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Elevated concentrations of inflammation-related proteins in postnatal blood predict severe developmental delay at two years in extremely premature infants

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Abstract

Objective—To evaluate the hypothesis that elevated levels of inflammation-related proteins in early postnatal blood predict impaired mental and motor development among extremely preterm infants.

Study design—We measured concentrations of 25 inflammation-related proteins in blood collected on postnatal days 1, 7, and 14 from 939 infants born before 28 weeks gestation. An elevated level was defined as a concentration in the highest quartile for gestational age and day of blood collection. We identified impaired development at 24 months of age using the Bayley Scales of Infant Development. The primary outcomes were scores on the Mental or Motor Scale below 55 (more than 3 standard deviations below the mean).

Results—For 17 of the 25 inflammation-related proteins, one or more statistically significant association ($p < 0.01$) was found between an elevated blood level of the protein and a developmental impairment. Elevations on multiple days were more often associated with

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*A list of members of the ELGAN Study Investigators is available at www.jpeds.com (Appendix).

The authors declare no conflicts of interest.

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developmental impairment than elevations present for only one day. The highest number of elevations was found in day-14 blood.

Conclusions—In extremely preterm infants, elevated levels of inflammation-related proteins in blood collected on postnatal days 7 and 14, especially when sustained, are associated with impaired mental and motor development at age two years.

Keywords

cytokines; developmental disability; prematurity; Bayley Scales of Infant Development; neurodevelopmental outcome

Preterm newborns are at increased risk for long-term impairments, especially cognitive impairment.(1) Factors contributing to the underlying brain damage probably include infection and inflammation.(2) Experimental inflammation-induced brain damage is mediated, in part, by inflammatory cytokines and other inflammation-related proteins.(3) Elevated levels of such proteins are present in preterm infants who had brain damage identified with neuroimaging,(4) but we are aware of only one study describing associations between such proteins and clinical dysfunctions at age 2 years. In that study of 67 infants born before 32 weeks gestation, levels of proinflammatory and modulatory cytokines in blood obtained during the first 72 postnatal hours were associated with motor, but not cognitive, impairment.(5) We evaluated the hypothesis that elevated levels of inflammation-related proteins in blood collected on day 1, 7, and 14 after extremely preterm birth predict impaired mental and motor development at 24 months of age.

Methods

The Extremely Low Gestational Age Newborn (ELGAN) Study was designed to identify characteristics and exposures that increase the risk of structural and functional neurological disorders in children born before 28 weeks gestation.(6) During the years 2002-2004, we invited the participation of women who delivered before 28 weeks gestation at any of 14 participating institutions. The study was approved by the Institutional Review Boards at each site.

Gestational age

The 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, we used (in order of preference) fetal ultrasound at 14 or more weeks (29%), last menstrual period without fetal ultrasound (7%), and gestational age recorded in the log of the neonatal intensive care unit (1%).

Blood protein measurements

Blood spot collection

Drops of blood were collected on 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). Dried blood spots were stored at -70°C in sealed bags with desiccant until processed.

Elution of proteins from blood spots

For protein elution, 12mm punched biopsies of the frozen blood spots were submerged in 300 µL phosphate buffered saline containing 0.1% Triton X100 and 0.03% Tween-20

(Fisher, Hampton, NH) ,vortexed for 30 sec, and incubated on a shaker for 1h at 4°C. The buffer and biopsy were then transferred over the filter of a SpinX tube, centrifuged at 2000 x g followed by collection of the filtered eluted blood. An additional wash of the punch was performed in 100 µL for a final elution volume of 400 µL.

Protein measurements

Proteins were measured in duplicate using the Meso Scale Discovery (MSD) multiplex platform and Sector Imager 2400 (MSD, Gaithersburg, MD). Multiplex assays, measuring up to 10 proteins simultaneously, were optimized to allow detection of each biomarker within the linearity range of the eluted samples. The total protein concentration in each eluted sample was determined by BCA assay and the measurements of each analyte normalized to mg total protein. More details about the procedures used to measure inflammation-related proteins are presented elsewhere.(7)

24-month developmental assessment

Developmental assessments at 24-months corrected age included the Bayley Scales of Infant Development-Second Edition (BSID-II),(8) and an assessment of gross motor function using the Gross Motor Function Classification System(9). Certified examiners administered and scored the BSID-II. All examiners were experienced users of the BSID-II and, specifically for the ELGAN Study, attended a one-day workshop where published guidelines for test administration and videotaped examinations were reviewed. Examiners were aware of the child's enrollment in the ELGAN Study and corrected age, but not the child's medical history.

When a child's visual or neurological impairments precluded assessment with the BSID-II, or more than 2 items were omitted or judged to be 'unscorable,' the child was classified as not testable on that scale. The Adaptive Behavioral Composite of the Vineland Adaptive Behavior Scales, obtained for 26 of 33 children who were considered not testable with the BSID-II Mental Scale (i.e., Mental Development Index), was used to approximate the Mental Scale score (10). Among infants not testable with the BSID-II Motor Scale (i.e., Psychomotor Development Index), 32 were assessed with the Vineland Adaptive Behavior Scales, and the Vineland Adaptive Behavior Scales Motor Skills Domain score was used to approximate the Motor Scale score.

The BSID-II manual defines a significant delay as a Mental or Motor Scale below 70, i.e., 2 standard deviations below the mean for the standardization sample. However, in very preterm infants, the predictive ability of a Mental Scale below 55 is higher than that of a score below 70(11). In addition, neonatal illness is more strongly associated with a score below 55 than with a score below 70.(12) Thus, we classified the BSID-II outcomes into 3 categories: Mental or Motor Scale less than 55 (more than 3 standard deviations below the mean), 55-69 (between 2 and 3 standard deviations below the mean), or greater than 69 (within 2 standard deviations of the mean or higher). The primary outcomes were scores on the Mental or Motor Scale below 55 (more than 3 standard deviations below the mean).

Data analysis

For analyses involving the BSID-II Mental Scale, we excluded infants with significantly impaired gross motor function, defined as an inability to walk independently (a Gross Motor Function Classification System level ≥ 1), because their failure on certain Mental Scale test items might have been attributable to impaired motor function, not impaired mental development.

For all analyses, we classified a protein biomarker concentration as elevated if it was in the highest gestational age- and postnatal day-specific quartile. To describe associations between gestational age- and postnatal day-specific elevations of protein concentrations and Mental or Motor Scale less than 55 or 55-69, we used multinomial logistic regression to estimate odds ratios. The referent group was infants with Mental or Motor Scale of 70 or greater. All models adjusted for gestational age. The aim of our etiological study was not to develop a prediction model for developmental impairment; hence we did not include all candidate predictors. To balance the risks of type 1 and type 2 errors, we chose to describe the precision of odds ratio estimates with 99% confidence intervals.

Results

Mothers (n=1249) of 1506 infants gave their informed consent. For this analysis, we limited the sample to the 939 children from whom blood was obtained for analyses of protein levels and whose development was assessed at 24 months of age. The overall follow up rate was 85% (1018/1200) and blood protein data were available for 92% (n=939) of the infants who were evaluated at 24 months. Thus of 1200 survivors, 182 were not included in this analysis because they did not return for follow up and 79 were not included because blood protein data were not available. (Figure; available at www.jpeds.com)

Eleven percent of the cohort scored below 55 on the Mental Scale (i.e., Mental Development Index), and 11% had scores of 55-69. On the Motor Scale (i.e., Psychomotor Development Index), 16% scored below 55, and 15% scored 55-69. Among children with a Mental Scale less than 55, 43% had a Motor Scale less than 55; among those with a Motor Scale less than 55, 55% had a Mental Scale less than 55. Low scores were especially likely among infants with gestational age 23-24 weeks or birth weight of 750 grams or less, and those born to mothers with preeclampsia. (Table I; available at www.jpeds.com).

Infants included in this study (i.e., those from whom we collected blood samples and who returned for developmental evaluations) were less likely than surviving infants who were not included in this study to have a mother with less than 12 years of formal education, received public insurance, and had cervical insufficiency (Table II; available at www.jpeds.com). Children in our sample were slightly more likely than others to have experienced late onset sepsis, necrotizing enterocolitis, chronic lung disease, ventricular enlargement or a parenchymal echolucent lesion on cranial ultrasound.

Blood proteins and the risk of developmental impairment

Associations between elevated protein levels and a Mental Scale below 55 were most evident in specimens collected on day 14 (13 proteins including cytokines and their receptors, chemokines, adhesion molecules, acute phase proteins, and growth factors), less in specimens collected on day 7 (4 proteins), and not evident in day-1 blood (Table III). A Mental Scale between 55 and 69 was not predicted by any protein elevation on any day.

No protein elevations in day-1 blood, one elevation in day-7 blood, and eight elevations in day-14 blood were associated with Motor Scale below 55. A Motor Scale between 55 and 69 was predicted by an elevated level of one protein in day-1 blood, no proteins in day-7 blood, and 4 proteins in day-14 blood (Table IV).

Single versus multiple days of protein elevations and the risk of developmental delay

For 12 proteins, an elevated concentration on two days a week apart predicted a Mental Scale below 55, whereas such a low Mental Scale was predicted by only four proteins elevated on a single day only (Table V). Three proteins elevated on multiple days were

associated with a Mental Scale 55-69, but no single-day elevation was associated with a score in that range (Table VI; available at www.jpeds.com).

For eight proteins, an elevated concentration on two days predicted a Motor Scale below 55, but only two single-day elevations were predictive of a score this low. Four of the five proteins whose elevated concentrations were associated with a Motor Scale 55-69 were elevated on multiple days (Table VI).

Late protein elevation

The two days of elevated protein concentrations in Table 3 might have been any combination of two or three of the three days blood was sampled (Table VII). Because most of the elevated concentrations that predicted low developmental indices occurred on day 14 (Table IV), we compared the strength of associations with elevated concentrations on day 14 and elevated concentrations on days 7 and 14.

For 11 proteins, elevated concentrations were predictive of a Mental Scale less than 55, regardless of whether the elevations were present only in day-14 blood or were present on both day 7 and day 14. However, for five other proteins, elevated concentrations predicted a Mental Scale below 55 only if identified on both days 7 and 14. A similar pattern was found for a Motor Scale below 55, which was associated with 6 protein elevations regardless of whether they were identified on day 14 only or days 7 and 14, and two elevations only if present on both days 7 and 14. Two protein elevations (for RANTES and IL-6R) were associated with a decreased risk of motor impairment.

Although no protein elevations present only on day 14 were associated with a Mental Scale 55-69, two protein elevations on day 7 and day 14 were associated with an increased risk, and two other protein elevations were associated with decreased risk (Table VIII; available at www.jpeds.com). Two protein elevations on both days 7 and 14 predicted a Motor Scale score of 55-69, and an elevated concentration of another protein on days 7 and 14 was associated with decreased risk, and two protein elevations predicted a Motor Scale of 55-69 regardless of whether they were identified on day 14 or days 7 and 14 (Table VIII).

Discussion

Extremely preterm infants who had elevated blood levels of inflammation-related proteins in the first 2 postnatal weeks were at increased risk for severely limited development two years later. This observation supports the concept that systemic inflammation near the time of birth contributes to developmental impairments after extremely preterm birth. It is also possible that the protein elevations reflect other putative risk factors for brain damage, such as intracranial hemorrhage, lung injury, necrotizing enterocolitis, sepsis, and blood gas abnormalities. Protein elevations in day-14 blood were most predictive, suggesting a role for postnatal inflammation.

Five proteins conveyed prognostic information only if they were elevated on both day 7 and 14, and protein elevations on multiple days were more often associated with developmental impairment than elevations present for only one day, suggesting that persistent or recurrent inflammation heightens the risk. (13) The increased risk associated with persistent or recurrent inflammation might represent sensitization, i.e., enhanced vulnerability of the developing brain to inflammation-related damage after a prior exposure to an inflammatory stimulus. (14) Postnatal inflammation followed by feedback loops is another possible explanation for finding that persistent postnatal inflammation predicts low MDI. (13)

Alterations in gene expression after endotoxin infusion into adult humans are extinguished within 24 hours.(15) Thus even if protein degradation occurs over a longer period in preterm neonates, protein elevations in blood collected on days 7 and 14 are probably are not a consequence of inflammation initiated before delivery that resolves soon after delivery. We offer three possible explanations for the observation that of the 13 proteins whose elevated concentrations predicted a Mental Scale below 55, nine were identified only on day 14. First, vulnerability of the neonatal brain to inflammation-induced damage might increase with advancing postnatal age, due to a postnatal decrease in the levels of protectors provided by the placenta or mother.(16) Second, high levels of inflammatory proteins in day-7 and day-14 blood might result from prenatal brain damage or damage in the first postnatal days. Blood concentrations of inflammation-related proteins can increase following stroke, indicating that a non-infectious stimulus leading to brain damage can result in a systemic inflammatory response.(17) Third, systemic inflammation in the second postnatal week might contribute to, or result from, damage to lung(18) or gut(19), either of which might contribute to the risk of developmental impairment.(12, 20)

Perinatal and early neonatal infections have been associated with elevated cytokines (21, 22) and with cognitive and motor limitations.(23) (24) Our finding that infants who had elevated concentrations of inflammation-related proteins during the first postnatal month are at increased risk of cognitive limitation raises the possibility that the systemic inflammatory response we identified is the intermediary between perinatal inflammatory stimuli and brain damage.(3, 25) In a prior study of inflammation-related proteins and developmental outcome, higher cord blood levels of tumor necrosis factor- α were associated with the risk of a BSID Motor Scale more than one standard deviation below the mean, but no association was found with low scores on the BSID Mental Scale.(5) However, that study included only 67 infants and defined developmental delay as a developmental index below 85, rather than the more severe impairment (index below 55) we considered.

Our study has several limitations. First, a child's BSID Mental Scale below 55 at 18-24 months is only modestly predictive of an intelligence quotient below 70 at 8 years of age (positive predictive value = 0.52).(11) Second, we measured only a few of the proteins that are differentially expressed with fetal systemic inflammation.(26) Similarly, we did not have information about many of proteins that might protect the brain of preterm infants, although we did identify several proteins (RANTES, IL-6, and VEGF) for which elevated concentrations were associated with a lower risk of impairment.

Our findings have implications for preventing developmental impairments in extremely preterm infants. Protein biomarkers in neonatal blood might serve as indicators of processes that underlie associations between clinical triggers for inflammation, such as necrotizing enterocolitis(27) and sepsis,(20) and subsequent developmental impairments. These biomarkers might also be useful for monitoring interventions that target inflammation-related brain damage. Our observations provide a potential explanation for associations, observed by others,(28, 29) between genetic polymorphisms in genes for cytokines and the risk of brain disorders in infants.

In conclusion, among extremely preterm infants, indicators of systemic inflammation during the second postnatal week are associated with increased risks of developmental impairment at age two years.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ELGAN	extremely low gestational age newborn
BSID-II	Bayley Scales of Infant Development- Second Edition

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Figure 1.
Origin of the study sample.

Table 1

Characteristics of the study sample. These are column percents. For example, of the 94 infants with MDI < 55, 24% were born at 23-24 weeks and 51% were born at 25-26 weeks.

		Mental Scale*			Motor Scale		
		<55	55-69	70+	<55	55-69	70+
Gestational age (weeks)	23-24	24	18	17	31	21	18
	25-26	51	45	47	45	47	47
Birth weight (g)	≤ 750	44	42	33	50	42	32
Birth weight Z-score	< -2	10	8	5	8	9	4
Delivery complication	Preeclampsia	18	14	12	20	8	12
	Fetal indication	6	8	3	3	5	4
	Preterm labor	37	44	46	42	51	45
	pPROM	27	18	22	22	20	23
	Abruption	5	11	12	6	11	12
	Cerv insufficiency	6	5	4	7	5	5
Placenta bacteria	Any organism	45	47	48	54	43	48
Placenta histology	Any inflammation	41	35	39	40	36	40
Umbilical cord vasculitis †	Yes	17	18	17	19	15	17
Inflamm chorion/decidua ††	Yes	36	30	36	36	34	36
Inflamm chorionic plate ‡	Yes	17	20	19	14	22	19
Antenatal corticosteroid	Complete	70	66	63	76	61	63
	Partial	22	27	26	16	28	26
Moderate/severe IVH	Yes	20	24	18	36	24	17
Ventricular enlargement	Yes	11	8	7	25	12	6
Echoluency	Yes	6	5	4	21	8	4
Cerebral palsy	Quadriplegia	2	0	1	30	5	1
	Diplegia	1	6	2	8	7	2
	Hemiplegia	5	2	1	5	3	1
GMFCS**	2+	0	0	0	28	3	0

		Mental Scale*			Motor Scale		
		<55	55-69	70+	<55	55-69	70+
Mental Scale*	<55				55	19	4
	55-69				9	27	8
Motor Scale	<55	43	8	4			
	55-69	27	40	11			
Head circumference Z-score	<-2	16	12	5	30	13	6
	≥-2, <-1	18	24	17	22	15	18
Column N		94	93	669	146	144	649

* when Gross Motor Function Classification System < 1

** GMFCS: Gross Motor Function Classification System

[†] grades 3, 4 and 5

^{††} grades 3 and 4

[‡] stage 3 and severity 3

Table II

Maternal and infant characteristics of children who survived to 24 months adjusted age, comparing those included in this study and those not included. These are column percents.

Maternal or infant characteristic		Included in study	Not included
Maternal education (years)	< 12	15	21
	12 (High school)	28	26
	>12 to <16	23	25
	16 (College grad)	19	18
	> 16	15	10
Mother's marital status	Married	59	51
Public insurance	Yes	38	45
Delivery complication	Preterm labor	45	43
	pPROM	22	21
	Preeclampsia	13	13
	Abruption	11	9
	Cervical insufficiency	5	10
	Fetal Indication	4	5
Sex	Male	53	51
Black race	Yes	27	28
Gestational age (weeks)	23-24	20	21
	25-26	46	46
	27	34	33
Birth weight Z-score *	< -2	5	5
	≥ -2, < -1	13	12
	> -1	82	84
Head circumference Z-score *	< -2	8	7
	≥ -2, < -1	23	20
	> -1	69	73
Umbilical cord vasculitis **	Yes	17	17
Inflammation of chorion/decidua †	Yes	36	43
Inflammation of chorionic plate ††	Yes	19	22
Chronic lung disease	Yes	52	44
Late onset sepsis	Yes	26	22
Necrotizing enterocolitis	Yes	12	10
Moderate/severe IVH	Yes	21	19
Ventricular enlargement	Yes	10	8
Echolucency	Yes	7	5
Maximum number of infants		939	261

* Yudkin standard ²⁴

**
grades 3, 4 and 5

†
grades 3 and 4

††
stage 3 and severity 3

Table 3

Odds ratios (99% confidence intervals) for a Mental Scale < 55 and a Mental Scale between 55 and 69 (when Gross Motor Function Classification System < 1) compared to a Mental Scale of 70 or more associated with the highest quartile of the distribution of the protein listed on the left (within gestational age category and day blood was obtained), and adjusted for gestational age. Bold indicates odds ratios significantly > 1 (p < 0.01).

	Mental Scale < 55* (n/N = 94/856)			Mental Scale 55-69* (n/N = 93/856)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Cytokines and their receptors						
IL-1 β	1.0 (0.5, 1.8)	0.9 (0.5, 1.8)	1.8 (0.9, 3.3)	1.0 (0.5, 1.8)	0.8 (0.4, 1.5)	1.2 (0.6, 2.3)
IL-6	1.2 (0.6, 2.4)	1.8 (1.00, 3.4)	2.8 (1.5, 5.2)	1.0 (0.5, 1.9)	1.3 (0.7, 2.5)	1.0 (0.5, 2.0)
IL-6R	1.0 (0.5, 1.9)	1.0 (0.5, 1.9)	1.0 (0.5, 2.0)	1.3 (0.7, 2.5)	0.6 (0.3, 1.3)	0.8 (0.4, 1.5)
TNF- α	1.5 (0.8, 2.7)	1.9 (0.98, 3.3)	2.2 (1.2, 4.2)	1.0 (0.5, 1.9)	1.5 (0.8, 2.8)	1.2 (0.6, 2.3)
TNF-R1	0.9 (0.5, 1.8)	1.3 (0.7, 2.4)	2.1 (1.1, 4.0)	0.7 (0.4, 1.5)	0.8 (0.4, 1.7)	1.1 (0.5, 2.3)
TNF-R2	1.2 (0.6, 2.4)	1.8 (0.97, 3.3)	2.3 (1.2, 4.3)	1.6 (0.9, 3.0)	1.2 (0.6, 2.3)	1.5 (0.8, 3.0)
Chemokines						
IL-8 (CXCL8)	1.5 (0.8, 2.9)	1.8 (0.98, 3.4)	2.8 (1.5, 5.3)	1.2 (0.6, 2.3)	1.1 (0.6, 2.3)	1.4 (0.7, 2.9)
MCP-1 (CCL2)	1.0 (0.5, 1.9)	1.1 (0.6, 2.2)	1.5 (0.8, 3.0)	0.8 (0.4, 1.7)	0.9 (0.4, 1.8)	0.8 (0.4, 1.7)
MCP-4 (CCL13)	0.5 (0.2, 1.1)	1.0 (0.5, 1.9)	1.3 (0.7, 2.5)	0.6 (0.3, 1.2)	0.8 (0.4, 1.7)	0.9 (0.5, 1.8)
MIP-1 β (CCL4)	1.0 (0.5, 2.0)	2.2 (1.2, 4.0)	2.0 (1.1, 3.8)	1.1 (0.6, 2.1)	1.8 (0.9, 3.4)	1.3 (0.6, 2.5)
RANTES (CCL5)	0.9 (0.5, 1.9)	1.2 (0.6, 2.3)	0.9 (0.5, 1.8)	1.1 (0.6, 2.2)	0.6 (0.3, 1.3)	0.4 (0.2, 1.02)
I-TAC (CXCL11)	0.6 (0.3, 1.2)	1.1 (0.6, 2.1)	1.4 (0.7, 2.8)	0.6 (0.3, 1.3)	0.5 (0.2, 1.1)	0.7 (0.4, 1.6)
Adhesion molecules						
ICAM-1 (CD54)	1.3 (0.7, 2.5)	2.2 (1.2, 4.0)	2.2 (1.2, 4.3)	1.1 (0.6, 2.2)	1.1 (0.5, 2.2)	1.2 (0.6, 2.5)
ICAM-3 (CD50)	0.6 (0.3, 1.3)	1.1 (0.6, 2.0)	2.0 (1.1, 3.8)	0.9 (0.5, 1.8)	0.8 (0.4, 1.7)	0.8 (0.4, 1.7)
VCAM-1 (CD106)	1.2 (0.6, 2.2)	1.7 (0.9, 3.1)	1.7 (0.9, 3.2)	1.4 (0.7, 2.6)	0.9 (0.4, 1.7)	0.9 (0.4, 1.8)
E-SEL (CD62E)	1.5 (0.8, 2.7)	1.6 (0.9, 3.0)	2.5 (1.3, 4.8)	1.1 (0.6, 2.1)	1.4 (0.7, 2.7)	1.3 (0.7, 2.5)
Matrix metalloproteinases (MMPs)						
MMP-1	0.9 (0.4, 1.7)	1.0 (0.5, 1.9)	0.7 (0.3, 1.4)	0.6 (0.3, 1.1)	0.9 (0.5, 1.8)	0.9 (0.4, 1.8)
MMP-9	0.6 (0.3, 1.3)	0.9 (0.5, 1.7)	1.4 (0.7, 2.7)	0.8 (0.5, 1.5)	1.1 (0.6, 2.1)	0.8 (0.4, 1.7)
Other indicators of inflammation						
CRP	1.2 (0.6, 2.4)	3.0 (1.6, 5.5)	2.2 (1.2, 4.2)	0.7 (0.3, 1.4)	1.5 (0.7, 2.7)	1.2 (0.6, 2.4)

	Mental Scale < 55* (n/N =94/856)			Mental Scale 55-69* (n/N =93/856)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
SAA	1.4 (0.8, 2.7)	2.0 (1.1, 3.7)	2.4 (1.3, 4.6)	1.2 (0.6, 2.3)	1.3 (0.6, 2.5)	1.0 (0.5, 2.1)
MPO	0.7 (0.3, 1.4)	1.5 (0.8, 2.7)	2.0 (1.1, 3.7)	1.0 (0.5, 1.8)	0.7 (0.3, 1.5)	0.8 (0.4, 1.6)
Growth factors, their receptors, and binding proteins						
VEGF	0.7 (0.4, 1.4)	1.4 (0.8, 2.6)	1.1 (0.6, 2.1)	0.8 (0.4, 1.6)	0.7 (0.3, 1.4)	0.5 (0.2, 1.1)
VEGF-R1	1.1 (0.6, 2.1)	0.9 (0.5, 1.8)	1.5 (0.8, 2.8)	1.0 (0.5, 1.9)	0.9 (0.5, 1.8)	1.1 (0.6, 2.1)
VEGF-R2	1.3 (0.7, 2.5)	1.7 (0.9, 3.1)	2.0 (1.1, 3.8)	1.4 (0.7, 2.5)	1.0 (0.5, 2.0)	1.2 (0.6, 2.4)
IGFBP-1	1.7 (0.9, 3.1)	1.8 (0.96, 3.3)	1.5 (0.8, 2.8)	1.1 (0.6, 2.1)	1.0 (0.5, 2.1)	0.9 (0.4, 1.8)

* when Gross Motor Function Classification System < 1

Abbreviations: IL-1 β : Interleukin-1 β ; IL-6 : Interleukin-6 ; IL-6R: interleukin-6 receptor; TNF- α : Tumor Necrosis Factor- α ; TNF-R1: Tumor Necrosis Factor- α receptor 1; TNF-R2: tumor necrosis factor- α -receptor-2; IL-8 : Interleukin-8 ; MCP-1: monocyte chemoattractant protein-1 (CCL2); MCP-4 : monocyte chemoattractant protein-4 (CCL13) ; MIP-1 β : Macrophage Inflammatory Protein-1 beta (CCL4); RANTES: regulated upon activation, normal T-cell expressed and (presumably) secreted; I-TAC : Interferon-inducible T cell Alpha-Chemoattractant ; ICAM-1: intercellular adhesion molecule-1; ICAM-3: Intercellular adhesion molecule-3 (CD54); VCAM-1: vascular cell adhesion molecule-1 (CD106); E-selectin (CD62E); MMP-1: Matrix MetalloProteinase-1; MMP-9: matrix metalloproteinase-9; CRP: C-Reactive Protein; SAA: serum amyloid A; MPO: myeloperoxidase; VEGF: Vascular Endothelial Growth Factor; VEGF-R1: Vascular Endothelial Growth Factor-Receptor 1; VEGF-R2: vascular endothelial growth factor-receptor-2; IGFBP-1: Insulin Growth Factor Binding Protein-1

Table 4

Odds ratios (99% confidence intervals) for a Motor Scale < 55 and a Motor Scale between 55 and 69 compared to a Motor Scale of 70 or more associated with the highest quartile of the distribution of the protein listed on the left (within gestational age category and day blood was obtained), and adjusted for gestational age. Bold indicates odds ratios significantly > 1 ($p < 0.01$).

	Motor Scale < 55 (n/N=146/939)			Motor Scale 55-69 (n/N =144/939)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Cytokines and their receptors						
IL- β	0.7 (0.4, 1.2)	1.0 (0.6, 1.7)	2.3 (1.4, 3.9)	1.0 (0.6, 1.7)	1.0 (0.6, 1.7)	1.3 (0.8, 2.3)
IL-6	1.4 (0.8, 2.4)	1.3 (0.9, 2.2)	2.1 (1.2, 3.7)	1.5 (0.9, 2.5)	1.0 (0.6, 1.8)	1.9 (1.1, 3.3)
IL-6R	0.8 (0.4, 1.4)	1.0 (0.5, 1.7)	0.7 (0.4, 1.3)	1.1 (0.6, 1.9)	1.4 (0.8, 2.4)	1.1 (0.6, 1.9)
TNF- α	1.1 (0.6, 1.8)	1.1 (0.6, 1.8)	2.0 (1.1, 3.4)	1.5 (0.9, 2.5)	1.0 (0.6, 1.8)	1.2 (0.7, 2.2)
TNF-R1	1.0 (0.5, 1.7)	1.2 (0.7, 2.2)	1.5 (0.9, 2.7)	1.2 (0.7, 2.1)	1.0 (0.6, 1.8)	1.5 (0.8, 2.6)
TNF-R2	1.0 (0.6, 1.8)	1.2 (0.7, 2.0)	1.4 (0.8, 2.5)	1.4 (0.8, 2.4)	1.1 (0.6, 1.9)	1.6 (0.9, 2.8)
Chemokines						
IL-8 (CXCL8)	0.9 (0.5, 1.6)	1.2 (0.7, 2.1)	3.0 (1.8, 5.3)	1.7 (1.03, 2.9)	1.1 (0.7, 2.0)	2.3 (1.3, 4.0)
MCP-1 (CCL2)	1.3 (0.7, 2.2)	1.5 (0.9, 2.5)	1.7 (0.97, 3.0)	1.0 (0.6, 1.8)	0.9 (0.5, 1.6)	1.1 (0.6, 2.1)
MCP-4 (CCL13)	0.7 (0.4, 1.3)	1.0 (0.6, 1.7)	1.1 (0.6, 2.0)	0.9 (0.5, 1.6)	0.8 (0.5, 1.4)	1.2 (0.7, 2.2)
MIP- β (CCL4)	1.1 (0.6, 1.9)	0.9 (0.5, 1.5)	1.5 (0.8, 2.6)	1.1 (0.6, 1.9)	1.1 (0.6, 1.9)	1.4 (0.8, 2.5)
RANTES (CCL5)	1.0 (0.6, 1.8)	0.7 (0.4, 1.2)	0.6 (0.3, 1.1)	1.0 (0.6, 1.8)	0.9 (0.5, 1.6)	0.4 (0.2, 0.9)
I-TAC (CXCL11)	0.7 (0.4, 1.2)	0.8 (0.5, 1.6)	1.1 (0.6, 2.0)	1.1 (0.6, 1.9)	0.8 (0.5, 1.5)	1.4 (0.8, 2.4)
Adhesion molecules						
ICAM-1 (CD54)	0.9 (0.5, 1.6)	1.7 (0.98, 2.8)	2.2 (1.2, 3.8)	1.5 (0.9, 2.5)	1.2 (0.7, 2.2)	2.0 (1.1, 3.5)
ICAM-3 (CD50)	0.6 (0.3, 1.2)	0.6 (0.3, 1.1)	1.7 (0.97, 2.9)	0.8 (0.4, 1.4)	1.3 (0.7, 2.2)	1.0 (0.6, 1.8)
VCAM-1 (CD106)	0.9 (0.5, 1.6)	0.7 (0.4, 1.2)	1.0 (0.6, 1.8)	1.3 (0.7, 2.2)	1.0 (0.6, 1.7)	0.8 (0.5, 1.5)
E-SEL (CD62E)	1.0 (0.6, 1.8)	0.9 (0.5, 1.6)	1.2 (0.7, 2.2)	1.3 (0.8, 2.2)	1.1 (0.6, 1.8)	1.1 (0.6, 1.9)
Matrix metalloproteinases (MMPs)						
MMP-1	0.6 (0.3, 1.1)	0.6 (0.3, 1.2)	0.6 (0.3, 1.2)	1.1 (0.6, 1.8)	1.0 (0.6, 1.8)	0.7 (0.4, 1.4)
MMP-9	0.8 (0.5, 1.5)	0.7 (0.4, 1.2)	1.2 (0.7, 2.1)	0.8 (0.4, 1.4)	1.2 (0.7, 2.0)	1.3 (0.7, 2.4)
Other indicators of inflammation						
CRP	1.2 (0.7, 2.1)	1.8 (1.1, 3.1)	1.8 (1.01, 3.2)	1.0 (0.6, 1.8)	1.5 (0.9, 2.6)	1.8 (1.02, 3.2)

	Motor Scale < 55 (n/N=146/939)			Motor Scale 55-69 (n/N =144/939)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
SAA	1.5 (0.8, 2.5)	1.2 (0.7, 2.2)	2.2 (1.2, 3.8)	1.4 (0.8, 2.4)	1.4 (0.8, 2.3)	1.5 (0.8, 2.7)
MPO	0.8 (0.5, 1.5)	1.4 (0.8, 2.4)	2.3 (1.3, 3.9)	0.9 (0.5, 1.6)	1.2 (0.7, 2.1)	1.2 (0.7, 2.1)
Growth factors, their receptors, and binding proteins						
VEGF	1.0 (0.6, 1.7)	0.8 (0.5, 1.4)	0.9 (0.5, 1.6)	1.1 (0.6, 1.8)	1.0 (0.6, 1.8)	1.0 (0.5, 1.7)
VEGF-R1	1.2 (0.7, 2.1)	0.9 (0.5, 1.6)	1.2 (0.7, 2.1)	1.0 (0.6, 1.8)	1.3 (0.7, 2.1)	1.2 (0.7, 2.0)
VEGF-R2	1.0 (0.6, 1.7)	0.9 (0.5, 1.6)	1.3 (0.7, 2.2)	1.3 (0.7, 2.1)	1.1 (0.7, 1.9)	1.2 (0.7, 2.2)
IGFBP-1	1.3 (0.7, 2.2)	1.2 (0.7, 2.1)	1.4 (0.8, 2.4)	1.1 (0.6, 1.9)	1.2 (0.7, 2.0)	1.4 (0.8, 2.6)

* when Gross Motor Function Classification System < 1

Abbreviations: IL-1 β : Interleukin-1 β ; IL-6 : Interleukin-6 ; IL-6R: interleukin-6 receptor; TNF- α : Tumor Necrosis Factor- α ; TNF-R1: Tumor Necrosis Factor-alpha receptor 1; TNF-R2: tumor necrosis factor-alpha-receptor-2; IL-8 : Interleukin-8 ; MCP-1: monocyte chemotactic protein-1 (CCL2); MCP-4 : monocyte chemoattractant protein-4 (CCL13) ; MIP-1 β : Macrophage Inflammatory Protein-1 beta (CCL4); RANTES: regulated upon activation, normal T-cell expressed and (presumably) secreted; I-TAC : Interferon-inducible T cell Alpha-Chemoattractant ; ICAM-1: intercellular adhesion molecule-1; ICAM-3: Intercellular adhesion molecule-3 (CD54); VCAM-1: vascular cell adhesion molecule-1 (CD106); E-SEL1: E-selectin (CD62E); MMP-1: Matrix MetalloProteinase-1; MMP-9: matrix metalloproteinase-9; CRP: C-Reactive Protein; SAA: serum amyloid A; MPO: myeloperoxidase; VEGF: Vascular Endothelial Growth Factor; VEGF-R1: Vascular Endothelial Growth Factor-Receptor 1; VEGF-R2: vascular endothelial growth factor-receptor-2; IGFBP-1: Insulin Growth Factor Binding Protein-1

Table V

Odds ratio (and 95% confidence interval) of the outcome listed at the top of each column among children who had a concentration in the top quartile (for gestational age and day specimen was obtained) of the protein(s) listed on the left on 1 day or 2 or more days relative to that of children who did not have an elevated concentration of the same protein(s). The multinomial regression models compare children whose scores were < 55 and those whose scores were between 55 and 69 to children whose scores were ≥ 70 , adjusting for gestational age (23-24, 25-26, 27 weeks). The sample consisted of children who had proteins measured on 2 separate days. The results for Mental Scale and Motor Scale 55-69 are shown in Supplemental Table 3. Bold indicates odds ratios significantly > 1 ($p < 0.01$).

	Mental Scale < 55* (n/N =91/805)		Motor Scale < 55 (n/N =136/881)	
	1 day only	≥ 2 days	1 day only	≥ 2 days
Cytokines and their receptors				
IL-1 β	1.1 (0.6, 1.8)	1.5 (0.8, 2.6)	1.2 (0.8, 1.9)	1.5 (0.9, 2.4)
IL-6	1.5 (0.9, 2.6)	3.2 (1.8, 5.7)	1.5 (0.98, 2.3)	2.3 (1.4, 3.8)
IL-6R	0.9 (0.5, 1.5)	1.1 (0.6, 1.8)	0.6 (0.4, 1.01)	0.7 (0.4, 1.1)
TNF- α	2.1 (1.2, 3.6)	2.7 (1.5, 4.7)	1.2 (0.8, 1.8)	1.6 (1.01, 2.6)
TNF-R1	1.8 (1.1, 3.0)	1.4 (0.8, 2.7)	1.4 (0.9, 2.1)	1.3 (0.8, 2.2)
TNF-R2	1.6 (0.9, 2.6)	2.2 (1.3, 3.9)	1.2 (0.8, 1.9)	1.3 (0.8, 2.1)
Chemokines				
IL-8	1.6 (0.9, 2.7)	3.5 (2.0, 6.2)	1.6 (1.05, 2.5)	2.1 (1.3, 3.5)
MCP-1	1.1 (0.7, 1.8)	1.4 (0.8, 2.5)	1.4 (0.9, 2.1)	1.8 (1.1, 2.9)
MCP-4	0.7 (0.4, 1.2)	0.8 (0.5, 1.5)	1.2 (0.8, 1.8)	0.8 (0.5, 1.3)
MIP-1 β	1.5 (0.9, 2.6)	2.5 (1.4, 4.2)	1.2 (0.8, 1.9)	1.2 (0.7, 1.9)
RANTES	0.9 (0.5, 1.5)	1.2 (0.7, 2.1)	0.9 (0.6, 1.3)	0.6 (0.4, 1.1)
I-TAC	1.2 (0.7, 1.9)	1.0 (0.5, 1.8)	0.8 (0.5, 1.3)	0.8 (0.5, 1.2)
Adhesion molecules				
ICAM-1	1.4 (0.8, 2.3)	2.5 (1.5, 4.3)	1.1 (0.7, 1.7)	1.8 (1.1, 2.9)
ICAM-3	1.2 (0.7, 2.0)	1.4 (0.8, 2.4)	0.7 (0.5, 1.2)	0.9 (0.5, 1.4)
VCAM-1	1.3 (0.8, 2.3)	1.9 (1.1, 3.3)	1.0 (0.6, 1.5)	0.8 (0.5, 1.3)
E-SEL	1.5 (0.9, 2.6)	2.4 (1.4, 4.1)	0.9 (0.6, 1.4)	1.2 (0.7, 1.9)
Matrix metalloproteinases (MMPs)				
MMP-1	0.8 (0.5, 1.5)	0.9 (0.5, 1.5)	0.4 (0.2, 0.8)	0.6 (0.4, 1.03)
MMP-9	1.1 (0.7, 1.8)	0.8 (0.5, 1.6)	0.8 (0.5, 1.2)	0.8 (0.5, 1.4)
Other indicators of inflammation				
CRP	1.7 (0.98, 2.8)	3.3 (1.9, 5.7)	1.6 (1.01, 2.4)	2.2 (1.3, 3.5)
SAA	1.3 (0.7, 2.2)	3.3 (1.9, 5.7)	1.4 (0.9, 2.1)	2.2 (1.4, 3.7)
MPO	1.9 (1.2, 3.2)	1.5 (0.8, 2.7)	1.1 (0.7, 1.8)	1.7 (1.1, 2.6)
Growth factors, their receptors, and binding proteins				
VEGF	1.2 (0.7, 2.0)	1.1 (0.7, 1.9)	1.1 (0.7, 1.8)	0.7 (0.5, 1.2)
VEGF-R1	1.4 (0.9, 2.4)	1.2 (0.7, 2.2)	1.5 (0.98, 2.3)	1.1 (0.7, 1.8)
VEGF-R2	1.9 (1.1, 3.3)	2.1 (1.2, 3.6)	1.3 (0.8, 1.9)	1.0 (0.6, 1.6)

	Mental Scale < 55* (n/N =91/805)		Motor Scale < 55 (n/N =136/881)	
	1 day only	≥ 2 days	1 day only	≥ 2 days
IGFBP-1	1.1 (0.6, 1.8)	2.2 (1.3, 4.0)	1.1 (0.7, 1.7)	1.6 (0.96, 2.7)

* when Gross Motor Function Classification System < 1

Abbreviations: IL-1 β : Interleukin-1 β ; IL-6 : Interleukin-6 ; IL-6R: interleukin-6 receptor; TNF- α : Tumor Necrosis Factor- α ; TNF-R1: Tumor Necrosis Factor-alpha receptor 1; TNF-R2: tumor necrosis factor-alpha-receptor-2; IL-8 : Interleukin-8 ; MCP-1: monocyte chemotactic protein-1 (CCL2); MCP-4 : monocyte chemoattractant protein-4 (CCL13) ; MIP-1 β : Macrophage Inflammatory Protein-1 beta) (CCL4); RANTES: regulated upon activation, normal T-cell expressed and (presumably) secreted; I-TAC : Interferon-inducible T cell Alpha-Chemoattractant ; ICAM-1: intercellular adhesion molecule-1; ICAM-3: Intercellular adhesion molecule-3 (CD54); VCAM-1: vascular cell adhesion molecule-1 (CD106); E-SEL1: E-selectin (CD62E); MMP-1: Matrix MetalloProteinase-1; MMP-9: matrix metalloproteinase-9; CRP: C-Reactive Protein; SAA: serum amyloid A; MPO: myeloperoxidase; VEGF: Vascular Endothelial Growth Factor; VEGF-R1: Vascular Endothelial Growth Factor-Receptor 1; VEGF-R2: vascular endothelial growth factor-receptor-2; IGFBP-1: Insulin Growth Factor Binding Protein-1

Table VI

Odds ratio (and 95% confidence interval) of the outcome listed at the top of each column among children who had a concentration in the top quartile (for gestational age and day specimen was obtained) of the protein(s) listed on the left on 1 day or 2 or more days relative to that of children who did not have an elevated concentration of the same protein(s). The multinomial regression models compared children whose scores were < 55 and those whose scores were between 55 and 69 to children whose scores ≥ 70 , adjusting for gestational age (23-24, 25-26, 27 weeks). The results for the groups whose scores were < 55 are presented in Table 3. The results here are for children whose scores were between 55 and 69. The sample consisted of children who had proteins measured on 2 separate days. Bold italic indicates odds ratios significantly < 1 and bold indicates odds ratios significantly > 1 ($p < 0.01$).

	Mental Scale 55-69* (n/N=85/805)		Motor Scale 55-69 (n/N=136/881)	
	1 day only	≥ 2 days	1 day only	≥ 2 days
Cytokines and their receptors				
IL-1 β	1.2 (0.7, 2.0)	1.0 (0.6, 1.9)	0.8 (0.5, 1.2)	1.1 (0.7, 1.8)
IL-6	1.1 (0.6, 1.8)	1.2 (0.6, 2.2)	1.2 (0.8, 1.8)	1.8 (1.1, 2.9)
IL-6R	0.9 (0.6, 1.6)	0.8 (0.5, 1.5)	0.9 (0.6, 1.4)	1.2 (0.8, 1.9)
TNF- α	1.3 (0.7, 2.1)	1.6 (0.9, 2.8)	1.1 (0.7, 1.7)	1.2 (0.8, 2.0)
TNF-R1	1.0 (0.6, 1.6)	0.8 (0.4, 1.5)	0.8 (0.5, 1.3)	1.4 (0.9, 2.3)
TNF-R2	1.1 (0.6, 1.8)	1.9 (1.1, 3.4)	1.5 (0.97, 2.3)	1.5 (0.9, 2.4)
Chemokines				
IL-8	0.9 (0.5, 1.6)	1.8 (1.02, 3.3)	1.6 (1.04, 2.4)	2.0 (1.2, 3.2)
MCP-1	0.7 (0.4, 1.2)	0.9 (0.5, 1.6)	0.6 (0.4, 0.9)	1.2 (0.8, 1.9)
MCP-4	1.0 (0.6, 1.7)	0.7 (0.4, 1.2)	0.9 (0.6, 1.4)	0.9 (0.5, 1.4)
MIP-1 β	1.4 (0.8, 2.4)	1.8 (1.04, 3.2)	1.1 (0.7, 1.6)	1.1 (0.7, 1.8)
RANTES	0.7 (0.4, 1.2)	0.7 (0.4, 1.3)	0.7 (0.5, 1.1)	0.6 (0.4, 1.1)
I-TAC	0.6 (0.3, 0.97)	0.5 (0.3, 0.99)	1.3 (0.9, 2.0)	1.0 (0.6, 1.6)
Adhesion molecules				
ICAM-1	1.0 (0.6, 1.7)	1.3 (0.7, 2.4)	1.4 (0.9, 2.2)	2.0 (1.2, 3.1)
ICAM-3	0.9 (0.5, 1.6)	0.8 (0.4, 1.4)	1.0 (0.6, 1.5)	1.0 (0.6, 1.6)
VCAM-1	1.4 (0.8, 2.4)	1.1 (0.6, 2.0)	1.1 (0.7, 1.7)	1.1 (0.7, 1.8)
E-SEL	0.9 (0.5, 1.6)	1.4 (0.8, 2.4)	0.7 (0.4, 1.1)	1.1 (0.7, 1.8)
Matrix metalloproteinases (MMPs)				
MMP-1	0.6 (0.3, 1.2)	0.7 (0.4, 1.2)	1.0 (0.6, 1.6)	1.0 (0.6, 1.5)
MMP-9	1.1 (0.7, 1.8)	0.7 (0.3, 1.4)	1.2 (0.8, 1.8)	1.0 (0.6, 1.7)
Other indicators of inflammation				
CRP	0.6 (0.3, 1.1)	1.3 (0.7, 2.3)	0.8 (0.5, 1.2)	1.6 (1.04, 2.6)
SAA	0.8 (0.4, 1.3)	1.7 (0.9, 3.0)	1.0 (0.6, 1.5)	1.7 (1.01, 2.7)
MPO	0.9 (0.5, 1.4)	0.7 (0.4, 1.3)	1.1 (0.7, 1.6)	1.1 (0.7, 1.7)
Growth factors, their receptors, and binding proteins				
VEGF	1.1 (0.7, 1.9)	0.4 (0.2, 0.8)	1.4 (0.9, 2.1)	1.0 (0.6, 1.6)
VEGF-R1	1.0 (0.6, 1.6)	1.0 (0.6, 1.8)	1.5 (0.96, 2.3)	1.2 (0.7, 1.9)

	Mental Scale 55-69* (n/N =85/805)		Motor Scale 55-69 (n/N =136/881)	
	1 day only	≥ 2 days	1 day only	≥ 2 days
VEGF-R2	1.6 (0.97, 2.8)	1.3 (0.7, 2.3)	0.7 (0.5, 1.2)	1.1 (0.7, 1.8)
IGFBP-1	0.9 (0.6, 1.5)	1.1 (0.5, 2.0)	1.3 (0.8, 1.9)	1.1 (0.6, 1.9)

* when Gross Motor Function Classification System < 1

Abbreviations: IL-1 β : Interleukin-1 β ; IL-6 : Interleukin-6 ; IL-6R: interleukin-6 receptor; TNF- α : Tumor Necrosis Factor- α ; TNF-R1: Tumor Necrosis Factor-alpha receptor 1; TNF-R2: tumor necrosis factor-alpha-receptor-2; IL-8 : Interleukin-8 ; MCP-1: monocyte chemotactic protein-1 (CCL2); MCP-4 : monocyte chemoattractant protein-4 (CCL13) ; MIP-1 β : Macrophage Inflammatory Protein-1 beta) (CCL4); RANTES: regulated upon activation, normal T-cell expressed and (presumably) secreted; I-TAC : Interferon-inducible T cell Alpha-Chemoattractant ; ICAM-1: intercellular adhesion molecule-1; ICAM-3: Intercellular adhesion molecule-3 (CD54); VCAM-1: vascular cell adhesion molecule-1 (CD106); E-SEL1: E-selectin (CD62E); MMP-1: Matrix MetalloProteinase-1; MMP-9: matrix metalloproteinase-9; CRP: C-Reactive Protein; SAA: serum amyloid A; MPO: myeloperoxidase; VEGF: Vascular Endothelial Growth Factor; VEGF-R1: Vascular Endothelial Growth Factor-Receptor 1; VEGF-R2: vascular endothelial growth factor-receptor-2; IGFBP-1: Insulin Growth Factor Binding Protein-1

Table VII

Odds ratios (and 95% confidence interval) of the outcome listed at the top of each set of columns among children who had a concentration in the top quartile (for gestational age and day specimen was obtained) of the protein(s) listed on the left on day 14 or days 7 and 14 relative to that of children who did not have an elevated concentration of the same protein(s). The multinomial regression models compare children whose scores were < 55 and those whose scores were between 55 and 69 to children whose scores were ≥ 70, adjusting for gestational age (23-24, 25-26, 27 weeks). The results for Mental Scale and Motor Scale 55-69 are shown in Supplemental Table 4. Bold italic indicates odds ratios significantly < 1 and bold indicates odds ratios significantly > 1 ($p < 0.01$).

	Mental Scale < 55* (n/N =61/587)		Motor Scale < 55 (n/N =92/638)	
	Day 14 only	Days 7 and 14	Day 14 only	Days 7 and 14
Cytokines and their receptors				
IL-1 β	1.5 (0.9, 2.8)	2.0 (1.03, 2.7)	2.3 (1.4, 3.9)	2.4 (1.4, 4.3)
IL-6	2.2 (1.1, 4.3)	4.8 (2.5, 9.1)	1.9 (1.04, 3.3)	2.6 (1.5, 4.6)
IL-6R	0.7 (0.3, 1.6)	1.1 (0.6, 2.1)	0.3 (0.1, 0.7)	1.1 (0.6, 2.0)
TNF- α	2.5 (1.3, 4.8)	3.1 (1.6, 5.9)	2.3 (1.3, 4.0)	1.9 (1.1, 3.3)
TNF-R1	2.1 (1.1, 4.0)	2.1 (1.1, 4.3)	1.8 (1.00, 3.1)	1.6 (0.9, 3.1)
TNF-R2	2.3 (1.2, 4.4)	3.1 (1.6, 6.0)	1.4 (0.8, 2.5)	1.7 (0.9, 3.0)
Chemokines				
IL-8	2.5 (1.3, 4.8)	4.9 (2.5, 9.9)	2.7 (1.6, 4.5)	3.5 (1.9, 6.4)
MCP-1	2.1 (1.1, 4.0)	1.5 (0.7, 3.0)	2.1 (1.2, 3.9)	1.8 (0.99, 3.3)
MCP-4	1.2 (0.6, 2.5)	1.3 (0.3, 1.8)	1.3 (0.7, 2.6)	1.1 (0.6, 1.9)
MIP-1 β	3.2 (1.6, 6.2)	2.5 (1.3, 4.9)	2.2 (1.3, 3.9)	1.1 (0.6, 2.1)
RANTES	0.9 (0.5, 1.9)	0.9 (0.5, 1.9)	0.5 (0.2, 0.9)	0.6 (0.3, 1.1)
I-TAC	1.4 (0.7, 2.8)	1.7 (0.9, 3.1)	1.0 (0.5, 1.9)	1.0 (0.6, 1.9)
Adhesion molecules				
ICAM-1	1.5 (0.7, 3.2)	3.3 (1.8, 6.0)	1.2 (0.6, 2.4)	3.2 (1.9, 5.2)
ICAM-3	1.9 (1.05, 3.6)	2.1 (1.1, 4.2)	1.3 (0.8, 2.2)	1.4 (0.8, 2.6)
VCAM-1	1.6 (0.8, 3.2)	2.2 (1.2, 4.1)	1.1 (0.6, 1.9)	0.9 (0.5, 1.6)
E-SEL	2.2 (1.1, 4.2)	3.1 (1.7, 5.7)	0.9 (0.5, 1.7)	1.4 (0.8, 2.5)
Matrix metalloproteinases (MMPs)				
MMP-1	0.7 (0.3, 1.7)	0.7 (0.3, 1.4)	0.7 (0.3, 1.5)	0.6 (0.3, 1.1)
MMP-9	1.3 (0.7, 2.4)	1.5 (0.7, 3.2)	1.1 (0.7, 1.9)	1.2 (0.6, 2.5)
Other indicators of inflammation				
CRP	2.1 (1.04, 4.3)	4.7 (2.4, 9.1)	1.7 (0.9, 3.1)	2.6 (1.4, 4.7)
SAA	2.1 (1.1, 4.0)	4.1 (2.1, 8.0)	2.3 (1.3, 3.8)	2.4 (1.3, 4.4)
MPO	2.4 (1.3, 4.4)	2.7 (1.3, 5.3)	1.8 (1.1, 3.1)	3.2 (1.8, 5.6)
Growth factors, their receptors, and binding proteins				
VEGF	1.0 (0.5, 2.1)	1.3 (0.7, 2.5)	0.8 (0.5, 1.5)	0.8 (0.4, 1.5)
VEGF-R1	1.7 (0.9, 3.1)	1.3 (0.7, 2.5)	1.3 (0.7, 2.2)	1.2 (0.7, 2.0)
VEGF-R2	2.0 (0.99, 3.9)	2.5 (1.4, 4.5)	1.3 (0.7, 2.3)	1.3 (0.8, 2.4)

	Mental Scale < 55* (n/N =61/587)		Motor Scale < 55 (n/N =92/638)	
	Day 14 only	Days 7 and 14	Day 14 only	Days 7 and 14
IGFBP-1	1.1 (0.5, 2.2)	3.0 (1.5, 5.9)	1.3 (0.7, 2.3)	1.7 (0.9, 3.3)

* when Gross Motor Function Classification System < 1

Abbreviations: IL-1 β : Interleukin-1 β ; IL-6 : Interleukin-6 ; IL-6R: interleukin-6 receptor; TNF- α : Tumor Necrosis Factor- α ; TNF-R1: Tumor Necrosis Factor-alpha receptor 1; TNF-R2: tumor necrosis factor-alpha-receptor-2; IL-8 : Interleukin-8 ; MCP-1: monocyte chemotactic protein-1 (CCL2); MCP-4 : monocyte chemoattractant protein-4 (CCL13) ; MIP-1 β : Macrophage Inflammatory Protein-1 beta) (CCL4); RANTES: regulated upon activation, normal T-cell expressed and (presumably) secreted; I-TAC : Interferon-inducible T cell Alpha-Chemoattractant ; ICAM-1: intercellular adhesion molecule-1; ICAM-3: Intercellular adhesion molecule-3 (CD54); VCAM-1: vascular cell adhesion molecule-1 (CD106); E-SEL1: E-selectin (CD62E); MMP-1: Matrix MetalloProteinase-1; MMP-9: matrix metalloproteinase-9; CRP: CReactive Protein; SAA: serum amyloid A; MPO: myeloperoxidase; VEGF: Vascular Endothelial Growth Factor; VEGF-R1: Vascular Endothelial Growth Factor-Receptor 1; VEGF-R2: vascular endothelial growth factor-receptor-2; IGFBP-1: Insulin Growth Factor Binding Protein-1

Table 8

Odds ratios (and 95% confidence interval) of the outcome listed at the top of each set of columns among children who had a concentration in the top quartile (for gestational age and day specimen was obtained) of the protein(s) listed on the left on day 14 or days 7 and 14 relative to that of children who did not have an elevated concentration of the same protein(s). The multinomial regression models compare children whose scores were < 55 and those whose scores were between 55 and 69 to children whose scores ≥ 70 , adjusting for gestational age (23-24, 25-26, 27 weeks). The results for the groups whose scores were < 55 are presented in Table 4. The results here are for children whose scores were between 55 and 69. Bold italic indicates odds ratios significantly < 1 and bold indicates odds ratios significantly > 1 ($p < 0.01$).

	Mental Scale 55-69* (n/N =65/587)		Motor Scale 55-69 (n/N =99/638)	
	Day 14 only	Days 7 and 14	Day 14 only	Days 7 and 14
Cytokines and their receptors				
IL-1 β	1.4 (0.7, 2.7)	0.7 (0.3, 1.7)	1.2 (0.7, 2.2)	1.5 (0.9, 2.8)
IL-6	0.9 (0.4, 1.9)	1.2 (0.5, 2.7)	2.1 (1.2, 3.6)	1.5 (0.8, 2.9)
IL-6R	1.1 (0.6, 2.2)	0.5 (0.2, 1.2)	0.7 (0.3, 1.3)	1.6 (0.9, 2.7)
TNF- α	0.9 (0.4, 1.9)	1.6 (0.8, 3.2)	1.4 (0.8, 2.5)	1.0 (0.5, 1.9)
TNF-R1	1.0 (0.5, 2.0)	1.2 (0.5, 2.6)	1.4 (0.9, 2.4)	1.4 (0.7, 2.5)
TNF-R2	0.9 (0.4, 1.9)	2.2 (1.1, 4.2)	1.7 (0.98, 3.1)	1.5 (0.8, 2.8)
Chemokines				
IL-8	1.0 (0.5, 2.0)	1.4 (0.6, 3.3)	2.0 (1.1, 3.4)	2.3 (1.2, 4.4)
MCP-1	0.5 (0.2, 1.4)	0.9 (0.5, 2.0)	1.1 (0.6, 2.1)	1.2 (0.7, 2.2)
MCP-4	0.8 (0.3, 1.8)	0.8 (0.4, 1.7)	1.3 (0.7, 2.5)	1.1 (0.6, 1.9)
MIP-1 β	1.6 (0.8, 3.3)	1.4 (0.7, 2.8)	1.1 (0.6, 2.2)	1.6 (0.9, 2.7)
RANTES	0.6 (0.2, 1.4)	0.1 (0, 0.6)	0.5 (0.2, 0.98)	0.4 (0.2, 0.9)
I-TAC	0.7 (0.3, 1.6)	0.5 (0.2, 1.2)	1.7 (0.9, 3.0)	1.1 (0.6, 2.0)
Adhesion molecules				
ICAM-1	1.0 (0.4, 2.1)	1.5 (0.8, 3.1)	2.1 (1.2, 3.8)	2.1 (1.2, 3.7)
ICAM-3	0.6 (0.3, 1.4)	0.7 (0.3, 1.7)	1.0 (0.5, 1.8)	1.1 (0.6, 2.2)
VCAM-1	1.0 (0.5, 2.2)	0.7 (0.3, 1.6)	0.8 (0.4, 1.6)	1.0 (0.5, 1.8)
E-SEL	1.2 (0.5, 2.6)	1.9 (0.9, 3.7)	0.9 (0.5, 1.7)	1.4 (0.8, 2.4)
Matrix metalloproteinases (MMPs)				
MMP-1	0.4 (0.1, 1.2)	0.5 (0.2, 1.2)	1.0 (0.5, 1.9)	0.7 (0.4, 1.3)
MMP-9	0.8 (0.4, 1.6)	0.6 (0.2, 1.6)	1.5 (0.9, 2.5)	1.3 (0.7, 2.8)
Other indicators of inflammation				
CRP	0.5 (0.2, 1.3)	2.4 (1.2, 4.6)	1.3 (0.7, 2.4)	2.5 (1.4, 4.4)
SAA	0.8 (0.3, 1.7)	1.8 (0.8, 3.8)	1.2 (0.6, 2.1)	2.1 (1.1, 3.9)
MPO	0.9 (0.5, 1.8)	0.6 (0.2, 1.6)	1.0 (0.6, 1.8)	1.5 (0.8, 2.8)
Growth factors, their receptors, and binding proteins				
VEGF	0.9 (0.5, 1.9)	0.2 (0.1, 0.8)	1.6 (0.9, 2.7)	0.7 (0.3, 1.4)
VEGF-R1	1.0 (0.5, 2.0)	1.1 (0.5, 2.1)	1.6 (0.9, 2.8)	1.3 (0.7, 2.4)
VEGF-R2	1.4 (0.7, 2.9)	1.2 (0.6, 2.4)	0.7 (0.4, 1.4)	1.6 (0.96, 2.7)

	Mental Scale 55-69* (n/N =65/587)		Motor Scale 55-69 (n/N =99/638)	
	Day 14 only	Days 7 and 14	Day 14 only	Days 7 and 14
IGFBP-1	1.0 (0.5, 1.9)	0.9 (0.4, 2.4)	1.6 (0.96, 2.8)	1.4 (0.7, 2.9)

* when Gross Motor Function Classification System < 1 Supplemental Table 4.

Abbreviations: IL-1 β : Interleukin-1 β ; IL-6 : Interleukin-6 ; IL-6R: interleukin-6 receptor; TNF- α : Tumor Necrosis Factor- α ; TNF-R1: Tumor Necrosis Factor-alpha receptor 1; TNF-R2: tumor necrosis factor-alpha-receptor-2; IL-8 : Interleukin-8 ; MCP-1: monocyte chemotactic protein-1 (CCL2); MCP-4 : monocyte chemoattractant protein-4 (CCL13) ; MIP-1 β : Macrophage Inflammatory Protein-1 beta) (CCL4); RANTES: regulated upon activation, normal T-cell expressed and (presumably) secreted; I-TAC : Interferon-inducible T cell Alpha-Chemoattractant ; ICAM-1: intercellular adhesion molecule-1; ICAM-3: Intercellular adhesion molecule-3 (CD54); VCAM-1: vascular cell adhesion molecule-1 (CD106); E-SEL1: E-selectin (CD62E); MMP-1: Matrix MetalloProteinase-1; MMP-9: matrix metalloproteinase-9; CRP: CReactive Protein; SAA: serum amyloid A; MPO: myeloperoxidase; VEGF: Vascular Endothelial Growth Factor; VEGF-R1: Vascular Endothelial Growth Factor-Receptor 1; VEGF-R2: vascular endothelial growth factor-receptor-2; IGFBP-1: Insulin Growth Factor Binding Protein-1