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The breadth and type of systemic inflammation and the risk of adverse neurological outcomes in extremely low gestational age newborns

Karl C. K. Kuban, MD^a, Thomas M. O'Shea, MD^b, Elizabeth N. Allred, MS^{c,d,e}, Raina N. Fichorova, MD, PhD^{c,f}, Tim Heeren, PhD^g, Nigel Paneth, MD^h, Deborah Hirtz, MDⁱ, Olaf Dammann, MD^j, and Alan Leviton, MD^{c,d} for the ELGAN Study Investigators

^aDepartment of Pediatrics, Boston Medical Center, Boston, MA, USA

^bWake Forest School of Medicine, Winston-Salem, NC, USA

^cHarvard Medical School, Boston, MA, USA

^dBoston Children's Hospital, Boston, MA, USA

^eHarvard School of Public Health, Boston, MA, USA

^fDepartment of Obstetrics, Gynecology & Reproductive Biology, Brigham and Women's Hospital, Boston, MA, USA

^gDepartment of Biostatistics, Boston University School of Public Health, Boston, MA, USA

^hDepartment of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA

ⁱNational Institute of Neurological Disorders and Stroke, Bethesda, MD, USA

^jDepartment of Public Health and Community Medicine, Tufts University School of Medicine, Boston MA, USA

Abstract

We hypothesized that the risk of brain damage in extremely preterm neonates increases with the breadth and type of systemic inflammation, indexed by the number of elevated inflammation-related proteins and the number of functional categories of inflammation-related proteins exhibiting an elevated concentration.

Methods—In blood from 881 infants born before 28 weeks gestation, we measured the concentrations of 25 inflammation-related proteins, representing 6 functional categories (cytokines, chemokines, growth factors, adhesion molecules, metalloproteinases, and liver-produced acute phase reactant proteins) on postnatal days 1, 7, and 14 and evaluated associations between the number and type of proteins whose concentrations were elevated on two separate occasions a week apart and the diagnoses of ventriculomegaly as a neonate, and, at 2 years,

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microcephaly, impaired early cognitive functioning, cerebral palsy, and autism risk as assessed with the Modified Checklist for Autism in Toddlers (M-CHAT) screen, and, in a subset of these children from 12 of 14 sites (n= 826), an attention problem identified with the Child Behavior Checklist (CBCL).

Results—The risk of abnormal brain structure and function overall was increased among children who had recurrent/persistent elevations of the 25 protein. The risk for most outcomes did not rise until at least four proteins in at least two functional categories were elevated. When we focused our analysis on 10 proteins previously found to be associated consistently with neurological outcomes, we found the risk of low Mental Development Index (MDI) on the Bayley Scales of Infant Development-II, microcephaly, and a CBCL-defined attention problem increased with higher numbers of these recurrently/persistently elevated proteins.

Interpretation—Increasing breadth of early neonatal inflammation, indexed by the number of protein elevations or the number of protein functional classes elevated, is associated with increasing risk of disorders of brain structure and function among infants born extremely premature.

Introduction

Inflammation is an antecedent of brain damage in very preterm newborns. Persistent/recurrent elevations of inflammation-related proteins in the blood of neonates in the first two postnatal weeks^{1,2} predict diffuse white matter damage in the first months of life,³ and, at 2 years of age, microcephaly,⁴ very low Mental Development Index on the Bayley Scales of Infant Development-II,¹ attention problem identified with the Child Behavior Checklist,⁵ and cerebral palsy.²

Previously, we showed that the inflammation signal was associated with adverse neurological findings based on the strength of the protein signal (whether the concentration of inflammation-related protein was in the highest quartile) and the duration of the signal (whether the elevation was present on more than one measurement over the course of at least one week).^{1-4,6} If inflammation is associated with increased risk of perinatal brain damage, then the risk might also increase in proportion to the breadth of inflammation, defined by the number of elevated blood levels of inflammation-related proteins and the number of functional categories represented by these proteins. In this study, we evaluated the extent to which the breadth of neonatal systemic inflammation and perinatal brain damage are related in a dose-response or threshold manner.

Methods

The ELGAN study was designed to identify characteristics and exposures that increase the risk of structural and functional neurologic disorders in ELGANs (the acronym for Extremely Low Gestational Age Newborns).⁷ During the years 2002–2004, women delivering before 28 weeks gestation at any of 14 participating institutions were asked to enroll in the study. At each site, enrollment and consent processes were approved by the institutional review board.

Mothers were approached for consent either upon antenatal admission or shortly after delivery, depending on clinical circumstance and institutional preference. 1249 mothers of 1506 infants consented. Approximately 260 women were either missed or did not consent to participate.

Of the 1200 ELGANs who survived to age 2 years, 1102 (92%) returned for follow up. While 939 (85%) of those who were followed had one or more protein measures, only 881 (80%) of the 1102 had protein measures on 2 days in the first two postnatal weeks and these children are the subjects of most parts of this report. Because not all children had an Modified Checklist for Autism in Toddlers (M-CHAT) or a Child Behavior Check List (CBCL), the samples for these assessments are smaller (M-CHAT, N=786; CBCL attention problems, N=826). Among children who survived to 2-years, those who had protein measures on 2 days did not differ from those who did not in mothers' marital or insurance status or in their own gestational age at birth, birth weight, birth weight Z-score or sex.

Gestational age estimates were based on a hierarchy of the quality of available information. Most desirable were estimates based on the dates of embryo retrieval or intrauterine insemination or fetal ultrasound before the 14th week (62%). When these were not available, reliance was placed sequentially on a fetal ultrasound at 14 or more weeks (29%), last menstrual period (7%), and gestational age recorded in the log of the neonatal intensive care unit (1%).

24-month neurologic assessment

Procedures to standardize the neurological examination and minimize examiner variability are presented elsewhere.⁸ The topographic diagnosis of cerebral palsy (quadripareisis, diparesis, or hemiparesis) was based on an algorithm using these data.⁹

24-month developmental assessment

Fully 85% of the 1200 surviving children returned for a developmental and neurological assessment, including head circumference measurement, at about 24-months corrected age. Of these children, 88% had their exam within the range of 23–28 months. Nine percent were examined between 16 and 23 months and 3% were examined between 28 and 44 months.

Certified examiners administered and scored the Bayley Scales of Infant Development-II,¹⁰ which yields a mental scale and a motor scale. For both scales, the standard deviation is 15; thus scores less than 55 are more than 3 standard deviations below the mean.

At the time of the 24-month developmental assessment, the parent or other caregiver completed the CBCL for Ages 1 ½–5 (CBCL/1½–5), a 99-item survey of child behavior problems.¹¹ The scoring system identifies attention problems according to each child's percentile based on a normative sample. Children were considered to have an attention problem if their T score was at or above the 93rd percentile.⁵

The M-CHAT asks the parent or other caregiver to report on 23 behaviors. A child screened positive if 2 of 6 “critical” items (Items 2, 7, 9, 13, 14, 15) or three of any of the 23 total items were abnormal. The M-CHAT results obtained from children with severe motor,

visual or hearing disability, the presence of which may independently lead to a positive screen, were excluded from this analysis.^{1-4,12}

Blood Protein measurements

Drops of whole blood were collected on (Schleicher & Schuell 903) filter paper on the first postnatal day (range: 1–3 days), the 7th postnatal day (range: 5–8 days), and the 14th postnatal day (range: 12–15 days). Twenty-five proteins were measured in the Laboratory of Genital Tract Biology, Brigham and Women's Hospital, using the Meso Scale Discovery multiplex platform and Sector Imager 2400 (Meso Scale Discovery, Gaithersburg, MD), which has been validated against ELISA. Details about the procedure for processing the blood spots and for measuring protein concentrations and absolute value ranges for proteins are explained elsewhere.^{1, 2,13}

We measured the following 25 inflammation-regulating proteins, categorized as cytokines: Interleukin (IL)-1, IL-6 and its receptor (IL-6R), tumor necrosis factor (TNF)-alpha and its receptors (TNF-alpha R1,2); adhesion molecules: intercellular adhesion molecule (ICAM)-1, ICAM-3, vascular cell adhesion molecule (VCAM-1), and E-selectin (E-SEL); growth factors: vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1,2), and insulin-like growth factor binding protein (IGFBP)-1; chemokines: IL-8, monocyte chemotactic protein (MCP)-1, monocyte chemoattractant protein-4 (MCP-4), macrophage inflammatory protein (MIP)-1beta, regulated upon activation, normal T-cell expressed, and (presumably) secreted (RANTES), and interferon-inducible T cell alpha-chemoattractant (I-TAC); metalloproteinases: matrix metalloproteinase (MMP)-1, MMP-9; liver-produced: serum amyloid A (SAA), C-reactive protein (CRP); and neutrophil activation: myeloperoxidase (MPO). We also examined a subset of 10 proteins (CRP, SAA, IL-1 β , IL-6, TNF- α , IL-8, ICAM-1, E-SEL, MMP-9, VEGF) that we have found to be associated consistently with structural and functional neurological outcomes.^{1,2,4,12}

Statistics

We evaluated two null hypotheses. The first postulates that the risks of indicators of perinatal brain damage do not vary with the number of persistently/recurrently elevated inflammation-related proteins in the blood. One set of analyses of the relationship between number of protein elevations and risk of adverse outcomes was based on data on all 25 proteins. Another set of analyses used data only for the subset of 10 proteins associated with structural and functional neurological outcomes.^{1,2,4,12} The second hypothesis postulates that the risks of indicators of brain damage do not vary with specific categories or the number of categories of inflammation-related proteins that are elevated in the blood.

We began by creating logistic regression models to quantify the risk of developmental disorders associated with different numbers of elevated inflammation-related proteins, first considering all 25 proteins (Table 1) and then considering the restricted subset of 10 proteins (Table 2). These models allowed us to calculate odds ratios and their 95% confidence intervals. The model for cerebral palsy subtypes is multinomial.

Subsequently, we evaluated the risk of adverse outcomes as a function of the number of each category of protein function (e.g., cytokines, chemokines, growth factors, adhesion

molecules, metalloproteinases, liver-produced acute phase reactants, which had one or more protein concentration in the highest quartile on 2 or more days (Table 3) as well as a function of the number of categories of protein function (Table 4). In all models, we adjusted for gestational age (23–24, 25–26, 27 weeks). In the microcephaly model we adjusted, additionally, for birth head circumference Z-score < -1 .

Results

Risk of developmental disorders associated with the number of proteins whose concentrations were repeatedly elevated

When we considered all 25 proteins, the risks of a CBCL-defined attention problem and microcephaly were increased when the concentrations of as few as 4–5 proteins were elevated on multiple occasions (Table 1), but the risk did not continue to increase with higher numbers of recurrently/persistently elevated proteins for microcephaly. The risks of neonatal ventriculomegaly and an MDI < 55 at age 2 years were increased when the concentrations of at least 6 proteins were elevated on multiple occasions. These risks did not increase further if the concentrations of nine or more proteins were recurrently/persistently elevated.

Analyses restricted to 10 proteins for which we previously have found associations with adverse outcomes^{1,2,4,12} suggested a dose-response relationship between the number of proteins whose concentrations were recurrently/persistently elevated and microcephaly at age 2 years and a CBCL-defined attention problem (Table 2). The increased risks of neonatal ventriculomegaly, diparetic and hemiparetic forms of cerebral palsy, and MDI scores 3 or more SD below the mean, were associated with repeatedly/elevated concentrations of at least 5 proteins. No dose-response relationship was seen with lower numbers of repeatedly elevated proteins.

Categories of proteins that exhibited persistently/repeatedly elevated concentrations and risk of adverse outcomes

The risk of ventriculomegaly and an MDI < 55 was significantly increased if a cytokine, chemokine, or liver-produced (SAA/CRP) protein had a concentration in the top quartile on two separate occasions (Table 3). The risk of an MDI < 55 was significantly increased if a chemokine, adhesion molecule, or liver-produced protein had a concentration in the top quartile on two separate days. The risk of an attention problem was increased if the protein whose concentration was recurrently/persistently elevated was a cytokine, chemokine or adhesion molecule.

The risk of an MDI < 55 was significantly increased when three or more of the protein-function categories had recurrently/persistently elevated concentrations, whereas the risk of ventriculomegaly required recurrently/persistently elevated concentrations of a minimum of 4 categories (Table 4).

Discussion

Previously, we characterized the inflammation signal associated with adverse neurological findings based on the strength of the protein signal (whether the concentration of inflammation-related protein was in the highest quartile) and the duration of the signal (whether the elevation was present on more than one measurement over the course of at least one week).^{1-4,6} In this report, we found that 5 or more elevated protein concentrations identified on multiple days a week or more apart predicted neonatal ventriculomegaly, and, at 2 years, very low Mental Development Index, microcephaly, hemiparetic and diparetic forms of cerebral palsy, and a CBCL-identified attention problem. These observations suggested the presence of a threshold effect of risk. In analyses based on 10 proteins that we have found to be associated consistently with adverse brain outcomes, however, the likelihood of low MDI, microcephaly, and an attention problem were lowest when there was an elevation of 0 or 1 protein, intermediate when 2 to 4 proteins were elevated, and highest when 5 or more proteins were elevated, suggesting a possible dose-response effect.

The lack of a dose-response association of inflammation with risk of brain damage when all 25 proteins were considered probably reflects the tendency for many proteins involved in various aspects of the inflammation process to vary together.^{14,15} In addition, a strong initial inflammatory insult initiates a cascade of events in which some mediators, such as IL-8, E-SEL and ICAM-1, occur downstream from others, including IL-1 β and TNF- α , and consequently peak later¹⁶. This induction of multiple components of the inflammatory systems, is an example of a non-linear system in which changes in components of the inflammatory system alter relationships with other components.¹⁷ In keeping with the concept of non-linearity, the inflammatory response tends to be complex and involve cytokines, chemokines, integrins, metalloproteinases and adhesion molecules that contribute to cell trafficking, debris removal, and repair,¹⁸⁻²⁰ and growth factors and other protein classes that modulate inflammation-related proteins.²¹ Indeed, an inflammatory stimulus given to rodents influences the expression of thousands of genes,²² while severe trauma can result in what has been called a “genomic storm” that includes many inflammation components.²³

In light of these findings we would expect the concentrations of most inflammation-related proteins to vary with one another. That is what we found in this sample of very preterm newborns.¹⁵ Elevated concentrations of 17 of the 25 proteins (including cytokines, chemokines, adhesion molecules, matrix metalloproteinases, a growth factor, its receptors, and a growth factor’s binding protein) were each associated with elevated concentrations of 15 or more other proteins.

Because of the complexity and interrelatedness of inflammation components, which may obscure subtleties of dose-response relationships, we attempted to parse out signal from background inflammation noise, and found some support for a dose-response pattern. When the analysis was restricted to the 10 proteins we previously identified as associated with low MDI, cerebral palsy, microcephaly, and an attention problem,^{1,2,4,12} the likelihood of adverse neurological outcomes was lowest when there was an elevation of 0 or 1 protein, intermediate when 2 to 4 proteins were elevated, and highest when 5 or more proteins were

elevated. These observations suggest a dose-response pattern. It may be that there is a dose-response contribution to inciting an inflammatory response that, once initiated, amplifies to involve many proteins and systems.

The involvement of a broad number of persistently elevated concentrations of inflammation-related proteins and protein categories further supports the notion that the inflammatory process is broad. Perinatal inflammation stimulates toll-like receptors and microglial activity, promotes apoptosis, and prompts oxidative and excitotoxic processes, all associated with brain injury.^{24–30} Inflammation also has the capacity to incite epigenetic changes³¹, some of which have been associated with adverse neurological outcomes of the type seen in the ELGAN cohort, including altered brain development,^{32–35} behavior and psychiatric disorders,^{36,37} autism,^{38,39} cerebral palsy,⁴⁰ diffuse white and gray matter injury,⁴¹ as well as impaired brain repair capacity.^{42,43}

Inflammation-related proteins were not associated with M-CHAT positivity. As a screen for autism at 2 years, the M-CHAT has low sensitivity and imprecise specificity.⁴⁴ The resulting non-differential misclassification can be expected to bias towards the null and, consequently, may have limited our ability to discern a difference. In contrast, attention disorder, which at 2 years is a more reliable predictor of similar disorders in later childhood, is associated with a strong, persistent and broad exposure to inflammation-related proteins.

Finally, whereas the risk for having diparesis and hemiparesis appears to be predicted by recurrent elevations of inflammation-related proteins in neonatal blood, quadriparesis was not. The underlying mechanisms for development of quadriparesis might differ from the other two forms of cerebral palsy. For example, genetic or epigenetic factors^{45,46,47} might have more impact on the risk of quadriparesis.

Because inflammation can have beneficial as well as adverse effects, we acknowledge that the mere number of protein elevations is unlikely to convey all the risk information we seek.⁴⁸ With much larger array systems becoming increasingly available for analyzing protein concentrations, as well as gene activation, future studies are likely to provide information that we could not.

Strengths of this study

Our study has several strengths. First, we included a large number of infants. Second, we collected our data prospectively. Third, attrition in the first two years was modest. Fourth, examiners at two years were not aware of the medical histories of the children they examined, thereby minimizing “diagnostic suspicion bias.” Fifth, we minimized observer variability in assessments of motor function.^{8,9} Sixth, our protein data are of high quality, with high content validity.^{13,49,50}

Limitations of this study

As with all observational studies, we are unable to distinguish between causation and association as explanations for what we found. Second, although we sampled a wide range of inflammation-associated proteins, including specific proteins known to be associated with neurological damage, we did not evaluate all known inflammation-associated proteins. The

proteins were selected on the basis of likely involvement in the fetal/neonatal inflammatory response and the accuracy with which they could be measured reliably in whole blood spots using the Meso Scale Discovery multiplex platform.

We examined associations between multiple developmental outcomes and several different measures of the breadth and type of systemic inflammation, which raises the issue of multiple comparisons. At the 95% confidence level, we would expect 6 of the 120 ORs calculated to be statistically significant by chance alone. In contrast, we found that 37 ORs identified significant relationships, presenting a consistent pattern of increased developmental risk with increased evidence of inflammation. Thus, our findings are unlikely to be due to chance alone.

Conclusion

Indicators of brain damage in very preterm infants are associated with recurrently/persistently elevated concentrations of inflammation-related proteins during the first two postnatal weeks. The association overall appears to reflect a threshold pattern. In a subset of a smaller number of proteins, however, this threshold effect might be preceded by a dose-response pattern of risk.

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Table 1

Odds ratios of the indicator of brain damage on the left associated with the number of proteins (maximum 25) whose concentrations were elevated on two days a week apart (listed at the top of the column). All models are adjusted for gestational age (23–24, 25–26, 27 weeks) and the microcephaly model is adjusted, additionally, for birth head circumference Z-score < −1.

	Number of proteins found to be persistently/recurrently elevated concentrations				
	0–1	2–3	4–5	6–8	9
Ventriculomegaly	1.0	1.8 (0.9, 3.5)	1.8 (0.8, 3.8)	2.7 (1.3, 5.8)	2.7 (1.4, 5.3)
Any cerebral palsy	1.0	0.8 (0.4, 1.5)	1.2 (0.6, 2.3)	1.2 (0.6, 2.4)	1.3 (0.7, 2.4)
Quadripareisis	1.0	0.8 (0.3, 1.9)	1.5 (0.7, 3.5)	1.4 (0.6, 3.4)	0.6 (0.2, 1.7)
Diparesis	1.0	0.6 (0.2, 1.9)	1.0 (0.3, 3.2)	0.8 (0.2, 2.9)	1.9 (0.8, 4.6)
Hemiparesis	1.0	1.4 (0.3, 5.7)	0.6 (0.1, 5.2)	1.3 (0.2, 7.4)	2.5 (0.7, 9.2)
MDI < 55	1.0	1.3 (0.7, 2.2)	2.1 (1.1, 3.7)	2.9 (1.6, 5.2)	1.9 (1.1, 3.4)
PDI < 55	1.0	1.2 (0.7, 2.0)	1.2 (0.7, 2.2)	1.3 (0.7, 2.5)	1.1 (0.6, 1.8)
Microcephaly	1.0	1.7 (0.8, 3.3)	2.2 (1.1, 4.6)	2.2 (1.03, 4.6)	1.8 (0.9, 3.5)
M-CHAT screen positive ^M	1.0	1.1 (0.6, 1.8)	1.0 (0.6, 1.9)	1.1 (0.6, 2.1)	0.9 (0.5, 1.6)
Attention problem ^A	1.0	1.8 (0.8, 4.2)	4.6 (2.1, 10)	3.6 (1.5, 8.9)	3.5 (1.6, 7.7)
Maximum column N	280	201	127	103	170

^M when GMFCS < 1 and no vision or hearing disability

^A Child Behavior Checklist-defined

MDI: Mental Development Index

PDI: Psychomotor Development Index

M-CHAT: Modified Checklist for Autism in Toddlers

Table 2

Odds ratios of the indicator of brain damage on the left associated with the number of proteins^P whose concentrations were elevated on two days a week apart (listed at the top of the column) among the 10 proteins associated with these adverse outcomes in previous analyses (listed in Table 1). All models are adjusted for gestational age (23–24, 25–26, 27 weeks) and the microcephaly model is adjusted, additionally, for birth head circumference Z-score < -1.

	Number of proteins ^P found to be persistently/recurrently elevated concentrations		
	0–1	2–4	5+
Ventriculomegaly	1.0	1.2 (0.7, 2.0)	3.4 (2.0, 5.9)
Any cerebral palsy	1.0	1.0 (0.5, 1.6)	2.6 (1.5, 4.5)
Quadriparesis	1.0	1.1 (0.5, 2.1)	1.5 (0.7, 3.5)
Diparesis	1.0	0.7 (0.2, 1.9)	3.6 (1.6, 8.2)
Hemiparesis	1.0	0.8 (0.2, 3.1)	4.1 (1.4, 12)
MDI < 55	1.0	1.4 (0.9, 2.2)	2.6 (1.6, 4.2)
PDI < 55	1.0	1.4 (0.9, 2.1)	1.5 (0.9, 2.5)
Microcephaly	1.0	1.7 (1.0, 2.8)	2.4 (1.3, 4.3)
M-CHAT screen positive ^M	1.0	1.0 (0.7, 1.6)	1.1 (0.6, 2.0)
Attention problem ^A	1.0	2.2 (1.3, 3.8)	2.8 (1.4, 5.2)

^P Proteins considered are CRP, SAA, IL-1 β , IL-6, TNF- α , IL-8, ICAM-1, E-SEL, MMP-9, and VEGF

^M when GMFCS < 1 and no vision or hearing disability

^A Child Behavior Checklist-defined

Table 3

The odds ratio (95% confidence interval) of the entity listed on the left associated with any protein of the type listed at the top of the column whose concentrations were in the highest quartile on two or more of the days assessed. (Maximum total N = 881). All models are adjusted for gestational age (23–24, 25–26, 27 weeks) and the microcephaly model is adjusted, additionally, for birth head circumference Z-score < −1.

Brain disorder indicator	Cytokine	Chemokine	Adhesion molecule	Metallo-proteinase	Liver-produced (SAA/CRP)
Ventriculomegaly	1.9 (1.2, 3.1)	2.3 (1.4, 3.9)	1.7 (1.1, 2.6)	1.4 (0.9, 2.2)	1.9 (1.2, 3.1)
Any cerebral palsy	1.1 (0.7, 1.7)	1.2 (0.8, 1.9)	1.5 (0.97, 2.3)	0.9 (0.6, 1.5)	1.6 (0.97, 2.5)
Quadripareisis	1.0 (0.5, 1.7)	1.2 (0.7, 2.3)	1.5 (0.9, 2.8)	0.5 (0.2, 1.00)	1.3 (0.7, 2.4)
Diparesis	1.1 (0.5, 2.2)	1.1 (0.5, 2.3)	1.6 (0.8, 3.3)	1.7 (0.8, 3.5)	2.0 (0.9, 4.1)
Hemiparesis	1.7 (0.6, 4.7)	1.4 (0.5, 3.8)	1.2 (0.5, 3.2)	1.5 (0.6, 4.1)	1.7 (0.6, 4.8)
MDI < 55	1.5 (1.04, 2.2)	1.9 (1.3, 2.8)	2.0 (1.4, 2.9)	0.8 (0.5, 1.2)	2.1 (1.4, 3.1)
PDI < 55	1.2 (0.8, 1.7)	1.2 (0.8, 1.7)	1.2 (0.8, 1.7)	0.8 (0.6, 1.3)	1.6 (1.1, 2.4)
Microcephaly	1.6 (1.01, 2.5)	1.3 (0.8, 2.0)	1.4 (0.9, 2.3)	1.1 (0.7, 1.8)	1.7 (1.1, 2.7)
M-CHAT screen pos ^M	0.9 (0.6, 1.3)	1.0 (0.7, 1.5)	1.0 (0.7, 1.4)	0.8 (0.5, 1.2)	0.8 (0.5, 1.3)
Attention problem ^A	2.2 (1.3, 3.7)	2.2 (1.3, 3.8)	2.2 (1.4, 3.6)	1.3 (0.8, 2.2)	1.4 (0.8, 2.4)
Maximum column total	467	505	383	274	220

^M when GMFCS < 1 and no vision or hearing disability

^A Child Behavior Checklist-defined

Table 4

The odds ratio (95% confidence interval) of the entity listed on the left associated with the number of systems (cytokine, chemokine, adhesion molecule, metallo-proteinase, liver-produced) that had at least one protein whose concentrations were in the highest quartile on two or more of the days assessed. The referent group consists of all children who did not have any protein whose concentration was elevated on two days. All models are adjusted for gestational age (23–24, 25–26, 27 weeks) and the microcephaly model is adjusted, additionally, for birth head circumference Z-score < –1. (Maximum total N = 881)

Brain disorder	Number of systems				
	0	1	2	3	4–5
Ventriculomegaly	1.0	1.5 (0.6, 3.8)	2.2 (0.9, 5.2)	2.3 (0.95, 5.6)	4.0 (1.8, 8.9)
Any cerebral palsy	1.0	1.0 (0.5, 1.9)	0.9 (0.5, 1.9)	1.1 (0.5, 2.2)	1.5 (0.8, 2.9)
Quadripareisis	1.0	1.2 (0.5, 3.0)	0.9 (0.3, 2.4)	1.4 (0.6, 3.6)	1.2 (0.5, 3.0)
Diparesis	1.0	0.6 (0.2, 2.3)	1.1 (0.4, 3.5)	0.6 (0.1, 2.3)	1.9 (0.7, 5.2)
Hemiparesis	1.0	0.9 (0.2, 4.7)	0.6 (0.1, 3.9)	1.1 (0.2, 5.5)	1.9 (0.5, 7.6)
MDI < 55	1.0	1.1 (0.6, 2.2)	1.4 (0.7, 2.7)	2.6 (1.4, 4.9)	2.3 (1.3, 4.3)
PDI < 55	1.0	0.9 (0.5, 1.7)	1.0 (0.5, 1.9)	1.4 (0.8, 2.6)	1.1 (0.7, 2.1)
Microcephaly	1.0	0.9 (0.4, 2.1)	1.3 (0.6, 2.8)	1.5 (0.7, 3.4)	2.0 (0.95, 4.1)
M-CHAT + screen ^M	1.0	0.7 (0.4, 1.2)	0.9 (0.5, 1.5)	1.0 (0.6, 1.8)	0.6 (0.3, 1.1)
Attention problem ^A	1.0	0.4 (0.1, 1.6)	2.9 (1.2, 7.2)	3.2 (1.3, 8.1)	2.8 (1.2, 6.7)
Maximum column N	165	187	175	157	197

^M when GMFCS < 1 and no vision or hearing disability

^A Child Behavior Checklist-defined