mechanism for how hypoxia in FGR can lead to a pro-inflammatory response that then causes brain injury.[15] Infants born with both VLBW and FGR may be especially at risk for brain injury due to their small size and inability to respond adequately to perinatal insults, combined with decreased blood flow to the brain due to placental insufficiency. Our study describes an association between increased levels of IL-6, IL-8, and IL-10 and the development of FGR and brain injury in a population of VLBW neonates.

VEGF was found to be significantly decreased in VLBW neonates with FGR but did not differ based on the presence of brain injury. VEGF is a growth factor activated by tissue hypoxia that promotes endothelial cell survival by inhibiting the apoptosis pathway and increasing vascular permeability.[16] Our findings in VLBW FGR neonates are supported by a previous study showing lower VEGF concentrations in neonatal blood on the day 1 of life in

infants with a birth weight z-score < −1 compared to infants whose birth weight z-score was ≥ −1.[17]

We did not find any significant differences in GFAP levels when comparing VLBW FGR babies to normally grown infants, but brain injured VLBW FGR infants had a significant increase in the repeated measures analysis from birth through day 5 when compared to non-brain injured neonates. Our findings are corroborated by several other studies showing increases in GFAP in low birth weight (<2500g) infants with PWMI from days 1–4,[6] and increases in term infants with HIE and other abnormal neurological outcomes from hour 6 of life to day 4, compared to controls.[7,18,19] GFAP may be an ideal biomarker for brain injury in neonates since it is released into the blood stream after astrocyte death and seems to be elevated in the early days of life across neonates born at various gestational ages with various forms of brain injury.

Limitations of this study include a small sample size for VLBW neonates with brain injury even though it involved screening all NICU admissions over a 10.5 year period. Although there were only 11 neonates with FGR and brain injury and 96 with FGR without brain injury in this cohort of VLBW neonates examined at our single institution over a 10.5 year period we were able to find significant differences in biomarkers after correcting for multiple comparisons using the Bonferroni method using a p value cutoff of 0.00625 as shown in Table 4 for IL-6, IL-8, IL-10 and GFAP. Power is an issue for negative studies that fail to find any significant differences, and the question becomes would a larger study have found significant differences that the smaller study failed to find, but here we are able to identify significant differences in biomarkers in this sample size. Limited infant serum quantity from discard specimens resulted in not all infants having biomarker measurements at each time point. Our biomarker assays were unable to detect biomarker levels that were lower than their lower limits of detection, so we used 0.500 as a stand-in level for undetectable biomarker levels, which is reflected in the geometric means. Although we were able to detect highly significant differences between the brain injured and noninjured neonates, there were not enough brain injured neonates over this 10.5 year period to precisely determine clinically useful cutoffs for identification of injury. Strengths of the study include an exploration into a specific population, VLBW FGR neonates, that thus far has not been the focus of biomarker studies. Additionally, the collection of a continuous stream of biomarker levels and maternal and neonatal clinical data over a 10.5 year period provides insight into how these biomarkers relate to growth restriction and brain injury.

There is debate about whether umbilical cord blood gas analysis at delivery should be used on some or all births (selectively or universally). [20] The 26th Royal College of Obstetricians and Gynaecologists' study group on Intrapartum Fetal Surveillance (1993) recommended measurement of the acid-base status of the umbilical artery and vein cord blood after delivery as "a measure of the fetal response to labour", however, despite the College recommendation and the relative ease of the procedure, its value is still debated. [20,21] Both the artery and vein are sampled to ensure that the artery has been sampled. Although some studies have advocated paired blood gas analysis in all deliveries,[20,21] there is not a uniform agreed upon way to do this. During the 10.5 year period of our study we did not have a policy that required paired samples at birth in all deliveries, but when they were performed

they were checked to make sure that they were physiologic in that the arterial samples was more acidotic with a lower pH and pO_2 and higher pCO_2. Base deficit is a calculated value derived from measured values of pH and pCO_2 in blood. Only the arterial pH and base deficit are used in the criteria to link metabolic acidosis with neonatal neurologic injury.[22] The VLBW non-FGR neonates in our study had a median (interquartile range) pH of 7.29 (7.25, 7.34) which is similar to a previous study of 1,105 preterm infants who had a median arterial pH at birth of 7.29 with 5%tile=7.14 and 95%tile=7.40.[23] For these reasons we believe that the arterial pH and base deficit given here provide meaningful information.

Our study analyzed a panel of biomarker candidates that could contribute to early identification of brain injury in VLBW FGR neonates. IL-6, IL-8, IL-10 may be involved in a common pathway contributing to both the development of fetal growth restriction and later brain injury, and GFAP may help identify brain injury within this growth restricted group. Our study provides novel information about these biomarkers in a less well-studied population of VLBW infants that are high risk and most vulnerable to brain injury. Future research is needed into how these biomarkers correlate with not only the presence of brain injury, but also the severity of brain injury and neurodevelopmental outcomes later in life, enhancing their ability to provide prognostic information and shape clinical care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Bivariate analysis of maternal and neonatal variables for VLBW neonates with FGR vs without FGR given as median (interquartile range) or number (percent).

	VLBW FGR N= 107	VLBW non-FGR N= 379	P-value
Maternal age	28.0 (23.0, 35.0)	29.0 (23.0, 34.0)	0.73
Gravidity	2 (1, 4)	3 (1, 4)	0.21
Parity	1 (0, 2)	1 (0, 2)	0.26
Gestational age (weeks)	29.9 (27.4, 31.9)	27.3 (25.0, 29.3)	<0.001 *
Race			0.35
White	35 (32.7%)	120 (31.7%)	
Black	65 (60.7%)	221 (58.3%)	
Hispanic	3 (2.8%)	15 (4.0%)	
Asian	4 (3.7%)	10 (2.6%)	
Other	0 (0.0%)	13 (3.4%)	
Cesarean delivery	92 (86.0%)	219 (57.8%)	<0.001 *
Preeclampsia	81 (75.7%)	62 (16.4%)	<0.001 *
Oligohydramnios	34 (31.8%)	16 (4.2%)	<0.001 *
PPROM	5 (4.7%)	166 (43.8%)	<0.001 *
Abruption	8 (7.5%)	58 (15.3%)	0.037 *
Meconium	5 (4.7%)	10 (2.6%)	0.28
Clinical chorioamnionitis	3 (2.8%)	65 (17.2%)	<0.001 *
Histologic chorioamnionitis	4 (3.7%)	149 (39.3%)	<0.001 *
Histologic funisitis	1 (0.9%)	86 (22.7%)	<0.001 *
Histologic placental infarcts	44 (41.5%)	36 (10.0%)	<0.001 *
Birth weight (grams)	970 (630, 1230)	990 (776, 1220)	0.11
Male gender	48 (44.9%)	200 (52.8%)	0.15
Nonreassuring fetal heart rate	44 (41.1%)	68 (18.0%)	<0.001 *
1 min Apgar <7	75 (70.1%)	298 (79.9%)	0.032 *
5 min Apgar <7	39 (36.4%)	186 (49.6%)	0.016 *
Arterial cord pH	7.23 (7.19, 7.28)	7.29 (7.25, 7.34)	<0.001 *
Arterial cord base deficit (mM)	4.0 (2.0–6.5)	3.0 (1.0–4.0)	<0.001 *
Initial neonatal WBC count (k/mm^3)	5.6 (4.0, 7.8)	8.3 (5.7, 13.0)	<0.001 *
Initial neonatal hematocrit (%)	46.0 (39.9, 51.5)	42.4 (38.0, 46.9)	<0.001 *
Initial neonatal platelet count (k/mm^3)	135.0 (93.5, 185.0)	199.5 (152.0, 253.0)	<0.001 *
Initial nucleated RBC (count/100 WBC)	3.2 (1.2, 11.0)	1.6 (0.7, 3.6)	<0.001 *
Respiratory distress	82 (76.6%)	349 (92.1%)	<0.001 *

	VLBW FGR N= 107	VLBW non-FGR N= 379	P-value
Blood infection	15 (14.0%)	56 (14.8%)	0.84
CSF Infection	1 (0.9%)	8 (2.1%)	0.43
Seizures	0 (0.0%)	23 (6.1%)	0.009*
Death	4 (3.7%)	27 (7.1%)	0.21
PWMI	5 (4.7%)	48 (12.7%)	0.019*
IVH	26 (24.3%)	149 (39.3%)	0.004*
Severe IVH (Grades 3 and 4)	4 (3.7%)	52 (13.7%)	0.004*

* Indicates $p \leq 0.05$

VLBW = very low birth weight

FGR = fetal growth restriction

PWMI = periventricular white matter injury

IVH = intraventricular hemorrhage

Table 2.

Daily biomarker levels from cord blood at birth to day 5 of life in VLBW neonates with FGR vs without FGR, given as geometric mean (95% confidence interval), adjusted for gestational age. Interaction p-values indicate whether changes in biomarkers differ between groups based on day of sampling from repeated measures analysis.

Biomarker	VLBW FGR N= 107 neonates	VLBW non-FGR N= 379 neonates	Interaction P-value	P-value
IL-6			<0.001*	
Cord Blood	5.6 (3.4–9.3), n=98	14.7 (11.3–19.1), n=338		0.001*
Day 1	14.4 (7.4–27.9), n=55	12.1 (8.7–16.7), n=211		0.64
Day 2	27.5 (16.8–45.1), n=70	15.2 (11.8–19.5), n=248		0.041
Day 3	17.3 (11.0–27.2), n=61	8.9 (7.2–11.1), n=244		0.012
Day 4	8.6 (6.2–11.8), n=64	4.8 (4.1–5.6), n=228		0.002*
Day 5	5.2 (4.1–6.6), n=71	3.5 (3.1–3.9), n=219		0.004*
IL-8			0.001*	
Cord Blood	111.5 (80.8–154.0), n=98	81.9 (69.3–96.7), n=338		0.10
Day 1	173.2 (118.6–253.0), n=55	95.8 (79.4–115.5), n=211		0.007
Day 2	277.4 (210.1–366.2), n=70	132.0 (114.6–152.0), n=248		<0.001*
Day 3	251.0 (182.9–344.3), n=61	123.2 (106.0–143.2), n=244		<0.001*
Day 4	178.7 (138.1–231.0), n=64	102.2 (89.7–116.4), n=228		<0.001*
Day 5	111.5 (88.5–140.4), n=71	85.3 (75.2–96.6), n=219		0.052
IL-10			<0.001*	
Cord Blood	2.4 (1.6–3.6), n=98	2.7 (2.2–3.4), n=338		0.52
Day 1	4.5 (2.7–7.5), n=55	2.4 (1.8–3.0), n=211		0.025
Day 2	4.5 (2.9–7.2), n=70	1.6 (1.3–2.0), n=247		<0.001*
Day 3	2.0 (1.2–3.3), n=61	0.77 (0.61–0.98), n=243		0.001*
Day 4	0.82 (0.52–1.30), n=64	0.39 (0.31–0.49), n=227		0.005*
Day 5	0.41 (0.27–0.63), n=71	0.35 (0.28–0.44), n=219		0.50
BDNF			0.38	
Cord Blood	447.5 (340.5–588.2), n=98	534.5 (464.1–615.7), n=338		0.27
Day 1	740.4 (543.4–1008.8), n=55	856.6 (735.2–998.1), n=211		0.41
Day 2	826.5 (632.5–1080.1), n=70	855.4 (746.7–979.9), n=248		0.84
Day 3	663.1 (519.4–846.4), n=61	772.0 (687.3–867.0), n=244		0.28
Day 4	784.1 (617.0–996.4), n=64	788.6 (698.5–890.4), n=228		0.97
Day 5	801.3 (635.5–1010.4), n=71	851.1 (750.5–965.2), n=219		0.66
VEGF			<0.001*	
Cord Blood	3.8 (2.16–6.8), n=98	16.6 (12.3–22.3), n=337		<0.001*
Day 1	3.3 (1.5–7.2), n=55	41.9 (28.5–61.5), n=211		<0.001*

Biomarker	VLBW FGR N= 107 neonates	VLBW non-FGR N= 379 neonates	Interaction P-value	P-value
Day 2	12.6 (6.2–25.5), n=70	89.6 (62.5–128.4), n=247		<0.001 *
Day 3	59.1 (35.9–97.3), n=60	199.216 (157.5–252.0), n=244		<0.001 *
Day 4	132.7 (101.6–173.2), n=64	299.701 (261.8–343.1), n=228		<0.001 *
Day 5	146.5 (116.0–185.0), n=71	328.114 (289.1–372.4), n=219		<0.001 *
GFAP			0.19	
Cord Blood	0.094 (0.066–0.134), n=99	0.088 (0.074–0.106), n=343		0.78
Day 1	0.086 (0.053–0.139), n=56	0.086 (0.068–0.108), n=221		0.99
Day 2	0.094 (0.060–0.147), n=70	0.076 (0.061–0.095), n=253		0.41
Day 3	0.099 (0.061–0.161), n=62	0.073 (0.058–0.092), n=253		0.28
Day 4	0.106 (0.065–0.173), n=71	0.078 (0.061–0.101), n=235		0.30
Day 5	0.110 (0.069–0.177), n=71	0.085 (0.066–0.110), n=227		0.36
NRGN			0.31	
Cord Blood	0.016 (0.011–0.023), n=99	0.023 (0.019–0.028), n=343		0.11
Day 1	0.017 (0.010–0.029), n=56	0.021 (0.017–0.027), n=221		0.48
Day 2	0.014 (0.009–0.022), n=70	0.013 (0.011–0.017), n=253		0.84
Day 3	0.015 (0.009–0.024), n=62	0.014 (0.011–0.017), n=253		0.66
Day 4	0.016 (0.010–0.026), n=71	0.014 (0.011–0.018), n=235		0.56
Day 5	0.019 (0.012–0.031), n=71	0.015 (0.011–0.019), n=227		0.32
Tau			0.031	
Cord Blood	650.1 (365.7–1155.5), n=26	262.3 (206.2–333.5), n=134		0.005 *
Day 1	462.0 (189.5–1126.6), n=10	255.5 (185.8–351.3), n=74		0.22
Day 2	512.0 (305.3–858.6), n=20	348.0 (275.4–439.7), n=90		0.19
Day 3	622.2 (310.7–1245.9), n=20	247.2 (182.7–334.6), n=93		0.02
Day 4	443.3 (232.2–846.5), n=19	263.3 (198.1–350.0), n=89		0.16
Day 5	337.5 (170.9–666.6), n=19	239.5 (174.6–328.7), n=81		0.38

* Indicates $p \leq 0.00625$

IL = interleukin

BDNF = brain derived neurotrophic factor

VEGF = vascular endothelial growth factor

GFAP = glial fibrillary acidic protein

NRGN = neurogranin

Table 3.

Bivariate analysis of maternal and neonatal variables for VLBW FGR neonates with brain injury vs without brain injury given as median (interquartile range) or number (percent).

	VLBW FGR with brain injury N=11	VLBW FGR without brain injury N=96	P-value
Maternal age	35.0 (25.0, 37.0)	28.0 (22.0, 33.0)	0.046 *
Gravida	2 (1, 4)	2 (1, 4)	0.75
Parity	0 (0, 2)	1 (0, 1.5)	0.87
Gestational age	28.4 (27.0, 31.1)	30.2 (27.4, 32.1)	0.16
Race			0.35
White	5 (45%)	30 (31%)	
Black	5 (45%)	60 (63%)	
Hispanic	1 (9%)	2 (2%)	
Asian	0 (0%)	4 (4%)	
Other	0 (0%)	0 (0%)	
Cesarean delivery	11 (100%)	81 (84%)	0.16
Preeclampsia	8 (73%)	73 (76%)	0.81
Oligohydramnios	5 (45%)	29 (30%)	0.30
PPROM	1 (9%)	4 (4%)	0.46
Abruption	3 (27%)	5 (5%)	0.008 *
Meconium	1 (9%)	4 (4%)	0.46
Clinical chorioamnionitis	1 (9%)	2 (2%)	0.18
Histologic chorioamnionitis	1 (10%)	3 (3%)	0.28
Histologic funisitis	4 (40%)	40 (42%)	0.75
Histologic placental infarcts	4 (40%)	40 (42%)	0.92
Birth weight (grams)	760 (570, 860)	995 (675, 1235)	0.082
Male gender	5 (45%)	54 (56%)	0.50
Nonreassuring fetal heart rate	6 (55%)	38 (40%)	0.34
1 min Apgar <7	11 (100%)	64 (67%)	0.022 *
5 min Apgar <7	8 (73%)	31 (32%)	0.008 *
Arterial cord pH	7.1 (7.0, 7.2)	7.2 (7.2, 7.3)	0.13
Arterial cord base deficit (mM)	6.5 (6.0–13.5)	4.0 (2.0–6.0)	0.034 *
Initial neonatal WBC count (k/mm^3)	5.2 (4.2, 13.0)	5.7 (3.9, 7.8)	0.58
Initial neonatal hematocrit (%)	41.3 (35.3, 47.2)	46.6 (40.3, 52.1)	0.052
Initial neonatal platelet count (k/mm^3)	127.0 (95.0, 140.0)	138.0 (92.0, 187.0)	0.42
Initial nucleated RBC (count/100 WBC)	1.7 (0.52, 2.5)	2.6 (1.0, 8.5)	0.003 *
Respiratory distress	10 (91%)	72 (75%)	0.24
Blood infection	5 (45%)	10 (10%)	0.002 *
CSF Infection	0 (0%)	1 (1%)	0.73

	VLBW FGR with brain injury N=11	VLBW FGR without brain injury N=96	P-value
Seizures	0 (0.0%)	0 (0.0%)	--
Death	4 (36%)	0 (0%)	<0.001 *
PWMI	5 (45%)	0 (0%)	<0.001 *
IVH	6 (55%)	20 (21%)	0.014 *
Severe IVH (Grades 3 and 4)	4 (36%)	0 (0%)	<0.001 *

* Indicates $p \leq 0.05$

PPROM = preterm premature rupture of membranes

WBC = white blood cell

RBC = red blood cell

PWMI = periventricular white matter injury

IVH = intraventricular hemorrhage

Table 4.

Repeated measures analysis of each biomarker from cord blood through day 5 of life in VLBW FGR neonates with brain injury vs without brain injury, given as geometric mean (95% confidence interval).

Biomarker	VLBW FGR with brain injury N=11	VLBW FGR without brain injury N=96	P-value
IL-6 (pg/mL)	24.0 (11.2–51.5), n=10	6.2 (4.8–8.0), n=96	0.001 *
IL-8 (pg/mL)	287.9 (174.2–475.7), n=10	125.9 (106.2–149.2), n=96	0.002 *
IL-10 (pg/mL)	3.4 (1.6 –7.5), n=10	1.0 (0.80–1.3), n=96	0.004 *
BDNF (pg/mL)	932.6 (581.6–1495.3), n=10	772.8 (659.6–905.5), n=96	0.46
VEGF (pg/mL)	10.5 (2.9 –38.0), n=10	30.1 (19.7–46.1), n=96	0.128
GFAP (ng/mL)	0.250 (0.098–0.637), n=11	0.063 (0.046–0.087), n=96	0.006 *
NRGN (ng/mL)	0.011 (0.004–0.031), n=11	0.018 (0.013–0.025), n=96	0.41
Tau (pg/mL)	725.8 (379.5–1388.4), n=8	341.0 (224.8–517.3), n=20	0.055

* Indicates $p \leq 0.00625$

IL = interleukin

BDNF = brain derived neurotrophic factor

VEGF = vascular endothelial growth factor

GFAP = glial fibrillary acidic protein

NRGN = neurogranin