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Systemic inflammation, intraventricular hemorrhage, and white matter injury

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Conflict of interest

The authors have no financial or personal relations that could pose a conflict of interest.

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Abstract

To see if the systemic inflammation profile of 123 infants born before the 28th week of gestation who had intraventricular hemorrhage (IVH) without white matter injury (WMI) differed from that of 68 peers who had both IVH and WMI, we compared both groups to 677 peers who had neither. Cranial ultrasound scans were read independently by multiple readers until concordance. The concentrations of 25 proteins were measured with multiplex arrays using an electrochemiluminescence system. Infants who had IVH and WMI were more likely than others to have elevated concentrations of CRP and IL-8 on days 1, 7, and 14, and elevated concentrations of SAA and TNF-alpha on 2 of these days. IVH should probably be viewed as two entities, IVH unaccompanied by WMI, and IVH accompanied by WMI. Each entity is associated with inflammation, but IVH accompanied by WMI has a stronger inflammatory signal than IVH unaccompanied by WMI.

Keywords

Infant; premature; hemorrhage; brain injuries; acute; inflammation

Introduction

Intraventricular hemorrhage (IVH) seen on an early ultrasound scan of an extremely preterm newborn predicts developmental impairments (O'Shea et al., 2012; O'Shea et al., 2005), and ultrasound indicators of cerebral white matter injury (WMI) predict impairments even more (Kuban et al., 2009; O'Shea et al., 2008). Both IVH and indicators of WMI, however, tend to occur together more commonly than would be expected if they occurred independently (Kuban et al., 1999; O'Shea et al., 2012).

In a sample of infants born before the 28th week of gestation, we found that elevated blood concentrations of inflammation-associated proteins during the first two postnatal weeks convey information about an increased risk of ultrasound-documented WMI (Leviton et al., 2011b), as well as developmental impairment (Laughon et al., 2011) and reduced head circumference at age two years (Leviton et al., 2011c). Here we try to disentangle the association of systemic inflammation with IVH from its association with ultrasound-documented WMI.

Methods

The ELGAN Study

The 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 (O'Shea et al., 2009). During the years 2002-2004, we invited the participation of

women who delivered before 28 weeks gestation at any of 14 participating institutions; 1249 mothers of 1506 infants consented. The study was approved by the Institutional Review Boards at each site.

Proteins were measured in early postnatal blood spots only from infants whose development was assessed at approximately 2 years post-term equivalent. For this report, we limited the sample to the 868 infants who also had both an early cranial ultrasound scan (between days 1 and 14) for diagnosis of IVH and a late scan (between day 15 and the end of week 40) for diagnosis of WMI [Table 1].

Neonatal and maternal data

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%).

Ultrasound protocol scans

Routine scans were performed by technicians at each of the participating 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. Protocol 1 scans were obtained between the 1st and 4th day; protocol 2 scans were obtained between the 5th and 14th day, and protocol 3 scans were obtained between the 15th day and 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 (Kuban et al., 2007). In addition, 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 IVH, moderate/severe lateral ventricle enlargement, a hyperechoic (echodense) lesion in the parenchyma, or a hypoechoic (echolucent) lesion in the parenchyma, the films were sent to a third (tie-breaking) reader who did not know what the other readers reported.

IVH was diagnosed when hyperechoic material was seen in the lateral ventricles or was adherent to the choroid plexus. For the current analysis, we defined WMI as the presence of a parenchymal echolucent (*i.e.*, hypoechoic) lesion on any scan, and/or ventriculomegaly on the last protocol scan that was deemed moderate or severe by comparison to illustrative

templates (Kuban et al., 2007). To have multinomial analyses compare children who had IVH but no WMI, and children who had IVH with WMI to children without either IVH or WMI we needed to exclude the 39 children who had WMI but no IVH. We did include the 39 children who had a ventricular shunt for three reasons. First, we did not want to exclude any group of children with IVH. Second, the rates of white matter damage were not appreciably increased (only 4 had ventriculomegaly on the last scan and only 1 had an echolucent lesion). Third, we did not want to reduce the sample size inappropriately.

Blood spot collection

Drops of blood were collected on filter paper on the first postnatal day (range: 1-3 days), the seventh postnatal day (range: 5-8 days), and the 14th postnatal day (range: 12-15 days). All blood was from the remainder after specimens were obtained for clinical indications. Dried blood spots were stored at -70°C in sealed bags with desiccants until processed.

Elution of proteins from blood spots

For protein elution, 12mm punched biopsies of the frozen blood spots were submerged in 300 μ L phosphate buffered saline containing 0.1% Triton X100 (Sigma-Aldrich, St. Louis, MO) and 0.03% Tween-20 (Fisher, Hampton, NH), vortexed for 30 seconds, and incubated on a shaker for 1 hour at 4°C. The buffer and biopsy were then transferred over the filter of a SpinX tube (Corning Fisher), centrifuged at 2000 x g and the filtered eluted blood collected. An additional wash of the punch was performed in 100 μ L for a final elution volume of 400 μ L.

Protein measurements

Proteins were measured in duplicate in the Laboratory of Genital Tract Biology of the Department of Obstetrics, Gynecology and Reproductive Biology at Brigham and Women's Hospital, Boston using the Meso Scale Discovery (MSD) multiplex platform and Sector Imager 2400 (MSD, Gaithersburg, MD), a system that has been validated by comparisons with traditional ELISA and produces measurements that have high content validity (Fichorova et al., 2011; Hecht et al., 2011; Leviton et al., 2011a; McElrath et al., 2011). The 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 MSD Discovery Workbench Software was used to convert relative luminescent units into protein concentrations (pg/mL) using interpolation from several log calibrator curves. Split quality control blood pools tested on each plate showed inter-assay variation of <10-20% for each protein. The total protein concentration in each eluted sample was determined by BCA assay (Thermo Scientific, Rockford, IL) using a multi-label Victor 2 counter (Perkin Elmer, Boston, MA). The measurements of each analyte were then normalized as pg specific protein per mg total protein

The following are the 25 proteins: C-Reactive Protein (CRP), Serum Amyloid A (SAA), Myeloperoxidase (MPO), Interleukin-1 β ($\square\square$ IL -1 β), Interleukin-6 (IL-6), Interleukin-6 Receptor (IL-6R), Tumor Necrosis Factor- α (TNF- α) $\square$, Tumor Necrosis Factor Receptor-1 (TNF-R1), Tumor Necrosis Factor Receptor-2 (TNF-R2), Interleukin-8 (IL-8; CXCL8), Monocyte Chemotactic Protein-1 (MCP-1; CCL2), Monocyte Chemotactic Protein -4

(MCP-4; CCL13), Macrophage Inflammatory Protein-1 β (MIP-1 β ; CCL4), Regulated upon Activation, Normal T-cell Expressed, and [presumably] Secreted (RANTES; CCL5), Interferon-inducible T cell Alpha-Chemoattractant (I-TAC; CXCL11), Intercellular Adhesion Molecule -1 (ICAM-1; CD54), Intercellular Adhesion Molecule -3 (ICAM-3; CD50), E-selectin (CD62E), Vascular Cell Adhesion Molecule-1 (VCAM-1; CD106), Matrix Metalloproteinase-1 (MMP-1), Matrix Metalloproteinase-9 (MMP-9), Vascular Endothelial Growth Factor (VEGF), Vascular Endothelial Growth Factor Receptor-1 (VEGF-R1; Flt-1), Vascular Endothelial Growth Factor Receptor-2 (VEGF-R2; KDR), and Insulin Growth Factor Binding Protein-1 (IGFBP-1).

Because we were most interested in high concentrations and not merely a shift in the mean or median, we classified a concentration as elevated if it was in the top quartile for gestational age among the specimens collected on each protocol day.

Data analyses

We evaluated the generalized null hypothesis that the risks of IVH unaccompanied by WMI and IVH accompanied by WMI are not associated with blood protein concentrations in the highest quartile. We also explored to what extent a concentration in the top quartile on two days at least one week apart was associated with greater increased risk than evident if the protein elevation was limited to only one of the protocol days. For this exploration, we excluded the 54 children who had only one set of protein measurements.

Because our outcomes are mutually exclusive and each is appropriately compared to the same referent group (*i.e.*, children who had neither IVH nor WMI), we used multinomial logistic regression for our evaluations of each protein individually. The only adjustment was for gestational age category (23-24, 25-26, 27 weeks).

The risks of IVH with and without WMI associated with a protein concentration in the highest quartile are presented as odds ratios and 99% confidence intervals. We selected this confidence interval rather than the conventional 95% confidence interval because we wanted to modify our analyses for multiple comparisons (25 proteins measured at 3 times), while not appreciably increasing the risk of a type 2 (false negative) error.

Results

Derivation of the sample (Table 1)

Of the 868 newborns who had proteins measured in at least one blood specimen, 123 had isolated IVH and 68 had both IVH and WMI. Of the 814 who had measurements of proteins in two or more specimens a week or more apart, 60 had isolated IVH and 116 had both IVH and WMI.

Sample description (Table 2)

Children who had both IVH and WMI were more likely than others to have been born at a very low gestational age and birth weight, and also more likely to have had an organism recovered from their placenta. They were also much more likely than others to develop cerebral palsy, and to have very low indices on the Bayley Scales of Infant Development.

Individual days (Table 3)

Compared to newborns who developed neither IVH nor WMI, those who developed isolated IVH were more likely to have a day-1 blood concentration of VCAM-1 and MMP-1 in the highest quartile. In contrast, newborns who developed IVH accompanied by WMI were more likely to have a blood concentration in the highest quartile of CRP, SAA, TNF-alpha, IL-8, MCP-1, and MIP-1beta.

On day 7, newborns who had isolated IVH and those who had IVH accompanied by WMI were more likely than children with neither to have a concentration of IL-8 in the top quartile. In other ways, these two groups differed. For example, newborns with only IVH were more likely to have elevated concentrations of MCP-1, while newborns with both IVH and WMI more likely to have elevated concentrations of CRP, IL-1beta, TNF-alpha, I-TAC, and ICAM-1.

On day 14, newborns who had isolated IVH and those who had IVH accompanied by WMI were more likely than children with neither to have a concentration of MMP-9 in the top quartile. Newborns with only IVH were also more likely to have concentrations in the top quartile of MPO, and MCP-1. In contrast, newborns with both IVH and WMI were more likely to have elevated concentrations of CRP, SAA, IL-8, ICAM-1, and ICAM-3.

One and multiple days (Table 4)

Newborns who had IVH without WMI were no more likely than others to have an elevated concentration of any protein on only one day. They were, however, more likely to have concentrations of IL-8 in the top quartile on two or more days a week apart. IL-8, when elevated for two or more days, was the inflammation-related protein most strongly associated with IVH without WMI, as well as with IVH accompanied/followed by WMI, whether elevated once or on two occasions separated by one week.

In contrast, newborns who had IVH with WMI were more likely to have elevated concentrations of IL-8, MCP-4, and ICAM-3 on only one day, and elevated concentrations on two or more days a week apart of CRP, SAA, IL-1beta, IL-6, IL-8, MCP-1, MIP-1beta, ICAM-1, and IGFBP-1.

Discussion

Compared to infants with neither IVH nor WMI, infants who had IVH unaccompanied by WMI were more likely to have elevated concentrations (i.e., in the top quartile for each day and gestational age) of VCAM-1 and MMP-9 on day 1, IL-8 and MCP-1 on day 7, and MPO, MCP-1, and MMP-9 on day 14. This modest inflammatory profile is in contrast to what was seen for IVH accompanied by WMI. The risk of that entity was increased if the child had elevated day-1 concentrations of CRP, SAA, TNF-alpha, IL-8, MCP-1, MIP-1beta, elevated day-7 concentrations of CRP, IL-1beta, TNF-alpha, IL-8, I-TAC, ICAM-1, and elevated day-14 concentrations of CRP, SAA, IL-8, ICAM-1, ICAM-3, and MMP-9.

These findings lead to two inferences. One is that IVH should probably be viewed as two entities, IVH unaccompanied by WMI, and IVH accompanied by WMI. The other is that each entity is associated with inflammation, but IVH accompanied by WMI is associated with a stronger and more persistent (or recurring) inflammatory signal than IVH unaccompanied by WMI.

Inflammation and IVH

Because most IVH is evident during the first postnatal week (Paneth et al., 1993), blood concentrations of inflammation-related proteins on days 7 and 14 might reflect the consequences of IVH rather than provide information about antecedents. Thus, we focus on day-1 concentrations to draw inferences about what might have contributed to isolated IVH.

Evidence that inflammation places a preterm newborn at increased risk of IVH derives from several sources. First, newborns whose placenta was inflamed were at heightened risk of IVH (Kaukola et al., 2006; Moscuza et al., 2011; Zanardo et al., 2008). Second, IVH risk has also been increased among preterm newborns whose mother had clinical chorioamnionitis (Jackson et al., 2006). Third, *Ureaplasma urealyticum* found in the placenta parenchyma (Olomu et al., 2009), and in blood collected soon after birth (Viscardi et al., 2008), has been associated with increased risk of IVH. Fourth, preterm infants who had elevated cord or early postnatal blood concentrations of inflammation-related proteins, including IL-6 and IL-8 were at increased risk of IVH (Hansen-Pupp et al., 2005; Hansen-Pupp et al., 2007; Heep et al., 2003; Kassal et al., 2005; Krediet et al., 2006). Nevertheless, IVH has not always been associated with elevated concentrations of inflammation-related proteins in cord or early postnatal blood (Kallankari et al., 2010).

Inflammation and WMI

Systemic inflammation has been associated repeatedly with structural and functional indicators of perinatal WMI (Dammann and O'Shea, 2008; Glass et al., 2008; Laughon et al., 2011; Leviton et al., 2010; Leviton et al., 2011c; Martin et al., 2010; Shah et al., 2008).

In adults, the half-life of many cytokines is very brief, but the half-life in premature newborns is unknown. Here we report that the risk of IVH accompanied by WMI was increased if on two days a week apart the child had elevated concentrations of CRP, SAA, IL-1 β , IL-6, IL-8, MCP-1, MIP-1B, ICAM-1, and IGFBP-1. Either the half-life of each of these proteins is much longer in preterm newborns than adults, or our subjects were more likely to have recurrent, rather than sustained, inflammation.

Models of relationships among antecedents, IVH, and WMI

We are not sure how the weak inflammation signal associated with IVH and the stronger inflammation signal associated with WMI are related. We consider 3 generic models (Figure 1).

Because IVH very frequently antedates WMI, it is possible that IVH contributes to WMI. Model A postulates that a (weak) inflammatory stimulus contributes to the occurrence of

IVH, which, in turn, perhaps by inciting a stronger inflammatory (and/or excitotoxic) response, contributes to WMI (Figure 1A) (Vinukonda et al., 2010).

What is called “grade IV IVH” was originally interpreted as the result of an extension of the hemorrhage from the ventricle into the adjacent white matter (Papile et al., 1978). However, periventricular hyperechoic lesions adjacent to a ventricle containing blood might have more likely “causes” than extension of a hemorrhage or hemorrhagic infarction (Adler et al., 2010; Paneth et al., 1994).

Intracerebral hemorrhage promotes the up-regulation of inflammatory proteins in adjacent white matter (Power et al., 2003). The blood need not gain access to the white matter directly to result in WMI. More likely in some cases, blood in the ventricle stretches and thereby disrupts the ependymal barrier allowing intraventricular contents (including proteins that can promote inflammation and injury) access to the surrounding parenchyma (Cherian et al., 2003; Xue et al., 2005). Blood components injected into the white matter parenchyma of adult rodents up-regulate NMDA receptor subunit expression (Nakamura et al., 2005), thereby possibly increasing the risk of excitotoxic damage.

In model B, the inflammation stimulus contributes to both IVH and WMI, but the WMI occurrence is not dependent on IVH Figure 1B. A modification of this model, indicated by the dotted line, allows IVH to contribute to WMI occurrence.

Unlike the previous models, the models in Figure 1C postulate two relatively separate stimuli, one that contributes to the occurrence of IVH, and the other to WMI occurrence. In one variation of this model, IVH does not influence WMI risk, whereas in the other variation, IVH does contribute to WMI occurrence.

Strengths and limitations

Our study has several strengths. First, we included a large number of infants providing power to perceive a doubling of IVH risk associated with an elevated concentration of inflammation-related proteins. Second, we enrolled infants based on gestational age and not birth weight, thereby reducing confounding associated with fetal growth restriction (Arnold et al., 1991). Third, we minimized observer variability as best we could in the interpretation of ultrasound scans (Kuban et al., 2007). Fourth, our protein data are of high quality and high content validity (Fichorova et al., 2011; Hecht et al., 2011; Leviton et al., 2011a; McElrath et al., 2011)

Two characteristics of this study limit the inferences we can draw. First, our reliance on ultrasound to identify white matter damage likely failed to identify all the white matter damage that might have been documented with MRI (Leijser et al., 2010). Second, children had to survive until age 2 years and return for a developmental assessment to be included in this sample. Consequently, children who died with IVH are not included. To the extent that these children differed from survivors, this requirement might have introduced bias.

Conclusion

IVH should probably be viewed as two entities, IVH unaccompanied by WMI, and IVH accompanied by WMI. Although each entity is associated with inflammation, IVH accompanied by WMI is associated with a stronger inflammatory signal than is IVH unaccompanied by WMI.

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Figure 1.

Models of the relationships among an inflammation stimulus, intraventricular hemorrhage (IVH) and white matter injury (WMI). The dotted lines are not needed to define the models, but do raise the possibility that IVH might provide supplemental information about WMI risk.

Table 1

Sample derivation

	Yes	No
Enrolled	1506	
Had the 3 rd ("late") protocol head ultrasound scan ^a	1268	238
Had the 1 st &/or 2 nd protocol head ultrasound scan ^{b c}	1255	13
Survived to 2 years	1153	102
Had protein measured on 1+ of 3 protocol days ^d	868	285
Neither IVH ^e nor WMI ^f	677	
IVH without WMI	123	
IVH with WMI	68	
Had protein measured on 2+ of 3 protocol days	814	54
Neither IVH nor WMI	638	
IVH without WMI	116	
IVH with WMI	60	

^a protocol 3 scans were obtained between the 15th day and the 40th week

^b protocol 1 scans were obtained between the 1st and 4th days

^c protocol 2 scans were obtained between the 5th and 14th days

^d the protocol days for blood specimens are postnatal days 1, 7, and 14

^e intraventricular hemorrhage

^f white matter injury

Table 2

Percent of children classified by their ultrasound lesions who also had the disorders and dysfunctions listed on the left. These are column percents.

		Neither	IVH only	IVH + WMI	Row N
Gestational age (weeks)	23-24	17	26	44	176
	25-26	46	52	38	404
Birth weight (g)	500-749	36	40	46	322
Birth weight Z-score	< -2	6	3	3	48
Indication for delivery	Preeclampsia	15	9	7	115
	Fetal indication	4	2	1	31
Placenta bacteriology	Any organism	45	55	62	377
Placenta histology	Any inflammation	39	37	49	320
Head circumference	< -2	9	12	21	89
Z-score	-2, < -1	18	16	28	159
Cerebral palsy	Quadriplegia	2	4	31	41
	Diplegia	3	4	7	28
	Hemiplegia	1	2	12	16
GMFCS [§]	2+	2	4	22	33
MDI [‡]	< 55	12	15	34	123
	55-69	10	17	7	97
PDI [#]	< 55	11	19	43	126
	55-69	14	21	12	132
Column N		677	123	65	868

[§]GMFCS – Gross Motor Function Classification System

[‡]MDI – Mental Development Index

[#]PDI – Psychomotor Development Index

Table 3

Odds ratios (99% confidence intervals) for IVH without and with accompanying white matter injury (WMI) (defined as an echolucent lesion of the white matter parenchyma) 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. Odds ratios significant at $p < .01$ are in bold typeface.

	Day 1		Day 7		Day 14	
	IVH	IVH + WMI	IVH	IVH + WMI	IVH	IVH + WMI
CRP*	1.4 (0.7, 2.5)	2.4 (1.2, 4.9)	1.3 (0.7, 2.4)	2.1 (1.01, 4.3)	1.0 (0.6, 2.0)	2.5 (1.1, 5.3)
SAA	1.1 (0.6, 2.0)	2.9 (1.4, 6.0)	1.1 (0.6, 2.1)	1.6 (0.7, 3.3)	0.9 (0.4, 1.7)	2.2 (1.05, 4.8)
MPO	1.2 (0.7, 2.2)	0.6 (0.2, 1.4)	1.0 (0.5, 1.8)	1.4 (0.6, 3.0)	2.0 (1.1, 3.5)	1.8 (0.8, 3.9)
IL-1•	0.8 (0.4, 1.6)	2.0 (0.96, 4.1)	1.0 (0.5, 1.9)	2.2 (1.05, 4.5)	1.0 (0.5, 1.9)	1.5 (0.7, 3.3)
IL-6	1.3 (0.7, 2.3)	2.0 (0.9, 3.1)	1.5 (0.8, 2.7)	2.0 (0.96, 4.2)	0.9 (0.5, 1.8)	1.4 (0.6, 3.2)
IL-6R	1.3 (0.7, 2.4)	0.5 (0.2, 1.4)	0.7 (0.4, 1.4)	0.7 (0.3, 1.8)	0.9 (0.5, 1.7)	1.1 (0.5, 2.4)
TNF- α	1.3 (0.7, 2.3)	2.2 (1.1, 4.5)	1.5 (0.8, 2.6)	2.1 (1.03, 4.4)	1.3 (0.7, 2.3)	1.8 (0.8, 4.0)
TNF-RI	1.3 (0.7, 2.4)	1.3 (0.6, 2.9)	1.0 (0.5, 1.8)	1.5 (0.7, 3.3)	1.3 (0.7, 2.4)	1.5 (0.7, 3.3)
TNF-R2	1.3 (0.7, 2.4)	1.5 (0.7, 3.3)	1.1 (0.6, 2.0)	1.5 (0.7, 3.2)	1.1 (0.6, 2.0)	1.0 (0.4, 2.3)
IL-8 (CXCL8)	1.4 (0.8, 2.6)	3.0 (1.4, 6.1)	1.8 (1.02, 3.2)	3.4 (1.6, 6.7)	1.7 (0.9, 3.1)	3.2 (1.5, 7.0)
MCP-1 (CCL2)	0.8 (0.4, 1.5)	2.5 (1.2, 5.2)	1.8 (1.04, 3.2)	1.7 (0.8, 3.6)	1.8 (1.02, 3.3)	1.6 (0.7, 3.6)
MCP-4 (CCL13)	1.0 (0.6, 1.9)	1.5 (0.7, 3.1)	0.9 (0.5, 1.8)	1.3 (0.6, 2.6)	1.1 (0.6, 2.1)	1.3 (0.6, 3.0)
MIP-1B (CCL4)	1.8 (0.99, 3.2)	2.3 (1.1, 4.7)	0.9 (0.5, 1.6)	1.2 (0.6, 2.6)	0.9 (0.5, 1.7)	1.6 (0.7, 3.5)
RANTES (CCL5)	1.5 (0.8, 2.6)	0.8 (0.3, 1.9)	0.7 (0.4, 1.4)	0.6 (0.2, 1.4)	0.5 (0.3, 1.1)	0.8 (0.3, 2.0)
I-TAC (CXCL11)	1.0 (0.5, 1.9)	1.0 (0.5, 2.2)	1.2 (0.7, 2.3)	2.2 (1.04, 4.5)	1.1 (0.6, 2.2)	1.1 (0.5, 2.5)
ICAM-1 (CD54)	1.1 (0.6, 2.1)	1.6 (0.8, 3.4)	1.1 (0.6, 2.0)	2.1 (1.02, 4.4)	1.2 (0.6, 2.2)	2.6 (1.2, 5.7)
ICAM-3 (CD50)	1.3 (0.7, 2.3)	0.8 (0.3, 1.9)	1.4 (0.8, 2.5)	1.4 (0.7, 3.2)	1.8 (1.00, 3.3)	3.2 (1.5, 6.9)
VCAM-1 (CD106)	2.5 (1.4, 4.4)	1.2 (0.6, 2.8)	0.9 (0.5, 1.6)	1.1 (0.5, 2.4)	1.2 (0.6, 2.2)	0.5 (0.2, 1.4)
E-SEL (CD62E)	1.1 (0.6, 2.0)	1.4 (0.6, 3.0)	1.4 (0.8, 2.5)	1.6 (0.7, 3.5)	1.0 (0.5, 1.9)	1.2 (0.5, 2.6)
MMP-1	1.9 (1.1, 3.4)	1.1 (0.5, 2.4)	1.1 (0.6, 2.0)	0.9 (0.4, 2.0)	1.1 (0.6, 2.0)	1.1 (0.5, 2.6)
MMP-9	1.0 (0.5, 11.8)	0.5 (0.2, 1.2)	1.3 (0.7, 2.4)	1.2 (0.6, 2.7)	2.1 (1.2, 3.8)	2.4 (1.1, 5.2)
VEGF	1.0 (0.5, 1.9)	1.6 (0.7, 3.3)	0.9 (0.5, 1.7)	1.0 (0.4, 2.2)	1.1 (0.6, 2.0)	1.9 (0.9, 4.2)
VEGF-RI	0.8 (0.4, 1.6)	2.0 (0.95, 4.1)	1.4 (0.8, 2.5)	2.0 (0.95, 4.3)	1.0 (0.5, 1.8)	1.5 (0.7, 3.3)

	Day 1		Day 7		Day 14	
	IVH	IVH + WMI	IVH	IVH + WMI	IVH	IVH + WMI
VEGF-R2	1.7 (0.98, 3.1)	1.7 (0.8, 3.6)	1.2 (0.7, 2.2)	1.5 (0.7, 3.2)	1.0 (0.5, 1.9)	1.0 (0.4, 2.3)
IGFBP-1	1.3 (0.7, 2.4)	2.0 (0.97, 4.3)	0.8 (0.4, 1.5)	1.8 (0.8, 3.8)	1.1 (0.6, 2.1)	1.2 (0.5, 2.7)

* For abbreviations, see methods section of text.

Table 4

Odds ratio (and 95% confidence interval) for IVH without and with accompanying WMI among children who had the concentration in the top quartile (for gestational age and day specimen was obtained) of the protein(s) listed on the left on one or at least two days relative to that of children who did not have an elevated concentration of the same protein(s). The sample for these analyses consists of children who had proteins measured in blood collected on two separate days. The models are adjusted for gestational age (23-24, 25-26, 27 weeks). **Odds ratios significant at $p < .01$ are in bold.**

	IVH unaccompanied by WMI		IVH accompanied by WMI	
	1 day only	> 2 days	1 day only	> 2 days
CRP *	1.2 (0.6, 2.1)	1.6 (0.8, 3.1)	1.3 (0.5, 3.4)	3.7 (1.6, 8.7)
SAA	0.8 (0.5, 1.5)	1.1 (0.5, 2.2)	2.2 (0.9, 5.1)	3.4 (1.4, 8.6)
MPO	1.2 (0.6, 2.2)	1.5 (0.8, 3.0)	0.7 (0.3, 1.6)	1.3 (0.5, 3.1)
IL-1 β	1.0 (0.6, 1.8)	1.0 (0.4, 2.1)	2.1 (0.9, 4.8)	2.8 (1.1, 7.4)
IL-6	1.3 (0.7, 2.2)	1.4 (0.7, 2.8)	1.8 (0.8, 4.2)	2.8 (1.1, 7.0)
IL-6R	1.0 (0.6, 1.9)	0.9 (0.4, 1.8)	0.9 (0.4, 2.2)	0.7 (0.2, 1.8)
TNF- α	1.1 (0.6, 2.1)	1.8 (0.9, 3.6)	1.1 (0.4, 2.7)	4.0 (0.7, 9.2)
TNF-R1	0.9 (0.5, 1.7)	1.3 (0.7, 2.6)	1.8 (0.8, 4.0)	1.9 (0.7, 4.9)
TNF-R2	1.0 (0.6, 1.9)	1.2 (0.6, 2.5)	1.2 (0.5, 2.9)	1.9 (0.8, 4.7)
IL-8 (CXCL8)	1.4 (0.8, 2.6)	2.5 (1.2, 5.1)	3.5 (1.3, 9.3)	11 (3.9, 29)
MCP-1 (CCL2)	1.7 (0.9, 3.0)	1.8 (0.9, 3.6)	1.5 (0.6, 3.5)	2.9 (1.2, 7.0)
MCP-4 (CCL13)	0.8 (0.4, 1.5)	1.0 (0.5, 1.9)	2.3 (1.03, 5.3)	1.6 (0.6, 4.1)
MIP-1 β (CCL4)	1.2 (0.7, 2.3)	1.1 (0.5, 2.2)	1.1 (0.5, 2.8)	2.4 (1.03, 5.6)
RANTES (CCL5)	1.1 (0.6, 2.0)	0.8 (0.4, 1.7)	0.9 (0.4, 2.0)	0.6 (0.2, 1.7)
I-TAC (CXCL11)	1.1 (0.6, 2.0)	1.2 (0.6, 2.4)	1.0 (0.4, 2.3)	1.7 (0.7, 4.0)
ICAM-1 (CD54)	0.9 (0.5, 1.6)	1.3 (0.7, 2.6)	1.5 (0.6, 3.6)	3.8 (1.6, 9.0)
ICAM-3 (CD50)	1.7 (0.9, 3.2)	1.8 (0.9, 3.4)	2.4 (1.01, 5.6)	2.2 (0.9, 5.5)
VCAM-1 (CD106)	1.5 (0.8, 2.7)	1.6 (0.8, 3.1)	1.0 (0.4, 2.4)	1.0 (0.4, 2.6)
E-SEL (CD62E)	1.2 (0.7, 2.2)	1.3 (0.6, 2.6)	1.3 (0.5, 3.1)	2.2 (0.9, 5.3)
MMP-1	1.1 (0.6, 2.2)	1.6 (0.8, 2.9)	0.9, 0.3, 2.3)	1.1 (0.5, 2.8)
MMP-9	1.7 (0.9, 3.0)	1.7 (0.9, 3.6)	1.4 (0.7, 3.2)	1.5 (0.6, 4.1)
VEGF	1.0 (0.5, 1.8)	1.0 (0.5, 1.9)	1.7 (0.7, 4.0)	2.1 (0.9, 4.9)
VEGF-R1	0.8 (0.4, 1.5)	1.1 (0.6, 2.3)	2.2 (0.9, 5.1)	2.4 (0.9, 6.2)
VEGF-R2	0.9 (0.5, 1.7)	1.5 (0.8, 2.8)	1.2 (0.5, 2.8)	1.6 (0.7, 4.0)
IGFBP-1	1.4 (0.8, 2.5)	0.9 (0.4, 2.1)	1.5 (0.6, 3.4)	2.8 (1.1, 7.2)

* For abbreviations, see methods section of text.