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Circulating biomarkers in extremely preterm infants associated with ultrasound indicators of brain damage

Alan Leviton^a, Elizabeth N. Allred^a, Raina N Fichorova^b, T. Michael O'Shea^c, Lynn A Fordham^c, Karl KC Kuban^d, and Olaf Dammann^e

^aBoston Children's Hospital, and Harvard Medical School, Boston, MA, USA

^bBrigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

^cUniversity of North Carolina, Chapel Hill, NC, USA

^dBoston Medical Center and Boston University School of Medicine, Boston, MA, USA

^eTufts University School of Medicine, Boston, MA

Abstract

Aim—To assess to what extent the blood concentrations of proteins with neurotrophic and angiogenic properties measured during the first postnatal month convey information about the risk of sonographically-identified brain damage among very preterm newborns.

Methods—Study participants were 1219 children who had a cranial ultrasound scan during their stay in the intensive care nursery and blood specimens collected on 2 separate days at least a week apart during the first postnatal month. Concentrations of selected proteins in blood spots were measured with electrochemiluminescence or with a multiplex immunobead assay and the risks of cranial ultrasound images associated with top-quartile concentrations were assessed.

Results—High concentrations of multiple inflammation-related proteins during the first 2 postnatal weeks were associated with increased risk of ventriculomegaly, while high concentrations of just 3 inflammation-related proteins were associated with increased risk of an echolucent/hypoechoic lesion (IL-6, IL-8, ICAM-1), especially on day 7. Concomitant high concentrations of IL6R and bFGF appeared to modulate the increased risks of ventriculomegaly and an echolucent lesion associated with inflammation. More commonly high concentrations of putative protectors/repair-enhancers did not appear to diminish these increased risks.

Conclusion—Our findings provide support for the hypothesis that endogenous proteins are capable of either protecting the brain against damage and/or enhancing repair of damage.

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

Each of the authors declares that she/he has no conflict of interest.

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Key terms

Neurotrophic factors; Cytokines; Inflammation; Infant; Premature/blood; angiogenesis

1. Introduction

Over the past two decades, survival rates have increased dramatically for neonates born extremely preterm.^(1, 2) Nevertheless, neurodevelopmental impairments continue to affect more than one quarter of survivors.^(3, 4) Perinatal brain injury, as identified on neonatal brain ultrasound, is a strong predictor of adverse neurodevelopmental outcome.^(5–7)

The “deprivation of succor” hypothesis offers one explanation for why extremely preterm newborns are at increased risk of limitations of brain development, and of brain damage. It postulates that the mother and/or placenta provide the fetus of proteins needed for brain maturation, and that birth before the newborn can synthesize adequate amounts of these proteins deprives the brain of needed brain-maturation enhancers.^(8, 9) These proteins with neurotrophic properties, which are often growth factors, can stimulate oligodendrocyte progenitor cell proliferation,⁽¹⁰⁾ induce oligodendrocyte progenitors “to undergo continuous self-renewal,”⁽¹¹⁾ minimize oligodendrocyte progenitor apoptosis,⁽¹²⁾ and rescue motor neurons from axotomy.^(13–15) These findings have helped fuel the recognition that growth factors might be therapeutic for neurologic disorders.^(16–22)

We are not aware of any studies of extremely preterm humans that have evaluated the relationship between the concentrations of growth factors in the circulation during the weeks following birth and any indicator of brain damage. The ELGAN Study provided an opportunity to fill this void.

2. Methods

The ELGAN study is a multi-center prospective, observational study of the risk of structural and functional neurologic disorders in infants born before the 28th week of gestation.⁽²³⁾ The sample for this study is comprised of the 1219 children who had a cranial ultrasound scan during their stay in the intensive care nursery and also provided blood specimens collected on 2 separate days at least a week apart during the first postnatal month. Enrollment and consent procedures for this follow up study were approved by the institutional review boards of all participating institutions.

2.1 Newborn variables

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, reliance was placed on a 14 weeks fetal ultrasound (29%), LMP without fetal ultrasound (7%), and gestational age recorded in the log of the NICU (1%). The birthweight Z-score is the number of standard deviations the infant’s birthweight is above or below the median weight of infants at the same gestational age in referent samples not delivered for preeclampsia or fetal indications.^(24, 25)

2.2 Ultrasound protocol scans

Routine scans were performed by technologists at all participating institutions using high frequency transducers (7.5 and 10 MHz). Ultrasound studies always included the six standard axial views and five sagittal views using the anterior fontanel as the sonographic window ⁽²⁶⁾.

Of the 1506 infants enrolled, 1445 had had at least one set of protocol ultrasound scans and 895 had all three sets. Of these 1219 had proteins measured in blood obtained on 2 days a week apart. The three sets of protocol cranial ultrasound scans were defined by the postnatal day on which they were obtained. 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.

A standardized interpretation manual and data collection form were created ⁽²⁷⁾. Templates of moderate/severe ventriculomegaly were included in the manual. Observer variability was low.⁽²⁷⁾

All ultrasound scans were read by two independent readers who were not provided with clinical information. Each set of scans was first read by the study sonologist at the institution of the infant's birth. The images, usually as digital images on a CD imbedded in the software eFilm WorkstationTM (Merge Healthcare/Merge eMed, Milwaukee, WI) were sent to a sonologist at another ELGAN study institution for a second reading. The eFilm software allowed the second reader to review the images, 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 interpretation of moderate/severe ventriculomegaly (VM) or an echolucent lesion, the images were sent to a third (tie-breaking) reader who did not know what the readers reported.

2.3 Blood spot collection and measurement

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), the 14th postnatal day (range: 12–15 days), the 21st postnatal day (range: 19–23 days), and the 28th postnatal day (range: 26–29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at –70 °C in sealed bags with a desiccant until processed. Details about the elution of proteins from the blood spots are provided elsewhere.⁽²⁸⁾ 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) and the measurements of each analyte normalized to mg total protein.

2.4 Proteins measured

The Genital Tract Biology Laboratory at the Brigham and Women's Hospital in Boston Massachusetts eluted all blood spots as previously described and measured all proteins reported here. The laboratory used the Meso Scale Discovery to measure: C-Reactive Protein (CRP), SAA, Myeloperoxidase (MPO) Interleukin-1 β (IL-1 β), IL-6, IL-6 Receptor (IL-6R), Tumor Necrosis Factor- α (TNF- α), TNF Receptor-1 (TNFR-1), TNFR-2, IL-8

(CXCL8), Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES; CCL5), Intercellular Adhesion Molecule -1 (ICAM-1; CD54), Vascular Cell Adhesion Molecule-1 VCAM-1; CD106), Vascular Endothelial Growth Factor (VEGF), VEGF Receptor-1 (VEGFR-1, also known as sFLT-1), VEGFR-2 (KDR), Insulin-Like Growth Factor-1 (IGF) Binding Protein-1 (IGFBP-1), thyroid stimulating hormone (TSH), Metalloproteinase (MMP)-9, and erythropoietin (EPO).

A multiplex immunobead assay manufactured by R&D Systems (Minneapolis, MN) and a MAGPIX Luminex reader (R&D Systems) were used to measure angiopoietin-1 (Ang-1), Ang-2, placenta growth factor (PIGF), Neurotrophin-4 (NT-4), Brain Derived Neurotrophic Factor (BDNF), and basic Fibroblastic Growth Factor (bFGF). ELISA (R&D Systems) was used to measure IGF-1.

During the first two postnatal weeks, the 10th, 50th, and 90th centiles of the concentrations of many inflammation-related proteins tended to increase with increasing interval from birth, while these centiles of the concentrations decreased with increasing gestational age between 23 and 27 weeks.^(29, 30) Consequently, we stratified by gestational age category (23–24, 25–26, 27 weeks), birth weight Z-score (<−1 and −1), and postnatal day of blood collection (1, 7, 14, 21 and 28). The protein concentrations were analyzed in two sets measured years apart with the “earlier” (days 1, 7, and 14) specimens measured in 2010, and the “later” (days 21 and 28) in 2014 – 2015. The distributions of each were similar, but not identical. Consequently, we divided our sample into 30 groups defined by gestational age category (23–24, 25–26, 27 weeks), postnatal day of blood collection (1, 7, 14, 21 and 28), and measurement set (2009–2010, 2015). Because we were interested in the contribution of both high and low concentrations, and the concentrations of most proteins did not follow a normal distribution, the distribution of each protein’s concentration was divided into quartiles among children in each of the 30 groups (3 gestational age groups, 5 collection days, early and late epoch).

2.5 Data analyses

We began with the assumption that the effects of a high concentration of a potentially brain-damaging (pro-inflammatory) protein might not be apparent in the presence of a high concentration of a potentially brain-protecting protein (*i.e.*, one with neurotrophic properties), and similarly, the effects of a high concentration of a brain-protecting protein might not be apparent in the presence of a low concentration of a potentially brain-damaging protein. This prompted us to conduct analyses examining the risk of brain damage in light of the concentrations of two proteins, one with pro-inflammatory properties, and one with neurotrophic properties.

We created multinomial logistic regression models that allowed us to adjust for gestational age category (23–24, 25–26, 27 weeks) and birth weight Z-score < −1, and calculate odds ratios and 95% confidence intervals of the risk of each ultrasound lesion among children who had a top quartile concentration of one or both proteins relative to the risk among children who had concentrations of both proteins below the 75th centile. This is indicated in the tables by odds ratios set to 1.0. Evidence that a neurotrophic protein appears to have protective effects is a statistically significant increased risk of the ultrasound lesion among

children whose blood had a top quartile concentration of the inflammation-related protein and a lower concentration of the angio-neurotroph, while the risk was not increased among children whose blood had a top quartile concentration of the inflammation-related protein and a top quartile concentration of the angio-neurotroph.

3. Results

3.1 Sample description (Supplement Table 1)

A total of 125 children had ventricular enlargement that was deemed moderate or severe, while 89 had an echolucent lesion. These numbers include the 38 children who had both.

3.2. ventriculomegaly

3.2.1 Individual proteins on individual days (Supplement Table 2)—Day 21 was the only day that did not have a top-quartile protein concentration associated with increased risk of ventriculomegaly. On the first postnatal day, children who had a top-quartile concentration of CRP, SAA, IL-6, TNF-R2, IL-8, ICAM-1, EPO, IGFBP-1, or VEGF-R2 were at increased risk of ventriculomegaly.

When found on more than one day, top-quartile concentrations of the following proteins were associated with increased risk of ventriculomegaly: CRP (days 1, 7, and 14), SAA (days 1 and 28), IL-6 (days 1, 7, and 14), TNF- α (days 7 and 14), IL-8 (days 1, 7, and 14), ICAM-1 (days 1 and 14), EPO (days 1, and 7), VEGF-R1 (days 7 and 14), and Ang-2 (days 7 and 14).

On days 1, 7, and 14, the top-quartile concentrations of CRP, IL-6, and IL-8 were associated with increased risk of ventriculomegaly. On 2 of these 3 days, top-quartile concentrations of the following proteins were also associated with increased ventriculomegaly risk: TNF- α (days 7 and 14), ICAM-1 (days 1 and 14), EPO (days 1 and 7), VEGF-R1 (days 7 and 14), and Ang-2 (days 7 and 14). Top-quartile concentrations of SAA on 2 days (days 1 and 28) and top-quartile concentrations on only one day of IL-1 β (day 7), TNF-R1 (day 7), IGFBP-1 (day 1), and PIGF (day 7) were also associated with heightened risk. In contrast, top-quartile concentrations of IL-6R (day 21), MMP-9 (days 21 and 28), BDNF (day 21), and Ang-1 (day 21) were associated with reduced risk.

3.2.2 Individual proteins during the early and late epochs (Supplement Table 3)—Newborns who had a top-quartile concentration on 2 days during the first two postnatal weeks high concentrations were considered to have high concentrations during the early epoch, while such elevations during the third and fourth postnatal weeks were considered to be late epoch elevations. Consequently, CRP, SAA, IL-6, TNF- α , IL-8, ICAM-1, EPO, and VEGF-R1 were classified as having high concentrations during the early epoch. While these were associated with increased risk of ventriculomegaly, reduced risk was associated with top-quartile, late-epoch concentrations of MMP-9.

3.2.3 Sets of proteins during the early epoch (Table 1)—The most common pattern in this table is characterized by increased risks of ventriculomegaly associated with an inflammation-related protein (column headings) regardless of the concentration of the

putative neurotrophic and/or angiogenic protein (listed on the left). We give the name “damage promoter resistant to reduction” to this pattern, which is exemplified by top-quartile concentrations (on two days of the early epoch) of TNF- α regardless of the concentrations of MMP-9, EPO, VEGF, VEGF-R1, VEGF-R2, NT-4, BDNF, IGF-1, PIGF, Ang-1, and Ang-2. It also occurred with high concentrations of IL-8 regardless of the concentrations of MMP-9, EPO, VEGF, VEGF-R1, VEGF-R2, BDNF, PIGF, Ang-1, and Ang-2, as well as with high concentrations of ICAM-1 regardless of the concentrations of EPO, VEGF, VEGF-R1, and VEGF-R2.

We have given the name, “protection in the face of adversity” to the pattern characterized by statistically-significantly increased risk of the ultrasound abnormality only when top-quartile concentrations of the inflammation-associated protein are not accompanied by top-quartile concentrations of the neurotrophic (protector/repair-enhancer) protein. This pattern occurred during the early epoch with two days of top-quartile concentrations of IL-6R, RANTES, and bFGF in the presence of top-quartile concentrations of TNF- α , IL-8, and ICAM-1. It was also seen with top-quartile concentrations of IL-8 and top-quartile concentrations of NT-4, and when top-quartile concentrations of ICAM-1 co-occurred with top-quartile concentrations of Ang-1.

Increased risks of ventriculomegaly occurred when top-quartile concentrations of ICAM-1 were accompanied by top-quartile concentrations of EPO, VEGF, and VEGF-R2. We apply the name “damage cause and/or consequence” to this pattern because it occurred only when the concentrations of both proteins were in the top quartile, which might indicate that both proteins contribute to damage, but could also reflect, (in part, perhaps) the consequences of damage.

3.2.4 Sets of proteins during the late epoch (Table 2)—No statistically significantly increased or decreased risk is seen with two days of top-quartile concentrations of an inflammation-related protein and neurotrophic and/or angiogenic protein during the second half of the first month.

3.3 echolucent/hypoechoic lesion

3.3.1 Individual proteins on individual days (Supplement Table 4)—On days 1 and 21, a top-quartile concentration of none of the 27 proteins was associated with increased risk of an echolucent lesion. On the seventh postnatal day, children who had a top-quartile concentration of IL-6, IL-8, and ICAM-1 were at increased risk of an echolucent lesion, while on day 14 a top-quartile concentration of only ICAM-1, and on day 28 a top-quartile concentration of only IL-8 were associated with increased risk.

Top-quartile concentrations of RANTES on days 1, 7, and 14 were associated with reduced risk. Top-quartile concentrations of other proteins were also associated with reduced risk (IL-6R, VCAM-1, and bFGF on day 1, and BDNF on day 21).

3.3.2 Individual proteins during the early and late epochs (Supplement Table 5)—Newborns who had top-quartile concentrations of IL-8, ICAM-1, and EPO on 2 of the days during the early epoch were at increased risk, while top-quartile concentrations of no

protein on both days of the late epoch were associated with either increased or decreased risk.

3.3.3 Sets of proteins during the early epoch (Table 3)—The “damage promoter resistant to reduction” pattern applies to what was seen with high concentrations of IL-8 regardless of the concentrations of EPO, VEGF-R1, VEGF-R2, and IGF-1, as well as with high concentrations of ICAM-1 regardless of the concentrations of VEGF-R1.

The “protection in the face of adversity” pattern occurred during the early epoch with two days of top-quartile concentrations of IL-6R and bFGF in the presence of a top-quartile concentration of TNF- α . This pattern was also exemplified by top-quartile concentrations of IL-6R, MMP-9, RANTES, NT-4, and bFGF in the presence of top-quartile concentrations of IL-8, as well as by top-quartile concentrations of IL-6R, MMP-9, and RANTES in the presence of top-quartile concentrations of ICAM-1.

The “damage cause and/or consequence” pattern was seen when top-quartile concentrations of TNF- α co-occurred with top-quartile concentrations of IGF-1 and Ang-1, when top-quartile concentrations of IL-8 co-occurred with top-quartile concentrations of IL-6R, MMP-9, RANTES, when top-quartile concentrations of IL-8 co-occurred with top-quartile concentrations of VEGF, BDNF, PIGF, and Ang-1, and when top-quartile concentrations of ICAM-8 co-occurred with top-quartile concentrations of EPO, VEGF, BDNF, and IGF-1.

The only early-epoch example of “protection in the absence of adversity” pattern occurred with top-quartile concentrations of IL-6R in the absence of top-quartile concentrations of ICAM-1.

3.3.4 Sets of proteins during the late epoch (Table 4)—As with the risk of ventriculomegaly associated with two days of high concentrations in the late epoch, so too, with the risk of an echolucent lesion associated with two days of high concentrations in the late epoch. No statistically significantly increased or decreased risks occurred, thereby depriving us of the opportunity to find any characteristic pattern.

4. Discussion

Our main finding is that the brain-damaging effects of high concentrations of an inflammation-related protein are sometimes not apparent in the presence of high concentrations of a protein with neurotrophic properties, and that the brain-protecting effects of high concentrations of a protein with neurotrophic properties are sometimes not apparent in the presence of high concentrations of an inflammation-related protein. We discuss our findings in light of four patterns of inter-relationships among two proteins and brain ultrasound images.

4.1 Modulation of damage promotion (or protection in the face of adversity) pattern

We have previously reported that elevated concentrations of some of the inflammation-related proteins were associated with increased risk of ventriculomegaly, and less so with an echolucent lesion.^(31, 32) Thus, we previously documented a damage-promoter pattern for

both ventriculomegaly and an echolucent lesion. What is new in this report is evidence that when the concentrations of some of these proteins are high, elevated concentrations of IL-6R, RANTES, and bFGF appear to modulate the expected increased risks of ventriculomegaly, and elevated concentrations of IL-6R, MMP-9, RANTES, and bFGF appear to modulate the expected increased risks of an echolucent lesion. Only one of these, bFGF, can be considered a growth factor. IL-6R has anti-inflammatory properties,⁽³³⁾ while MMP-9, plays a major role in synaptic plasticity,⁽³⁴⁾ and RANTES has angiogenic (repair) properties.⁽³⁵⁾ Among children born at term, low concentrations of RANTES were associated with increased risk of presumed developmental delay and separately of presumed autism,⁽³⁶⁾ although this has yet to be replicated.⁽³⁷⁾

4.2 Damage promoter resistant to reduction pattern

In the face of systemic inflammation, elevated concentrations of EPO, BDNF, IGF-1, and PIGF, did not appear to protect against the occurrence of ventriculomegaly or an echolucent-lesion. Each of these, EPO,^(38, 39) BDNF,⁽⁴⁰⁾ IGF-1,⁽⁴¹⁾ and PIGF,⁽⁴²⁾ have shown neuroprotective properties. We are not sure why they did not display neuroprotective properties here. Perhaps these are not failures of neuroprotection, but rather indicators of the amount of damage (see below).

4.3 Damage promoter or marker of brain damage (cause or consequence) pattern

In light of the evidence suggesting that high concentrations of growth factors should be able to protect the brain, or promote repair of brain damage,^(43, 44) our finding that sometimes top-quartile concentrations are associated with increased, rather than decreased risk of brain damage deserves explanation. Rather than discuss individual proteins, we provide an overview of potential mechanisms.

4.3.1. brain damage results in the release of indicators of inflammation and growth factors—Brain damage can be followed by increased concentrations peripherally of TNF,^(45–49) IL-6,⁽⁴⁹⁾ IL-8,⁽⁵⁰⁾ ICAM-1,^(48, 49) and MMP-9.⁽⁴⁸⁾ Brain damage also promotes the release and/or upregulation of the expression of neurotrophic proteins so that increased concentrations are evident in blood or CSF. These proteins include IGF-1,^(51–53) BDNF,^(54–57) bFGF,^(58, 59) NT-4,⁽⁶⁰⁾ VEGF,^(61–63) EPO,^(64–67). In light of these observations, some of what we found might be a consequence of brain damage rather than contributions to brain damage.

4.3.2. inflammation is often accompanied by increased concentrations of growth factors—In our cohort of extremely preterm newborns, those who had high concentrations of inflammation-related proteins during the first postnatal month also tended then to have high concentrations of putative neuroprotectors, including NT4,⁽⁶⁸⁾ BDNF,⁽⁶⁸⁾ bFGF,⁽⁶⁸⁾ VEGF,⁽⁶⁹⁾ VEGF-R1,⁽⁶⁹⁾ VEGF-R2,⁽⁶⁹⁾ PIGF,⁽⁶⁹⁾ Ang-1,⁽⁶⁹⁾ Ang-2,⁽⁶⁹⁾ EPO,⁽⁷⁰⁾ and TSH.⁽⁷¹⁾ Others, too, have found that high concentrations of inflammation-related proteins appear to be able to increase the concentrations of neuroprotectors, such as BDNF.^(72–79) And yet, inflammatory stimuli can also decrease BDNF concentrations and expression.^(80–83)

Inflammation and angiogenesis/vascular remodeling can promote/support each other,^(84–87) while PIGF also appears to promote inflammation.^(88–90) Then again, inflammation can contribute to blood-brain-barrier dysfunction,^(91–97) as can brain damage.^(98–103)

4.3.3 pleiotrophic effects—In addition to angiogenic proteins having the pro-inflammatory properties described above, they also have anti-inflammatory properties.⁽¹⁰⁴⁾ By definition, they are pleiotrophic. This applies to BDNF,^(105–107) bFGF,^(108, 109) IGF-1,⁽¹¹⁰⁾ and EPO,^(111, 112). The pleiotrophic properties can also include brain damage, which has been documented for IGF,^(113, 114) and EPO.^(115, 116) Consequently, simple explanations are unlikely to be adequate in our attempts to account for what we found.

Limitations and strengths—Perhaps our main weakness is the interrelatedness of early and late systemic inflammation, which limits our ability to tease apart the contributions of each to the occurrence of ultrasound-documented brain damage. We are also limited by the relatively small number of proteins measured. Inflammation is a broad and complex phenomenon,^(117, 118) and we have assessed a very small part of it.

We relied on blood specimens obtained for clinical indications. As their physiology became more stable, infants were less likely than their sicker peers to have blood drawn on days 14, 21, and 28. Consequently, selection bias probably occurred to some extent, especially for the late epoch. Another consequence of fewer children with protein measurements in the late epoch is a loss of power.

Our ability to perceive odds ratio as low as 1.8 as statistically significant attests to our power, at least for the early epoch. Other strengths include efforts to minimize observer variability in the assessment of ultrasound scans,⁽²⁷⁾ and protein data of high quality,⁽¹¹⁹⁾ and high content validity.^(29, 120–122)

4.4 Synthesis/inferences/conclusions

Our findings continue to support the inference that systemic inflammation, especially if sustained, is a correlate (either cause or consequence) of brain damage.⁽¹²³⁾ What is new is our documentation that high concentrations of selected neurotrophic factors are indeed associated with some diminution of the brain damage associated with high concentrations of inflammation-related proteins. We view this as support for the hypothesis that endogenous proteins are capable of either protecting the brain against adversity, or enhancing repair of the damage caused by the adversity.

On the other hand, we also found that in many situations, high concentrations of putative protectors did not reduce the risk of brain damage. We offer the possibility that this reflects the release of both damage promoters and protectors into the circulation as a consequence of damage to the white matter parenchyma, and to the blood-brain barrier.^(45–67)

In essence, the high concentrations of protectors seen in this situation are a consequence of brain damage, and do not necessarily reflect an inability to diminish damage or enhance early repair. In such situations, the greater the damage, the higher the concentrations of protectors, as well as damage promoters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Proteins with neurotrophic and angiogenic properties have the capacity to promote growth and maturation of brain cells, and to protect against damage or enhance repair.
- We found that extremely preterm newborns who have high blood concentrations of some inflammation-related proteins are sometimes at reduced risk of ultrasound indicators of brain damage if they also have high blood concentrations of specific proteins with neurotrophic and angiogenic properties.
- Elevated blood concentrations of several proteins with known neuroprotective capabilities were sometimes paradoxically associated with increased risk of brain damage. To what extent this observation reflects the consequences of brain damage remains unknown.

Table 1

Sample description

	Yes	No
Enrolled	1506	
Had a qualifying protocol ultrasound scan	1455	51
Had blood specimens collected on only one day	1220	235
Had blood specimens collected on 2 days	1219	1
Had ventriculomegaly alone	87	1041
Had both ventriculomegaly and an echolucent lesion	38	1041
Had an echolucent lesion alone	51	1041

Table 2

This is a summary of Appendix Tables 1–4, which assess one protein at a time for the early epoch (left 3 columns) and separately for the late epoch (right 3 columns). Upper case indicates increased risks of for ventriculomegaly (VM), and for an echolucent lesion (EL) on an ultrasound scan obtained during the first postnatal month that are associated top quartile concentrations of the protein that heads each column relative to the risk among infants whose concentration (within gestational age category and day blood was obtained) of that protein was below the top quartile. Capital letters indicate significantly ($p < 0.05$) increased risk, while *lower case italics* indicate reduced risk ($p < 0.05$). The risks were adjusted for gestational age and birth weight Z-score -1).

	Individual days					Epochs	
	Day 1	Day 7	Day 14	Day 21	Day 28	Early [*]	Late [†]
CRP	VM	VM	VM			VM	
SAA	VM				VM	VM	
MPO							
IL-1 β		VM					
IL-6	VM	VM, EL	VM			VM	
IL-6R	<i>el</i>			<i>vm</i>			
TNF- α		VM	VM			VM	
TNF-R1			VM				
TNF-R2	VM						
IL-8	VM	VM, EL	VM		EL	VM, EL	
RANTES	<i>el</i>	<i>el</i>	<i>el</i>				
ICAM-1	VM	EL	VM, EL			VM, EL	
VCAM-1	<i>el</i>						
MMP-9				<i>vm</i>	<i>vm</i>		<i>vm</i>
TSH							
EPO	VM	VM				VM, EL	
NT-4							
BDNF				<i>vm</i>			
bFGF	<i>el</i>						
IGF-1							
IGFBP-1	VM						

	Individual days					Epochs	
	Day 1	Day 7	Day 14	Day 21	Day 28	Early [*]	Late [‡]
VEGF							
VEGF-R1		VM	VM			VM	
VEGF-R2	VM						
PlGF		VM					
Ang-1				<i>vm</i>			
Ang-2		VM	VM				
Column total	1119	1140	1031	938	878	1151	767

^{*} Early epoch: days 1, 7, and 14

[‡] Late epoch: days 21 and 28

Table 3

This is a summary of Appendix Tables 5–8, which address two proteins at a time for the early epoch (left 3 columns) and separately for the late epoch (right 3 columns). The inflammation-related protein is identified at the top of each set of columns, and the neurotrophic proteins are identified on the left. The presence of VM or EL in a cell indicates that a top-quartile concentration of the neurotrophic protein on two days of the identified epoch modulated the increased risk associated with a top quartile concentration of the inflammation-related protein on two days of that epoch. **Bold** indicates odds ratios significantly > 1 ($p < 0.05$) and ***bold italic*** indicates odds ratios significantly < 1 ($p < 0.05$).

Proteins	Early epoch			Late epoch		
	TNF- α	IL-8	ICAM-1	TNF- α	IL-8	ICAM-1
IL-6R	VM, EL	VM, EL	VM, EL			
MMP-9	VM	VM, EL	VM, EL			
RANTES	VM	VM, EL	VM, EL			
EPO	VM	VM, EL	VM, EL			
VEGF	VM	VM, EL	VM, EL			
VEGF-R1	VM	VM, EL	VM, EL			
VEGF-R2	VM	VM, EL	VM			
NT-4	VM	VM, EL				
BDNF	VM	VM, EL	VM, EL			
bFGF	VM, EL	VM, EL	VM			
IGF-1	VM	VM				
PIGF	VM	VM, EL				
Ang-1	VM, EL	VM, EL	VM			
Ang-2	VM	VM				

^aThe quartiles were determined within gestational age category and day blood was obtained.

^bThe early epoch consists days 1, 7, and 14.

^cThe late epoch consists days 21 and 28

^dThe logistic regression models included variables for gestational age and birth weight Z-score < -1.

Odds ratios (95% confidence intervals) for an echolucent lesion on an ultrasound scan obtained during the first postnatal month comparing children with (+) and without (–) a top quartile^a blood concentration of each of two proteins measured in specimens obtained on two days of the early epoch^d to children without a top quartile concentration of both proteins.^c **Bold** indicates odds ratios significantly > 1 (p < 0.05).

Table 4

	Early epoch				Late epoch		
	TNF- α	IL-8	ICAM-1		TNF- α	IL-8	ICAM-1
IL-6R	–	EL	EL				
MMP-9	–		EL				
RANTES	–		EL				
EPO	–		EL				
VEGF	–						
VEGF-R1	–						
VEGF-R2	–						
NT-4	–		EL				
BDNF	–	EL	EL				
bFGF	–						
IGF-1	–						
PIGF	–						
Ang-1	–						
Ang-2	–						

^aThe quartiles were determined within gestational age category and day blood was obtained.

^bThe early epoch consists days 1, 7, and 14.

^cThe late epoch consists days 21 and 28

^dThe logistic regression models included variables for gestational age and birth weight Z-score < –1.