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Systemic inflammation on postnatal days 21 and 28 and indicators of brain dysfunction 2 years later among children born before the 28th week of gestation

Alan Leviton[a], **Elizabeth N. Allred**[a], **Raina N Fichorova**[b], **Karl Kuban**[c], **T. Michael O'Shea**[d], and **Olaf Dammann**[e,f] **for the ELGAN Study Investigators**

[a]Neuroepidemiology Unit, Department of Neurology, Boston Children's Hospital, Harvard University, Boston, Massachusetts 02115

[b]Laboratory of Genital Tract Biology, Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital Boston MA 02115

[c]Division of Neurology, Department of Pediatrics, Boston Medical Center and Boston University, Boston, MA 02118

[d]Department of Pediatrics, Wake Forest University, Winston-Salem, NC 27157

[e]Department of Public Health and Community Medicine, Tufts University School of Medicine, Boston, Massachusetts, 02111

[f]Perinatal Neuropidemiology Unit, Hannover Medical School, 30625 Hannover, Germany

Abstract

Background—Systemic inflammation during the first two postnatal weeks in extremely preterm newborns (< 28 weeks gestation) has been associated with an increased risk of

Corresponding author: Alan Leviton, Boston Children's Hospital, Au-414, 300 Longwood Avenue, Boston MA 02115-5724, alan.leviton@childrens.harvard.edu, telephone: 617 355-6491.

Participating institutions (site principal investigator)
Baystate Medical Center, Springfield MA (Bhavesh Shah)
Beth Israel Deaconess Medical Center, Boston MA (Camilia R. Martin)
Brigham & Women's Hospital, Boston MA (Linda J. Van Marter)
Children's Hospital, Boston MA (Alan Leviton)
Massachusetts General Hospital, Boston MA (Robert Insoft)
New England Medical Center, Boston MA (Cynthia Cole, John Fiascone)
U Mass Memorial Health Center, Worcester, MA (Francis Bednarek, deceased)
Yale-New Haven Hospital, New Haven CT (Richard Ehrenkranz)
Forsyth Hospital, Baptist Medical Center, Winston-Salem NC (T. Michael O'Shea)
University Health Systems of Eastern Carolina, Greenville NC (Stephen Engelke)
North Carolina Children's Hospital, Chapel Hill NC (Carl Bose,)
DeVos Children's Hospital, Grand Rapids MI (Mariel Portenga)
Sparrow Hospital, Lansing MI (Padmani Karna)
University of Chicago Hospital, Chicago IL (Michael D. Schreiber)
William Beaumont Hospital, Royal Oak MI (Daniel Batton)

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neurodevelopmental dysfunctions. Little is known, however, about the relationship between systemic inflammation during the third and fourth postnatal weeks and subsequent development.

Methods—We measured the concentrations of 16 inflammation-related proteins in blood spots collected on postnatal days 21 (N = 749) and 28 (N = 697) from infants born before the 28th week of gestation and assessed at age 2 years. We then sought the developmental correlates of top quartile concentrations for gestational age and day the specimen was collected. Odds ratios and 95% confidence intervals were calculated from regular or multinomial logistic regression models (as appropriate).

Results—Top quartile concentrations of CRP, IL-1β, IL-6, IL-6R, TNF-R2, IL-8, ICAM-1, and TSH on both days 21 and 28 were associated with ventriculomegaly (when in the NICU) and microcephaly at age 2 years. Top quartile concentrations of CRP, SAA, IL-6, TNF-R2, IL-8, and ICAM-1 were associated with Mental Development Index (MDI) of the Bayley-II < 55, while top quartile concentrations of CRP, TNF-α (inversely), IL-8, and ICAM-1 were associated with Psychomotor Development Index (PDI) < 55

Conclusion—Extremely preterm newborns who had systemic inflammation during the third and fourth postnatal weeks were at increased risk of ventriculomegaly during the months after birth, and of microcephaly, and low Bayley Scale scores at 2 years of age.

Keywords

Infant; premature; brain; developmental disabilities; inflammation

Introduction

The ELGAN Study of extremely low gestational age newborns (ELGANs)(*i.e.*, born before the 28th week of gestation) measured blood concentrations of inflammation-related proteins on postnatal days 1, 7, and 14. In this study, concentrations in the top quartile on two separate occasions a week apart were associated with increased risks of ventriculomegaly during the intensive care nursery stay,[1] and 2 years later with cerebral palsy,[2] low Bayley Scales of Infant Development-II,[3] an attention problem,[4] and microcephaly.[5]

These findings support the view that “intermittent or sustained systemic inflammation” contributes to brain damage in ELGANs.[6] The name “intermittent/sustained systemic inflammation” conveys the uncertainty that an elevated concentration on two separate days one week apart reflects sustained inflammation, two separate episodes of inflammation, or flare-ups of a low-level ongoing process. We could not distinguish among these possibilities because we did not have measurements of specimens collected at shorter intervals.

Recently, however, we were able to measure the concentrations of proteins in blood specimens collected from the same ELGAN subjects on postnatal days 21 and 28. This allowed us to consider that an elevated concentration on 4 days separated from each other by one week or more is unlikely to represent an intermittent process and more likely to reflect ongoing (sustained) inflammation.

Here we explore how well systemic inflammation at the end of the third and fourth weeks after birth of very preterm newborns conveys information about the risk of indicators of brain damage in the intensive care nursery and two years later.

Methods

Participants

During the years 2002–2004, women who gave birth before 28 weeks gestation at one of 14 participating hospitals in 5 states in the U.S. were invited to enroll. The individual institutional review boards approved the enrollment and consent.

Mothers were approached for consent either upon antenatal admission or shortly after delivery. A total of 1506 infants born to 1249 mothers were enrolled. The sample for the analyses of ventriculomegaly when the child was in the intensive care nursery is larger than the sample for the analyses that evaluated head circumference and function at age 2 years (Table 1).

Newborn variable

Gestational age was estimated based on date of embryo retrieval, intrauterine insemination, or fetal ultrasound before the 14th week (62%). When any of these were not available, the estimate was based on fetal ultrasound at week 14 or later (29%), last menstrual period (7%), or the gestational age recorded in the log of the Neonatal Intensive Care Unit (NICU) (1%).

Protocol ultrasound scans

Routine scans were performed by technicians at all of the hospitals using digitized high frequency transducers (7.5 and 10 MHz). Ultrasound studies always included the six standard quasi-coronal views and five sagittal views using the anterior fontanel as the sonographic window.[7] The three sets of protocol scans were defined by the postnatal day on which they were obtained (1st through 4th day; 5th through 14th day, and 15th day through the 40th week).

After creation of a manual and data collection form, observer variability minimization efforts included conference calls discussing aspects of images prone to different interpretations.[8] Templates of multiple levels of ventriculomegaly were included in the manual.

All ultrasound scans were read by two independent readers who were not provided clinical information. Each set of scans was first read by one study sonologist at the institution of the infant’s birth. The images, usually as electronic images on a CD imbedded in the software eFilm Workstation™ (Merge Healthcare/Merge eMed, Milwaukee, WI) were sent to a sonologist at another ELGAN study institution for a second reading. The eFilm program allowed the second reader to see what the first reader saw, and provided options to adjust and enhance the studies similar to the original reader, including the ability to zoom and alter gains. When the two readers differed in their recognition of moderate/severe

ventriculomegaly, the films were sent to a third (tie-breaking) reader who did not know what the readers reported.

24-month developmental assessment

Families were invited to bring their child for a developmental assessment close to the time when s/he would be 24-months corrected age. The full evaluation included a neurological examination, the Bayley Scales of Infant Development, Second edition, the Gross Motor Function Classification System, and the Modified-Checklist for Autism in Toddlers.

Fully 91% of surviving children returned for the developmental assessment. Of these children, 75% had their exam within the range of 23.5–27.9 months, 14% were assessed before 23.5 months, and 12% were assessed after 27.9 months.

Head circumference

The head circumference was measured as the largest possible occipital-frontal circumference and rounded to the closest 0.1 centimeter. All head circumferences are presented as Z-scores because newborns were assessed at different approximations of 24 months corrected age (range: 16–44 months corrected age, with 68% assessed at 23–25 months corrected age). Z-scores were based on standards in the CDC data set.[9]

Bayley Scales of Infant Development – Second Edition (BSID-II)[10]

Certified examiners administered and scored the BSID-II. Only 2% of examiners indicated at the time of the examination that they had more than a limited amount of information about the child. Before testing examiners were told the child's chronologic age. After completion of testing they were told the gestational age so that the mental development index (MDI) and psychomotor development index (PDI) could be age-adjusted as appropriate.

The child was classified as non-testable on a scale if her/his impairments prohibited standardized administration, or more than 2 items were judged to be 'not applicable.' Children considered non-testable were assigned their scores on scales 4 and/or 5 of the Vineland Adaptive Behavioral Composite which, like the BSID-II scales, have means of 100 and standard deviations of 14.[11]

Blood spot collection and protein measurement

Drops of blood were collected on filter paper on the first postnatal day (range: 1–3 days), the 7^{th} postnatal day (range: 5–8 days), the 14^{th} postnatal day (range: 12–15 days), the 21^{st} postnatal day (range: 19–23 days), and the 28^{th} postnatal day (range: 26–29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at −70°C in sealed bags with a desiccant until processed. Details about the elution of proteins from the blood spots are provided elsewhere.[12]

Each sample was analyzed in duplicate using the Meso Scale Discovery electrochemiluminescence multiplex platform and Sector Imager 2400. This platform has

been validated by comparisons with traditional ELISA,[13] and produces measurements that have high content validity[14–18]

Proteins measured

The Genital Tract Biology Laboratory at the Brigham and Women's Hospital in Boston Massachusetts measured the following 16 proteins: C-Reactive Protein (CRP), Serum Amyloid A (SAA), Myeloperoxidase (MPO), Interleukin-1 β (IL-1β), Interleukin-6 (IL-6), Interleukin-6 Receptor (IL-6R), Tumor Necrosis Factor-α (TNF-α), Tumor Necrosis Factor Receptor-2 (TNF-R2), Interleukin-8 (IL-8; CXCL8), Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES; CCL5), Intercellular Adhesion Molecule-1 (ICAM-1; CD54), Matrix Metalloproteinase-9 (MMP-9), Vascular Endothelial Growth Factor (VEGF), Vascular Endothelial Growth Factor Receptor-2 (VEGF-R2; KDR), thyroid-stimulating hormone (TSH), and Erythropoietin (EPO).

Because the volume of blood spots can vary, each protein measurement was normalized to milligrams of total protein. Measurements were made in duplicate, and the mean served as the basis for all tables and analyses.

The protein concentrations in the ELGAN study varied with gestational age, and with the postnatal day of specimen collection.[19, 20] In addition, one set of measurements was made in 2009–2010 and the second in 2015, and, while the distributions of each were similar, they were not identical. Consequently, we divided our sample into 30 groups defined by gestational age category (23–24, 25–26, 27 weeks), postnatal day of blood collection (1, 7, 14, 21 and 28), and measurement set (2009–2010, 2015). Because we were interested in the contribution of high concentrations, and the concentrations of most proteins did not follow normal distributions, the distribution of each protein's concentration was dichotomized into the highest quartile and the lower three quartiles in each of the 30 groups (3 gestational age groups, 5 collection days, 2 sets of measurements made years apart).

Data analyses

We evaluated the hypothesis that infants who had a protein concentration in the top quartile on one day were no more likely than their peers who had a lower concentration that day of having presumed structural indicators of brain damage, including ventriculomegaly while the child was in the intensive care nursery and microcephaly at age 2 years, as well as such functional indicators at age 2 years as low MDI and PDI.

For ventriculomegaly and microcephaly, we created logistic regression models to calculate odds (risk) ratios and 95% confidence intervals that infants who had a concentration in the highest quartile on one day were more likely than their peers with lower concentrations to have the indicator of brain damage. For the trichotomous variables of MDI and PDI (< 55, 55–69, and ≥70), we created multinomial regression models.[21, 22]

To help identify potential confounders, we sought associations between antecedents and elevated concentrations of individual proteins on days 21 and 28. Birth weight Z-score < −1 was the most consistently identified antecedent, and that is what we adjusted for when calculating all odds ratios and confidence intervals. Such correlates of the outcomes as male

sex and socioeconomic indicators were not associated with elevated protein concentrations and therefore these potential confounders are not confounders in these analyses.

To quantify how much elevated concentrations of inflammation-associated proteins in blood obtained at the end of weeks 3 and 4 supplement information provided by elevated concentrations during the first 2 weeks, we created time-oriented models.[1, 23–34] We offered the model information about elevated concentrations during the first two weeks as the first epoch, and information about elevated concentrations during the third and fourth weeks as the second epoch. Each model included those proteins whose top quartile concentrations on two separate days during the first two weeks predicted the neurodevelopmental entity. For ventriculomegaly these proteins were CRP, SAA, IL-6, TNF-α, TNF-R1, IL-8, MCP-1, MCP-4, ICAM-1, VEGF, and VEGF-R1, while for severe microcephaly they included CRP, SAA, IL-1β, IL-6, TNF-α, TNF-R2, IL-8, MCP-1, ICAM-1, E-SEL, and IGFBP-1. Bayley Scales-II mental development indices < 55 were predicted by repeatedly elevated concentrations of CRP, SAA, IL-6, TNF-α, TNF-R2, IL-8, MIP-1β, ICAM-1, VCAM-1, E-SEL, VEGF-R2, and IGFBP-1, while such low Bayley Scales-II motor development indices were predicted by repeated top quartile concentrations of CRP, SAA, MPO IL-6, TNF-α, IL-8, MCP-1, and ICAM-1.

We focus on those proteins whose concentrations in the top quartile on both days 21 and 28 were associated with increased risk of a structural or functional indicator of brain damage (column labeled “late” in Tables 2–5b). Often an association is seen for the combination of days, but not both days individually. We include in Tables 2–5b, odds ratios for the “early” set measurements to provide context for the time-oriented analyses in the column labeled “late in light of early” in, but do not comment on them further as they have been reported previously.[5, 35] They differ a bit from those published previously because of differences in the samples. Here, unlike the previous report, only those children who had measurements of proteins on both days 21 and day 28 (N = 599) are included.

Results

Ventriculomegaly (Table 2)

We present odds ratios associated with an elevated concentration on day 21 (N = 771) and separately on day 28 (N = 723). We also present risk information for measurements in the top quartile on both days 21 and 28 (N = 632)(column headed “late”). Concentrations of IL-6R in the highest quartile on day 21 and of MMP-9 on days 21 and 28, and both days were associated with reduced risk of ventriculomegaly.

Microcephaly (Table 3)

Elevated concentrations of CRP, SAA, TNF-R2, IL-8 and ICAM-1 on day 21 were associated with increased risk of microcephaly, while day-28 elevated concentrations of IL-6, IL-6R, TNF-R2, IL-8, ICAM-1, and VEGF-R2 were also associated with increased of microcephaly. Top quartile concentrations of CRP, IL-1β, IL-6, IL-6R, TNF-R2, IL-8, ICAM-1, and TSH on both days 21 and 28 were associated with increased risk of

microcephaly and a top quartile concentration of RANTES on both days was associated with reduced risk.

The increased risk of microcephaly associated with top quartile concentrations of TNF-R2, IL-8, and ICAM-1 on both days 21 and 28 supplemented risk information provided by earlier elevations of these proteins. The supplemental information provided by elevated concentrations of IL-6 very closely approached statistical significance at the $p < .05$ level.

Low MDI (Tables 4a and 4b)

Increased risk of an MDI more than 3 standard deviations below the expected mean (*i.e.*, <55, Table 4a) was associated with blood concentrations in the highest quartile concentrations of CRP, IL-6, TNF-R2, IL-8, and ICAM-1 on both days 21 and 28. TNF-α was the only protein whose concentration in the highest quartile on day 21 only was associated with increased risk of such a low MDI, while top quartile concentrations of SAA and VEGF-R2 on day 28 only were also associated with increased risk of an MDI <55. Top quartile concentrations on both days 21 and 28 of CRP, SAA, IL-6, IL-8, and ICAM-1 supplemented information about increased risk of a very low MDI provided by repeated elevated concentrations on earlier days.

Increased risk of an MDI between 2 and 3 standard deviations below the expected mean (*i.e.*, 55–69, Table 4b) was associated with blood concentrations in the highest quartile of TNF-α on both days 21 and 28, individually. Top quartile concentrations of IL-6, IL-8, ICAM-1 and VEGF-R2 were the only proteins whose concentration in the highest quartile on day 21 only were associated with increased risk of such a low MDI, while top quartile concentrations of CRP and SAA on day 28 only were associated with increased risk of an MDI between 55 and 69.

Children who had top quartile concentrations of CRP, IL-6, and TNF-α on both days 21 and 28 (“late” column) were at increased risk of MDI 55–69, while those who had a top quartile concentration of IL-6R on both days were at reduced risk.

Low PDI (Tables 5a and Table 5b)

Increased risk of a PDI < 55 was associated with blood concentrations in the highest quartile of ICAM-1 on each of days 21 and 28. IL-6, TNF-R2, and IL-8 were the only proteins whose concentrations in the highest quartile on day 28 only were associated with increased risk of such a low PDI.

Elevated CRP, IL-8, and ICAM-1 concentrations on both days 21 and 28 were associated with an increased risk of a PDI more than 3 standard deviations below the expected mean, while top quartile concentrations of TNF-alpha on both days 21 and 28 were associated with reduced risk of a very low PDI.

TNF-α and ICAM-1 were the only proteins whose blood concentrations on each of both days 21 and 28 were associated with increased risk of a PDI between two and three standard deviations below the normative mean (*i.e.*, 55 and 69). CRP, TNF-R2, IL-8, and VEGF-R2

were the only proteins whose blood concentrations on day 21 alone were associated with increased risk of a PDI between 55 and 69.

Elevated blood concentrations of CRP, TNF-alpha, IL-8, and ICAM-1 on both days 21 and 28 were associated with increased risk of a PDI between 55 and 69 (“late” column). Elevated blood concentrations of CRP on both days 21 and 28 supplemented risk information provided by earlier systemic inflammation.

Discussion

Our main finding is that very preterm newborns who have relatively high concentrations of inflammation-related proteins in their blood during the second half of the first postnatal month are at increased risk at age 2 years of microcephaly and low scores on assessments of mental and motor function. In most situations, this “late” inflammation provided risk information not available from specimens collected earlier.

Discrepancy between individual days and combination of days

We found that some proteins were associated with a developmental limitation when we defined exposure as an elevated concentration on both days 21 and 28, even when elevated concentrations on each of these days were not associated with the developmental limitation. This paradox is a consequence of requiring measurements on both days. Fully 771 infants had a day-21 specimen and 723 had a day-28 specimen, but only 632 had specimens from both days.

Time-oriented risk models

In this sample, “early” systemic inflammation (evident on days 7 and 14) predicts “late” systemic inflammation (evident on days 21 and 28). Precisely because children who had “early” systemic inflammation were more likely than others to have “late” systemic inflammation, we wanted to be able to determine if “late” inflammation added information about the risk of indicators of impaired development above and beyond that conveyed by early systemic inflammation. Indeed we found support for the hypothesis that systemic inflammation on days 21 and 28 adds risk information to that provided by earlier systemic inflammation. This raises the possibility that therapies to diminish late inflammation will be able to reduce the risk of brain damage in the most vulnerable.

Common set of proteins

Elevated concentrations of three proteins, CRP, IL-8, and ICAM-1, were most commonly associated with indicators of brain damage. CRP is an acute phase reactant, while IL-8 functions as a chemokine, as well as in other ways, and ICAM-1 is an adhesion molecule. We interpret their repeated involvement as indicative of a broad inflammatory response.[36]

Others have also found that elevated CRP concentrations in perinatal blood were associated with cognitive or motor limitations years later.[37–39] Elevated IL-8 concentrations in perinatal blood have also been associated with cognitive or motor limitations.[40–42] We are not aware of similar assessments of elevated concentrations of ICAM-1.

MMP-9 and reduced risk of ventriculomegaly

We are not sure why top quartile concentrations of MMP-9 were associated with reduced risk of ventriculomegaly. Low levels of MMP-9 have been associated with pro-inflammatory phenomena in women during mid pregnancy,[43] suggesting an inverse relationship with some other indicators of inflammation. In addition, other findings suggest that MMP-9 has anti-inflammatory properties.[44–46] These pleotropic properties also indicate that MMP-9, along with the tissue inhibitor of metalloproteinase-1, which helps regulate the activity of MMP-9, operate in complex ways, only some of which are probably related to inflammation. Indeed, the authors of one review wrote, “we still do not fully understand the complexity of MMP-9 mechanisms of action.”[47] In light of these limitations, we are unable to explain why we found that elevated concentrations of MMP-9 were associated with reduced risk of ventriculomegaly.

Obviously, what is measured in the blood might not reflect the concentration or availability in the brain. We are not aware of any studies in preclinical models of perinatal brain damage in immature vertebrates that compare concentrations of MMP-9 in the brain to what is measured in the blood.

Sustained inflammation

The extremely preterm newborn has many reasons to have prolonged/chronic inflammation. [6] These include persistence of an inflammatory stimulus,[48] hampered resolution of inflammation,[49, 50] a pro-inflammatory profile characteristic of extremely preterm newborns,[51, 52] limited ability to degrade inflammation-related proteins,[53-55] positive feedback loops between innate and adaptive immune systems,[56] epigenetic phenomena, [57] endoplasmic reticulum stress resulting in an unfolded protein response,[58] impairments of ubiquitylation,[59] and impairments of autophagy.[60]

Limitations and strengths

We relied on blood specimens obtained for clinical indications. As their physiology became more stable, some infants were less likely than their sicker peers to have blood drawn on days 21, and 28. Consequently, selection bias probably occurred to some extent. We are also limited by the relatively small number of proteins measured. Inflammation is a broad and complex phenomenon,[61, 62] and we have assessed a very small part of it. Finally, we are unable to distinguish between causation and association as explanations for what we found.

Our creating time-oriented risk models allowed us to document the added contribution of “late” inflammation to brain-damage risk. With more than 600 children and our classifying exposure as the top quartile, and such outcomes as MDI < 55 identified in 96 children, we have a power of 0.95 to appreciate an odds ratio of 1.75. Other strengths are the selection of infants based on gestational age, not birth weight,[63] prospective collection of all data, examiners who were not aware of the medical histories of the children they examined, thereby minimizing “diagnostic suspicion bias,”[64] efforts to minimize observer variability in the assessment of neurodevelopmental functions,[65] modest attrition, and finally, protein data of high quality [19,20], and high content validity.[14, 15, 19, 66]

Conclusions

Among very preterm newborns, systemic inflammation during the third and fourth postnatal weeks is associated with multiple structural and functional indicators of brain damage evident at age two years.

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Abbreviations

ELGAN	Extremely low gestational age newborn
NICU	Neonatal intensive care unit
BSID-II	Bayley Scales of Infant Development – Second Edition
MDI	mental development index of the BSID-II
PDI	psychomotor development index of the BSID-II
CRP	C-Reactive Protein
SAA	Serum Amyloid A
MPO	Myeloperoxidase
IL-1β	Interleukin-1 β
IL-6	Interleukin-6
IL-6R	Interleukin-6 Receptor
TNF-α	Tumor Necrosis Factor-α
TNF-R2	Tumor Necrosis Factor Receptor-2
IL-8	Interleukin-8 (CXCL8)
RANTES	Regulated upon Activation, Normal T-cell Expressed, and Secreted (CCL5)
ICAM-1	Intercellular Adhesion Molecule-1 (CD54)
MMP-9	Matrix Metalloproteinase-9
VEGF	Vascular Endothelial Growth Factor
VEGF-R2	Vascular Endothelial Growth Factor Receptor-2 (KDR)
TSH	thyroid-stimulating hormone
EPO	Erythropoietin

References

1. Leviton A, Kuban K, O'Shea TM, Paneth N, Fichorova R, Allred EN, et al. The Relationship between early concentrations of 25 blood proteins and cerebral white matter injury in preterm newborns: The ELGAN Study. J Pediatr. 2011; 158:897–903. e5. [PubMed: 21238986]

2. Kuban KC, O'Shea TM, Allred EN, Paneth N, Hirtz D, Fichorova RN, et al. Systemic Inflammation and Cerebral Palsy Risk in Extremely Preterm Infants. J Child Neurol. 2014; 29:1692–8. [PubMed: 24646503]

3. O'Shea TM, Allred EN, Kuban K, Dammann O, Paneth N, Fichorova R, et al. Elevated concentrations of inflammation-related proteins in postnatal blood predict severe developmental delay at two years in extremely premature infants. J Pediatr. 2012; 160:395–401. e4. [PubMed: 22000304]

4. O'Shea TM, Joseph RM, Kuban KCK, Allred EN, Ware J, Coster T, et al. Elevated blood levels of inflammation-related proteins are associated with an attention problem at age 24 months in extremely preterm infants. Pediatric Research. 2014

5. Leviton A, Kuban KC, Allred EN, Fichorova RN, O'Shea TM, Paneth N, et al. Early postnatal blood concentrations of inflammation-related proteins and microcephaly two years later in infants born before the 28th post-menstrual week. Early Hum Dev. 2011; 87:325–30. [PubMed: 21334149]

6. Dammann O, Leviton A. Intermittent or sustained systemic inflammation and the preterm brain. Pediatr Res. 2014

7. Teele, R.; Share, J. Ultrasonography of infants and children. Philadelphia: Saunders; 1991.

8. Kuban K, Adler I, Allred EN, Batton D, Bezinque S, Betz BW, et al. Observer variability assessing US scans of the preterm brain: the ELGAN study. Pediatr Radiol. 2007; 37:1201–8. [PubMed: 17901950]

9. CDC. CDC Growth Charts: United States. 2007. http://www.cdc.gov/nchs/data/nhanes/growthcharts/zscore/zhcageinf.xls

10. Bayley, N. Bayley Scales of Infant Development-II. Psychological Corporation; San Antonio, TX: 1993.

11. Sparrow SS, Cicchetti DV. Diagnostic uses of the Vineland Adaptive Behavior Scales. J Pediatr Psychol. 1985; 10:215–25. [PubMed: 4020603]

12. Fichorova RN, Beatty N, Sassi RRS, Yamamoto HS, Allred EN, Leviton A, et al. Systemic inflammation in the extremely low gestational age newborn following maternal genitourinary infections. American Journal of Reproductive Immunology. 2015; 73:162–74. [PubMed: 25164433]

13. Fichorova RN, Richardson-Harman N, Alfano M, Belec L, Carbonneil C, Chen S, et al. Biological and technical variables affecting immunoassay recovery of cytokines from human serum and simulated vaginal fluid: a multicenter study. Anal Chem. 2008; 80:4741–51. [PubMed: 18484740]

14. Hecht JL, Fichorova RN, Tang VF, Allred EN, McElrath TF, Leviton A. Relationship between neonatal blood protein profiles and placenta histologic characteristics in ELGANs. Pediatr Research. 2011; 69:68–73.

15. McElrath TF, Fichorova RN, Allred EN, Hecht JL, Ismail MA, Yuan H, et al. Blood protein profiles of infants born before 28 weeks differ by pregnancy complication. Am J Obstet Gynecol. 2011; 204:418, e1–e12. [PubMed: 21349490]

16. Leviton A, Hecht JL, Allred EN, Yamamoto H, Fichorova RN, Dammann O. Persistence after birth of systemic inflammation associated with umbilical cord inflammation. J Reprod Immunol. 2011; 90:235–43. [PubMed: 21722967]

17. Leviton A, O'Shea TM, Bednarek FJ, Allred EN, Fichorova RN, Dammann O. Systemic responses of preterm newborns with presumed or documented bacteraemia. Acta Paediatr. 2012; 101:355–9. [PubMed: 22085230]

18. Bose CL, Laughon MM, Allred EN, O'Shea TM, Van Marter LJ, Ehrenkranz RA, et al. Systemic inflammation associated with mechanical ventilation among extremely preterm infants. Cytokine. 2013; 61:315–22. [PubMed: 23148992]

19. Leviton A, Fichorova R, Yamamoto Y, Allred EN, Dammann O, Hecht J, et al. Inflammation-related proteins in the blood of extremely low gestational age newborns. The contribution of inflammation to the appearance of developmental regulation. Cytokine. 2011; 53:66–73. [PubMed: 20934883]

20. Leviton A, Allred EN, Yamamoto H, Fichorova RN. Investigators. ftES. Relationships among the concentrations of 25 inflammation-associated proteins during the first postnatal weeks in the blood of infants born before the 28th week of gestation. Cytokine. 2012

21. Dubin N, Pasternack BS. Risk assessment for case-control subgroups by polychotomous logistic regression. Am J Epidemiol. 1986; 123:1101–17. [PubMed: 3706280]
22. Liang KY, Zeger S. Longitudinal data analysis using generalized linear models. Biometrika. 1986; 73:13–22.
23. SanGiovanni JP, Allred EN, Mayer DL, Stewart JE, Herrera MG, Leviton A. Reduced visual resolution acuity and cerebral white matter damage in very-low-birthweight infants. Dev Med Child Neurol. 2000; 42:809–15. [PubMed: 11132254]
24. Van Marter LJ, Allred EN, Pagano M, Sanocka U, Parad R, Moore M, et al. Do clinical markers of barotrauma and oxygen toxicity explain interhospital variation in rates of chronic lung disease? The Neonatology Committee for the Developmental Network. Pediatrics. 2000; 105:1194–201. [PubMed: 10835057]
25. Laughon M, O'Shea MT, Allred EN, Bose C, Kuban K, Van Marter LJ, et al. Chronic lung disease and developmental delay at 2 years of age in children born before 28 weeks' gestation. Pediatrics. 2009; 124:637–48. [PubMed: 19620203]
26. Van Marter LJ, Kuban KCK, Allred EN, Bose C, Dammann O, O'Shea TM, et al. Does Bronchopulmonary Dysplasia Contribute to the Occurrence of Cerebral Palsy Among Infants Born Before 28 weeks of Gestation? Arch Dis Child Fetal Neonatal Ed. 2011; 96:F20–F9. [PubMed: 20736416]
27. Tolsma KW, Allred EN, Chen ML, Duker J, Leviton A, Dammann O, et al. Neonatal Bacteremia and Retinopathy of Prematurity: The ELGAN Study. Arch Ophthalmol. 2011; 129:1555–63. [PubMed: 22159674]
28. Downey LC, O'Shea TM, Allred EN, Kuban K, McElrath TF, Warner DD, et al. Antenatal and early postnatal antecedents of parent-reported attention problems at 2 years of age. J Pediatr. 2015; 166:20–5. [PubMed: 25241173]
29. Holm M, Msall ME, Skranes J, Dammann O, Allred E, Leviton A. Antecedents and correlates of visual field deficits in children born extremely preterm. Eur J Paediatr Neurol. 2015; 19:56–63. [PubMed: 25455711]
30. Leviton A, Paneth N, Reuss ML, Susser M, Allred EN, Dammann O, et al. Maternal infection, fetal inflammatory response, and brain damage in very low birthweight infants. Pediatric Research. 1999; 46:566–75. [PubMed: 10541320]
31. Leviton A, Pagano M, Kuban KC, Krishnamoorthy KS, Sullivan KF, Allred EN. The epidemiology of germinal matrix hemorrhage during the first half-day of life. Dev Med Child Neurol. 1991; 33:138–45. [PubMed: 2015981]
32. Leviton A, Kuban KC, Pagano M, Allred EN, Van Marter L. Antenatal corticosteroids appear to reduce the risk of postnatal germinal matrix hemorrhage in intubated low birth weight newborns. Pediatrics. 1993; 91:1083–8. [PubMed: 8502506]
33. Leviton A, Paneth N, Reuss ML, Susser M, Allred EN, Dammann O, et al. Hypothyroxinemia of prematurity and the risk of cerebral white matter damage. J Pediatr. 1999; 134:706–11. [PubMed: 10356138]
34. Leviton A, Dammann O, Allred EN, Kuban KCK, Pagano M, Van Marter LJ, et al. Antenatal corticosteroids and cranial ultrasound abnormalities. Am J Obstet Gynecol. 1999; 181:1007–17. [PubMed: 10521769]
35. Martin CR, Dammann O, Allred EN, Patel S, O'Shea TM, Kuban KC, et al. Neurodevelopment of extremely preterm infants who had necrotizing enterocolitis with or without late bacteremia. J Pediatr. 2010; 157:751–6. e1. [PubMed: 20598317]
36. Kuban KC, O'Shea TM, Allred EN, Fichorova RN, Heeren T, Paneth N, et al. The breadth and type of systemic inflammation and the risk of adverse neurological outcomes in extremely low gestation newborns. Pediatr Neurol. 2015; 52:42–8. [PubMed: 25459361]
37. Kaukola T, Kapellou O, Laroche S, Counsell SJ, Dyet LE, Allsop JM, et al. Severity of perinatal illness and cerebral cortical growth in preterm infants. Acta Paediatr. 2009; 98:990–5. [PubMed: 19302092]
38. Inomata K, Mizobuchi M, Tanaka S, Iwatani S, Sakai H, Yoshimoto S, et al. Patterns of increases in interleukin-6 and C-reactive protein as predictors for white matter injury in preterm infants. Pediatr Int. 2014; 56:851–5. [PubMed: 24832219]

39. Rose J, Vassar R, Cahill-Rowley K, Hintz SR, Stevenson DK. Neonatal Biomarkers of Inflammation: Correlates of Early Neurodevelopment and Gait in Very-Low-Birth-Weight Preterm Children. Am J Perinatol. 2015
40. Procianoy RS, Silveira RC. Association between high cytokine levels with white matter injury in preterm infants with sepsis. Pediatr Crit Care Med. 2012; 13:183–7. [PubMed: 21666535]
41. Carlo WA, McDonald SA, Tyson JE, Stoll BJ, Ehrenkranz RA, Shankaran S, et al. Cytokines and neurodevelopmental outcomes in extremely low birth weight infants. J Pediatr. 2011; 159:919–25. e3. [PubMed: 21798559]
42. Hansen-Pupp I, Hallin AL, Hellstrom-Westas L, Cilio C, Berg AC, Stjernqvist K, et al. Inflammation at birth is associated with subnormal development in very preterm infants. Pediatr Res. 2008; 64:183–8. [PubMed: 18391842]
43. Ozdamar O, Gun I, Mungen E, Atay V. The assessment of the relationship between amniotic fluid matrix metalloproteinase-9 and zinc levels with adverse obstetric outcomes. Archives of gynecology and obstetrics. 2014; 290:59–64. [PubMed: 24573505]
44. Yabluchanskiy A, Ma Y, Iyer RP, Hall ME, Lindsey ML. Matrix metalloproteinase-9: Many shades of function in cardiovascular disease. Physiology (Bethesda). 2013; 28:391–403. [PubMed: 24186934]
45. Zamilpa R, Ibarra J, de Castro Bras LE, Ramirez TA, Nguyen N, Halade GV, et al. Transgenic overexpression of matrix metalloproteinase-9 in macrophages attenuates the inflammatory response and improves left ventricular function post-myocardial infarction. Journal of molecular and cellular cardiology. 2012; 53:599–608. [PubMed: 22884843]
46. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes & development. 2000; 14:163–76. [PubMed: 10652271]
47. Deleon-Pennell KY, Altara R, Yabluchanskiy A, Modesti A, Lindsey ML. The circular relationship between matrix metalloproteinase-9 and inflammation following myocardial infarction. IUBMB life. 2015; 67:611–8. [PubMed: 26269290]
48. Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, et al. Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatric disease and treatment. 2015; 11:97–106. [PubMed: 25657582]
49. Kotecha S, Mildner RJ, Prince LR, Vyas JR, Currie AE, Lawson RA, et al. The role of neutrophil apoptosis in the resolution of acute lung injury in newborn infants. Thorax. 2003; 58:961–7. [PubMed: 14586049]
50. Nguyen CN, Schnulle PM, Chegini N, Luo X, Koenig JM. Neonatal neutrophils with prolonged survival secrete mediators associated with chronic inflammation. Neonatology. 2010; 98:341–7. [PubMed: 20551711]
51. Jones CA, Cayabyab RG, Kwong KY, Stotts C, Wong B, Hamdan H, et al. Undetectable interleukin (IL)-10 and persistent IL-8 expression early in hyaline membrane disease: a possible developmental basis for the predisposition to chronic lung inflammation in preterm newborns. Pediatr Res. 1996; 39:966–75. [PubMed: 8725256]
52. Blahnik MJ, Ramanathan R, Riley CR, Minoo P. Lipopolysaccharide-induced tumor necrosis factor-alpha and IL-10 production by lung macrophages from preterm and term neonates. Pediatr Res. 2001; 50:726–31. [PubMed: 11726731]
53. Wynn JL, Levy O. Role of innate host defenses in susceptibility to early-onset neonatal sepsis. Clin Perinatol. 2010; 37:307–37. [PubMed: 20569810]
54. Brochu ME, Girard S, Lavoie K, Sebire G. Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: An experimental study. J Neuroinflammation. 2011; 8:55. [PubMed: 21599903]
55. Gille C, Dreschers S, Leiber A, Lepiorz F, Krusch M, Grosse-Opphoff J, et al. The CD95/CD95L pathway is involved in phagocytosis-induced cell death of monocytes and may account for sustained inflammation in neonates. Pediatr Res. 2013; 73:402–8. [PubMed: 23269121]
56. Leviton A, Dammann O, Durum SK. The adaptive immune response in neonatal cerebral white matter damage. Ann Neurol. 2005; 58:821–8. [PubMed: 16250014]

57. Natoli G. Tuning up inflammation: how DNA sequence and chromatin organization control the induction of inflammatory genes by NF-kappaB. FEBS letters. 2006; 580:2843–9. [PubMed: 16530189]

58. Bueter W, Dammann O, Leviton A. Endoplasmic reticulum stress, inflammation, and perinatal brain damage. Pediatr Res. 2009; 66:487–94. [PubMed: 19668101]

59. Tokunaga F. Linear ubiquitination-mediated NF-kappaB regulation and its related disorders. Journal of biochemistry. 2013; 154:313–23. [PubMed: 23969028]

60. Agrawal V, Jaiswal MK, Mallers T, Katara GK, Gilman-Sachs A, Beaman KD, et al. Altered autophagic flux enhances inflammatory responses during inflammation-induced preterm labor. Scientific reports. 2015; 5:9410. [PubMed: 25797357]

61. Zak DE, Aderem A. Systems biology of innate immunity. Immunol Rev. 2009; 227:264–82. [PubMed: 19120490]

62. Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, et al. A genomic storm in critically injured humans. The Journal of experimental medicine. 2011; 208:2581–90. [PubMed: 22110166]

63. Arnold CC, Kramer MS, Hobbs CA, McLean FH, Usher RH. Very low birth weight: a problematic cohort for epidemiologic studies of very small or immature neonates. Am J Epidemiol. 1991; 134:604–13. [PubMed: 1951265]

64. Sackett DL. Bias in analytic research. J Chronic Dis. 1979; 32:51–63. [PubMed: 447779]

65. Kuban KCK, Allred EN, O'Shea TM, Paneth N, Pagano M, Leviton A, et al. An algorithm for diagnosing and classifying cerebral palsy in young children. J Pediatr. 2008; 153:466–72.e1. [PubMed: 18534210]

66. Fichorova RN, Onderdonk AB, Yamamoto H, MLD, Dubois AM, Allred E, et al. Microbe-specific modulation of inflammatory response in extremely low gestational age newborns. mBio. 2011; 2:e00280–10. [PubMed: 21264056]

Highlight

- Very preterm newborns who had systemic inflammation during the third and fourth postnatal weeks appear to be at increased risk of indicators of brain damage and dysfunction at age 2 years

Table 1

Sample description

	Yes	No
Enrolled	1506	
Had head cranial ultrasound in the NICU	1455	55
Had day-1 proteins measured	1109	346
Had day-7 proteins measured	1140	315
Had day-14 proteins measured	1031	424
Had day-21 proteins measured	938	517
Had day-28 proteins measured	878	577
Survived to 24 months	1200	255
Had 24 month developmental assessment	1102	98
Had day-1 proteins measured	973	129
Had day-7 proteins measured	986	116
Had day-14 proteins measured	890	212
Had day-21 proteins measured	809	293
Had day-28 proteins measured	750	351

Table 2

Odds ratios (95% Confidence Intervals) of **ventriculomegaly** associated with associated with concentrations in the top quartile on days 21 and 28, both days without regard to risk information provided by top quartile concentrations before day 21 (third column), and associated with concentrations in the top quartile on both the 21st and 28th postnatal days in light of associations with elevated concentrations of this protein on multiple occasions earlier, and adjusted for birth weight Z-score < −1 (fourth column). **Bolded (non-italicized) odds ratios and confidence intervals** indicate statistically significant increased risk (p <0.05). ***Bolded italicized odds ratios and confidence intervals*** indicate statistically significant reduced risk (p <0.05).

Protein	Day 21	Day 28	Early*	Late**	Late in light of early§
CRP	1.1 (0.6, 1.7)	1,4 (0.8, 2.2)	1.7 (0.98, 2.9)	1.2 (0.6, 2.4)	1.0 (0.5, 2.2)
SAA	0.9 (0.5, 1.4)	**1.6 (1.01, 2.6)**	1.2 (0.6, 2.2)	1.1 (0.6, 2.5)	1.1 (0.5, 2.4)
MPO	0.9 (0.5, 1.6)	0.7 (0.4, 1.2)	1.2 (0.6, 2.2)	0.7 (0.3, 1.5)	0.6 (0.3, 1.4)
IL-1β	0.8 (0.5, 1.4)	1.1 (0.7, 1.9)	1.4 (0.7, 2.5)	0.8 (0.4, 1.8)	0.8 (0.4, 1.7)
IL-6	1.4 (0.9, 2.3)	1.0 (0.6, 1.8)	**1.8 (1.02, 3.1)**	1.1 (0.5, 2.2)	0.9 (0.5, 2.0)
IL-6R	***0.5 (0.3, 0.9)***	0.8 (0.5, 1.4)	0.8 (0.4, 1.5)	0.6 (0.2, 1.3)	0.6 (0.2, 1.4)
TNF-α	1.4 (0.9, 2.3)	1.5 (0.9, 2.4)	**3.1 (1.9, 5.2)**	1.3 (0.7, 2.3)	0.9 (0.5, 1.7)
TNF-R2	0.6 (0.4, 1.1)	1.1 (0.6, 1.8)	1.5 (0.9, 2.7)	0.7 (0.3, 1.5)	0.6 (0.3, 1.4)
IL-8	1.1 (0.7, 1.9)	1.2 (0.7, 2.0)	**2.4 (1.4, 4.1)**	1.2 (0.6, 2.4)	0.9 (0.5, 1.9)
RANTES	0.7 (0.4, 1.2)	1.1 (0.6, 1.8)	0.6 (0.3, 1.3)	0.6 (0.3, 1.3)	0.7 (0.3, 1.6)
ICAM-1	0.9 (0.6, 1.6)	1.1 (0.7, 1.9)	1.3 (0.7, 2.3)	0.8 (0.4, 1.6)	0.7 (0.4, 1.5)
MMP-9	***0.5 (0.3, 0.96)***	***0.3 (0.2, 0.7)***	1.0 (0.5, 1.9)	***0.1 (0.02, 0.8)***	***0.1 (0.02, 0.8)***
VEGF	0.8 (0.5, 1.4)	0.6 (0.4, 1.1)	0.9 (0.5, 1.8)	0.6 (0.3, 1.5)	0.6 (0.3, 1.5)
VEGF-R2	0.9 (0.5, 1.5)	1.0 (0.6, 1.7)	1.1 (0.6, 2.0)	1.3 (0.7, 2.5)	1.3 (0.6, 2.5)
TSH	1.4 (0.8, 2.2)	1.4 (0.8, 2.3)	1.1 (0.6, 2.1)	1.6 (0.8, 3.1)	1.6 (0.8, 3.2)
EPO	1.3 (0.8, 2.0)	1.3 (0.8, 2.1)	**1.8 (1.03, 3.0)**	1.4 (0.7, 2.7)	1.3 (0.7, 2.5)
Max N	938	878	754	754	754

*Early: protein in the highest quartile on two or more of the first three days (days 1, 7, and 14)

**Late: protein in the highest quartile on both of the last two days (days 21 and 28)

§Information added by the late concentrations after adjusting for the early concentrations

Table 3

Odds ratios (95% Confidence Intervals) of a **2-year head circumference Z-score < −2** associated with concentrations in the top quartile on days 21 and 28, both days without regard to risk information provided by top quartile concentrations before day 21 (third column), and associated with concentrations in the top quartile on both the 21st and 28th postnatal days in light of associations with elevated concentrations of this protein on multiple occasions earlier, and adjusted for birth weight Z-score < −1 (fourth column).

Protein	Day 21	Day 28	Early*	Late**	Late in light of early§
CRP	**2.2 (1.4, 3.6)**	1.6 (0.9, 2.6)	1.4 (0.8, 2.4)	**3.3 (1.8, 6.1)**	**3.2 (1.7, 5.9)**
SAA	**1.7 (1.03, 2.7)**	1.0 (0.6, 1.7)	**1.8 (1.01, 3.2)**	1.7 (0.8, 3.5)	1.5 (0.7, 3.3)
MPO	0.7 (0.4, 1.3)	1.2 (0.7, 2.1)	**1.8 (1.01, 3.2)**	1.2 (0.6, 2.5)	1.2 (0.6, 2.4)
IL-1β	1.5 (0.9, 2.5)	1.4 (0.8, 2.3)	1.4 (0.8, 2.6)	**2.0 (1.03, 3.8)**	1.9 (0.97, 3.6)
IL-6	1.5 (0.9, 2.5)	**1.7 (1.01, 2.8)**	**1.8 (1.04, 3.3)**	**2.1 (1.1, 4.0)**	1.9 (0.96, 3.7)
IL-6R	1.0 (0.6, 1.7)	**1.7 (1.01, 2.8)**	1.6 (0.9, 2.8)	**2.0 (1.1, 3.8)**	1.9 (0.99, 3.5)
TNF-α	1.2 (0.7, 2.0)	1.2 (0.7, 2.0)	**1.9 (1.1, 3.3)**	1.1 (0.6, 2.0)	0.9 (0.5, 1.8)
TNF-R2	**2.1 (1.3, 3.4)**	**2.2 (1.3, 3.5)**	**2.0 (1.1, 3.4)**	**2.6 (1.4, 4.8)**	**2.4 (1.3, 4.5)**
IL-8	**1.9 (1.1, 3.1)**	**1.9 (1.2, 3.2)**	**2.2 (1.3, 3.8)**	**2.3 (1.3, 4.2)**	**2.0 (1.1, 3.6)**
RANTES	*0.6 (0.3, 1.01)*	0.7 (0.4, 1.2)	0.7 (0.4, 1.4)	***0.1 (0.01, 0.7)***	***0.1 (0.01, 0.7)***
ICAM-1	**2.5 (1.6, 4.0)**	**2.6 (1.6, 4.3)**	**2.2 (1.3, 3.8)**	**2.9 (1.7, 5.1)**	**2.6 (1.5, 4.5)**
MMP-9	0.9 (0.5, 1.6)	1.1 (0.7, 1.9)	1.5 (0.8, 2.9)	1.2 (0.6, 2.6)	1.2 (0.5, 2.5)
VEGF	0.6 (0.3, 1.1)	0.6 (0.3, 1.1)	1.0 (0.5, 1.9)	0.6 (0.2, 1.4)	0.6 (0.2, 1.4)
VEGF-R2	1.0 (0.6, 1.7)	**1.8 (1.1, 3.0)**	0.9 (0.5, 1.7)	1.5 (0.8, 2.8)	1.5 (0.8, 2.9)
TSH	1.1 (0.7, 1.8)	1.6 (0.9, 2.6)	1.3 (0.7, 2.4)	**2.0 (1.1, 3.7)**	**1.9 (1.01, 3.6)**
EPO	1.1 (0.6, 1.8)	1.0 (0.6, 1.8)	1.6 (0.9, 2.9)	1.5 (0.8, 2.9)	1.4 (0.7, 2.8)
Max N	771	723	620	620	620

*Early: protein in the highest quartile on two or more of the first three days (days 1, 7, and 14)

**Late: protein in the highest quartile on both of the last two days (days 21 and 28)

§Information added by the late concentrations after adjusting for the early concentrations

Table 4a

Odds ratios (95% Confidence Intervals) of an **MDI < 55** associated with concentrations in the top quartile on days 21 and 28, both days (without regard to risk information provided by top quartile concentrations before day 21) (third column), and associated with concentrations in the top quartile on both the 21st and 28th postnatal days in light of associations with elevated concentrations of this protein on multiple occasions earlier, and adjusted for birth weight Z-score < −1 (fourth column). ORs are from a multinomial logistic regression.

Protein	Day 21	Day 28	Early*	Late**	Late in light of early§
CRP	**1.7 (1.1, 2.6)**	**2.1 (1.3, 3.3)**	**2.3 (1.4, 3.7)**	**3.4 (1.8, 6.1)**	**3.1 (1.7, 5.7)**
SAA	1.1 (0.7, 1.8)	**1.8 (1.1, 2.8)**	**2.7 (1.6, 4.6)**	**2.2 (1.1, 4.3)**	**2.0 (1.01, 3.9)**
MPO	0.8 (0.5, 1.3)	0.8 (0.5, 1.3)	1.1 (0.6, 2.0)	0.7 (0.4, 1.5)	0.7 (0.3, 1.5)
IL-1β	1.3 (0.8, 2.0)	1.4 (0.9, 2.3)	1.6 (0.9, 2.8)	1.2 (0.6, 2.3)	1.1 (0.6, 2.1)
IL-6	**1.7 (1.1, 2.6)**	**1.9 (1.2, 2.9)**	**2.2 (1.3, 2.8)**	**2.8 (1.5, 5.1)**	**2.4 (1.3, 4.6)**
IL-6R	0.8 (0.5, 1.3)	1.0 (0.6,1.6)	1.1 (0.6, 1.9)	0.7 (0.4, 1.4)	0.7 (0.3, 1.3)
TNF-α	**1.7 (1.1, 2.6)**	1.3 (0.8, 2.1)	**1.8 (1.1, 3.1)**	1.6 (0.9, 2.8)	1.5 (0.9, 2.7)
TNF-R2	1.5 (0.98, 2.4)	**2.4 (1.5, 3.7)**	1.4 (0.8, 2.4)	**2.2 (1.2, 4.0)**	**2.1 (1.2, 3.9)**
IL-8	**2.4 (1.6, 3.8)**	**3.0 (1.9, 4.7)**	**2.4 (1.4, 4.1)**	**3.0 (1.7, 5.2)**	**2.6 (1.5, 4.6)**
RANTES	0.9 (0.6, 1.4)	0.7 (0.4, 1.2)	1.4 (0.8, 2.3)	0.8 (0.4, 1.7)	0.8 (0.4, 1.6)
ICAM-1	**1.9 (1.2, 3.0)**	**2.0 (1.3, 3.2)**	**2.4 (1.4, 3.9)**	**2.2 (1.3, 3.8)**	**1.9 (1.1, 3.3)**
MMP-9	0.9 (0.6, 1.5)	0.8 (0.5, 1.3)	0.8 (0.4, 1.5)	0.7 (0.3, 1.5)	0.7 (0.3, 1.6)
VEGF	0.9 (0.6, 1.5)	0.9 (0.5, 1.4)	0.8 (0.5, 1.5)	0.8 (0.4, 1.5)	0.8 (0.4, 1.6)
VEGF-R2	1.2 (0.7, 1.9)	**1.6 (1.02, 2.5)**	1.2 (0.7, 2.0)	1.0 (0.5, 1.8)	0.9 (0.5, 1.8)
TSH	1.1 (0.7, 1.7)	1.2 (0.8, 1.9)	1.1 (0.7, 1.9)	1.2 (0.6, 2.2)	1.1 (0.6, 2.2)
EPO	0.8 (0.5, 1.4)	1.1 (0.7, 1.8)	**1.8 (1.1, 3.0)**	1.2 (0.6, 2.3)	1.1 (0.6, 2.2)
Max N	749	697	599	599	599

*Early: protein in the highest quartile on two or more of the first three days (days 1, 7, and 14)

**Late: protein in the highest quartile on both of the last two days (days 21 and 28)

§Information added by the late concentrations after adjusting for the early concentrations

Table 4b

Odds ratios (95% Confidence Intervals) of an **MDI 55–69** associated associated with concentrations in the top quartile on days 21 and 28, both days (without regard to risk information provided by top quartile concentrations before day 21) (third column), and associated with concentrations in the top quartile on both the 21st and 28th postnatal days in light of associations with elevated concentrations of this protein on multiple occasions earlier, and adjusted for birth weight Z-score < −1 (fourth column). ORs are from a multinomial logistic regression.

Protein	Day 21	Day 28	Early*	Late**	Late in light of early§
CRP	1.6 (0.99, 2.6)	**1.9 (1.2, 3.2)**	1.4 (0.8, 2.5)	**2.5 (1.3, 5.1)**	**2.5 (1.2, 5.)**
SAA	1.3 (0.8, 2.0)	**1.7 (1.01, 2.8)**	**1.9 (1.05, 3.6)**	2.1 (1.00, 4.3)	1.9 (0.9, 4.1)
MPO	0.9 (0.6, 1.6)	1.0 (0.6, 1.7)	0.8 (0.4, 1.7)	0.6 (0.3, 1.5)	0.7 (0.3, 1.5)
IL-1β	1.3 (0.8, 2.1)	1.4 (0.8, 2.4)	0.9 (0.4, 1.8)	0.9 (0.5, 2.0)	0.9 (0.4, 2.0)
IL-6	**1.8 (1.1, 2.9)**	1.5 (0.9, 2.5)	1.0 (0.5, 2.0)	**2.2 (1.1, 4.4)**	**2.2 (1.1, 4.5)**
IL-6R	1.0 (0.6, 1.6)	0.6 (0.4, 1.2)	0.7 (0.4, 1.4)	***0.4 (0.2, 0.997)***	*0.4 (0.2, 1.04*
TNF-α	**1.7 (1.01, 2.7)**	**2.2 (1.3, 3.6)**	1.5 (0.8, 2.7)	**2.0 (1.1, 3.6)**	**1.9 (1.1, 3.5)**
TNF-R2	1.5 (0.9, 2.5)	1.4 (0.8, 2.4)	**2.1 (1.2, 3.6)**	1.5 (0.7, 3.0)	1.3 (0.6, 2.6)
IL-8	**2.2 (1.4, 3.6)**	1.7 (1.00, 2.8)	**1.9 (1.04, 3.5)**	**2.4 (1.3, 4.5)**	**2.2 (1.1, 4.1)**
RANTES	0.8 (0.4, 1.3)	0.8 (0.5, 1.4)	0.6 (0.3, 1.2)	0.6 (0.3, 1.5)	0.7 (0.3, 1.6)
ICAM-1	**2.0 (1.2, 3.2)**	1.1 (0.6, 1.9)	1.3 (0.7, 2.4)	1.2 (0.6, 2.3)	1.1 (0.6, 2.3)
MMP-9	1.0 (0.6, 1.6)	0.8 (0.4, 1.2)	1.1 (0.5, 2.1)	0.7 (0.3, 1.7)	0.7 (0.3, 1.6)
VEGF	0.9 (0.6, 1.6)	0.9 (0.5, 1.6)	0.6 (0.3, 1.2)	0.6 (0.3, 1.4)	0.7 (0.3, 1.5)
VEGF-R2	**2.0 (1.3, 3.3)**	1.5 (0.9, 2.4)	1.1 (0.6, 1.9)	1.4 (0.7, 2.7)	1.4 (0.7, 2.7)
TSH	1.1 (0.6, 1.8)	1.0 (0.6, 1.7)	0.7 (0.4, 1.4)	1.1 (0.5, 2.2)	1.2 (0.5, 2.5)
EPO	1.5 (0.9, 2.4)	1,2 (0.7, 2.0)	1.2 (0.7, 2.3)	1.7 (0.9, 3.4)	1.7 (0.9, 3.4)
Max N	749	697	599	599	599

*Early: protein in the highest quartile on two or more of the first three days (days 1, 7, and 14)

**Late: protein in the highest quartile on both of the last two days (days 21 and 28)

§Information added by the late concentrations after adjusting for the early concentrations

Table 5a

Odds ratios (95% Confidence Intervals) of an **PDI < 55** or a PDI 55–69 associated with concentrations in the top quartile on days 21 and 28, both days (without regard to risk information provided by top quartile concentrations before day 21) (third column), and associated with concentrations in the top quartile on both the 21st and 28th postnatal days in light of associations with elevated concentrations of this protein on multiple occasions earlier, and adjusted for birth weight Z-score < −1 (fourth column). ORs are from a multinomial logistic regression.

Protein	Day 21	Day 28	Early*	Late**	Late in light of early§
CRP	1.4 (0.9, 2.2)	1.4 (0.8, 2.2)	1.2 (0.7, 2.0)	**2.1 (1.1, 4.0)**	**1.2 (1.1, 4.0)**
SAA	0.9 (0.5, 1.4)	1.3 (0.9, 2.0)	1.3 (0.7, 2.4)	1.2 (0.6, 2.6)	1.2 (0.6, 2.5)
MPO	1.0 (0.6, 1.6)	1.6 (0.99, 2.5)	1.2 (0.7, 2.2)	0.9 (0.5, 1.9)	0.9 (0.5, 1.9)
IL-1β	1.0 (0.7, 1.7)	1.3 (0.8, 2.0)	1.1 (0.6, 2.0)	1.0 (0.5, 2.0)	1.0 (0.5, 2.0)
IL-6	1.4 (0.9, 2.3)	**1.7 (1.1, 2.7)**	1.7 (0.98, 3.0)	1.6 (0.8, 3.2)	1.5 (0.8, 3.0)
IL-6R	0.9 (0.6, 1.5)	1.5 (0.9, 2.4)	1.0 (0.6, 1.8)	1.5 (0.9, 2.8)	1,5 (0.8, 2.8)
TNF-α	1.0 (0.6, 1.6)	0.8 (0.5, 1.4)	1.2 (0.7, 2.1)	***0.5 (0.2, 0.98)***	***0.4 (0.2, 0.96)***
TNF-R2	1.4 (0.9, 2.2)	**1.8 (1.2, 2.9)**	1.1 (0.6, 1.9)	1.6 (0.8, 2.9)	1.6 (0.8, 2.9)
IL-8	1.5 (0.9, 2.4)	**2.3 (1.5, 3.6)**	1.0 (0.6, 1.9)	**2.1 (1.2, 3.8)**	**2.1 (1.2, 3.9)**
RANTES	0.8 (0.5, 1.3)	0.9 (0.5, 1.5)	0.9 (0.4, 1.6)	1.0 (0.5, 2.0)	1.0 (0.5, 2.1)
ICAM-1	**1.9 (1.2, 3.0)**	**2.9 (1.9, 4.7)**	1.5 (0.9, 2.6)	**2.9 (1.7, 5.1)**	**2.8 (1.6, 4.9)**
MMP-9	1.1 (0.7, 1.7)	0.8 (0.5, 1.3)	0.8 (0.4, 1.5)	0.7 (0.3, 1.7)	0.8 (0.3, 1.7)
VEGF	0.7 (0.4, 1.2)	0.8 (0.4, 1.3)	0.5 (0.3, 1.04)	0.8 (0.4, 1.7)	0.9 (0.4, 1.9)
VEGF-R2	0.9 (0.6, 1.5)	1.4 (0.9, 2.2)	0.7 (0.4, 1.3)	0.9 (0.5, 1.8)	1.0 (0.5, 1.9)
TSH	1.1 (0.7, 1.8)	1.3 (0.8, 2.1)	1.4 (0.8, 2.4)	1.7 (0.9, 3.2)	1.6 (0.9, 3.0)
EPO	0.7 (0.4, 1.2)	1.1 (0.6, 1.8)	**1.8 (1.05, 3.1)**	0.8 (0.4, 1.8)	0.8 (0.4, 1.7)
Max N	749	697	599	599	599

*Early: protein in the highest quartile on two or more of the first three days (days 1, 7, and 14)

**Late: protein in the highest quartile on both of the last two days (days 21 and 28)

§Information added by the late concentrations after adjusting for the early concentrations

Table 5b

Odds ratios (95% Confidence Intervals) of a **PDI 55–69** associated with with concentrations in the top quartile on days 21 and 28, both days (without regard to risk information provided by top quartile concentrations before day 21) (third column), and associated with concentrations in the top quartile on both the 21st and 28th postnatal days in light of associations with elevated concentrations of this protein on multiple occasions earlier, and adjusted for birth weight Z-score < −1 (fourth column). ORs are from a multinomial logistic regression.

Protein	Day 21	Day 28	Early*	Late**	Late in light of early§
CRP	**1.8 (1.2, 2.8)**	1.5 (0.96, 2.4)	**1.8 (1.1, 2.9)**	**2.3 (1.2, 4.4)**	**2.2 (1.1, 4.1)**
SAA	1.5 (0.96, 2.3)	1.0 (0.6, 1.6)	**2.2 (1.3, 3.8)**	1.4 (0.7, 2.8)	1.3 (0.6, 2.6)
MPO	1.2 (0.7, 1.9)	1.4 (0.9, 2.2)	1.2 (0.7, 2.1)	1.1 (0.6, 2.1)	1.1 (0.6, 2.1)
IL-1β	1.1 (0.7, 1.7)	1.2 (0.8, 2.0)	1.6 (0.9, 2.8)	1.0 (0.5, 1.9)	0.9 (0.4, 1.8)
IL-6	1.2 (0.8, 2.0)	1.0 (0.6, 1.6)	1.6 (0.9, 2.7)	1.3 (0.7, 2.3)	1.2 (0.6, 2.5)
IL-6R	1.3 (0.8, 2.0)	1.5 (0.9, 2.3)	1.1 (0.6, 1.8)	1.2 (0.7, 2.3)	1.2 (0.6, 2.3)
TNF-α	**1.8 (1.1, 2.8)**	**1.6 (1.1, 2.6)**	1.4 (0.9, 2.3)	**1.9 (1.1, 3.1)**	**1.8 (1.1, 3.1)**
TNF-R2	**1.6 (1.01, 2.5)**	0.9 (0.6, 1.5)	1.2 (0.7, 2.0)	1.0 (0.5, 1.9)	0.9 (0.5, 1.9)
IL-8	**2.0 (1.3, 3.1)**	1.5 (0.9, 2.4)	1.2 (0.7, 2.0)	**2.0 (1.1, 3.6)**	**2.0 (1.1, 3.6)**
RANTES	1.1 (0.7, 1.8)	1.0 (0.6, 1.6)	0.6 (0.3, 1.2)	0.9 (0.4, 1.8)	1.0 (0.5, 2.0)
ICAM-1	**1.9 (1.2, 2.9)**	**1.7 (1.04, 2.7)**	1.4 (0.8, 2.4)	**2.0 (1.2, 3.6)**	**1.9 (1.1, 3.5)**
MMP-9	1.0 (0.6, 1.6)	1.1 (0.7, 1.7)	1.0 (0.6, 1.9)	0.9 (0.4, 1.8)	0.8 (0.4, 1.8)
VEGF	0.9 (0.5, 1.4)	1.3 (0.8, 2.1)	0.8 (0.4, 1.4)	1.2 (0.6, 2.2)	1.3 (0.7, 2.3)
VEGF-R2	**1.6 (1.01, 2.4)**	1.4 (0.9, 2.2)	1.4 (0.9, 2.3)	1.4 (0.8, 2.5)	1.4 (0.9, 2.4)
TSH	1.0 (0.6, 1.5)	1.0 (0.6, 1.6)	0.9 (0.5, 1.6)	0.8 (0.4, 1.7)	0.9 (0.4, 1.8)
EPO	1.3 (0.8, 2.0)	1.5 (0.9, 2.3)	1.4 (0.8, 2.5)	1.4 (0.7, 2.6)	1.3 (0.7, 2.5)
Max N	749	697	599	599	599

*Early: protein in the highest quartile on two or more of the first three days (days 1, 7, and 14)

**Late: protein in the highest quartile on both of the last two days (days 21 and 28)

§Information added by the late concentrations after adjusting for the early concentrations