HHS Public Access

Author manuscript

*Int J Dev Neurosci*. Author manuscript; available in PMC 2019 May 01.

Published in final edited form as:

*Int J Dev Neurosci*. 2018 May ; 66: 45–53. doi:10.1016/j.ijdevneu.2018.01.001.

# Neonatal systemic inflammation and the risk of low scores on measures of reading and mathematics achievement at age 10 years among children born extremely preterm

**Alan Leviton**[1], **Olaf Dammann**[2], **Elizabeth N Allred**[1], **Robert M Joseph**[3], **Raina N Fichorova**[4], **T. Michael O'Shea**[5], and **Karl CK Kuban**[6]

[1]Boston Children's Hospital and Harvard Medical School, Boston, MA, USA

[2]Tufts University School of Medicine, Boston, MA, USA

[3]Boston University School of Medicine, Boston, MA, USA

[4]Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

[5]University of North Carolina School of Medicine, Chapel Hill NC, USA

[6]Boston Medical Center and Boston University School of Medicine, Boston, MA, USA

## Abstract

**Background—**Difficulties with reading and math occur more commonly among children born extremely preterm than among children born at term. Reasons for this are unclear.

**Methods—**We measured the concentrations of 27 inflammatory-related and neurotrophic/angiogenic proteins (angio-neurotrophic proteins) in multiple blood specimens collected a week apart during the first postnatal month from 660 children born before the 28$^{th}$ week of gestation who at age 10 years had an IQ ≥ 70 and a Wechsler Individual Achievement Test 3$^{rd}$ edition (WIAT-III) assessment. We identified four groups of children, those who had a Z-score ≤ −1 on the Word Reading assessment only, on the Numerical Operations assessment only, on both of these assessments, and on neither, which served as the referent group. We then modeled the risk of each learning limitation associated with a top quartile concentration of each protein, and with high and lower concentrations of multiple proteins.

**Results—**The protein profile of low reading scores was confined to the third and fourth postnatal weeks when increased risks were associated with high concentrations of IL-8 and ICAM-1 in the presence of low concentrations of angio-neurotrophic proteins. The profile of low math scores was very similar, except it did not include ICAM-1. In contrast, the profile of low scores on both assessments was present in each of the first four postnatal weeks. The increased risks associated

**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.

with high concentrations of TNF-α in the first two weeks and of IL-8 and ICAM-1 in the next two weeks were modulated down by high concentrations of angio-neurotrophic proteins.

**Conclusions—**High concentrations of angio-neurotrophic proteins appear to reduce/moderate the risk of each learning limitation associated with systemic inflammation. The three categories of limitations have protein profiles with some similarities, and yet some differences, too.

### Keywords

Developmental outcome; Reading; Mathematics; Inflammation; Very premature infant; Neuroprotection; angioneurins

## 1. Introduction

Compared to children born at term, those born very preterm are at increased risk of reading(1) and related limitations,(2) and math limitations.(3–7) These children are more likely than others to have structural and functional impairments of the brain.(8–12) We reasoned that if very preterm children who had systemic inflammation during the first postnatal month are more likely than others to have structural and/or functional abnormalities of the brain,(13–16) and children who have structural and/or functional abnormalities of the brain are more likely than others to have learning limitations,(8–12) then children who had systemic inflammation during the first postnatal month might be at increased risk of learning problems.

The “paucity of protectors” hypothesis offers one explanation for why extremely preterm newborns are at increased risk of limitations of brain development, and of brain damage. According to this hypothesis the mother and/or placenta provide the fetus with proteins needed for brain maturation, and birth before the newborn can synthesize adequate amounts of these proteins deprives the brain of needed brain-maturation enhancers.(17) These proteins with neurotrophic properties, which are often growth factors, are now recognized as having angiogenic properties,(18–20) and capable of reducing the risk of brain damage and/or promoting repair.(21, 22) Some of these proteins are viewed as mainly neurotrophic, while others are primarily considered angiogenic.(23, 24) Regardless, these proteins almost invariably have both neurotrophic and angiogenic properties, (24–32) prompting the names “angioneurins”(33–39) and “angioglioneurins.”(40) To emphasize the trophic properties of these proteins, we prefer the name “angio-neurotrophic protein.”

Data from the ELGAN (Extremely Low Gestational Age Newborn) Study provided an opportunity to explore this possibility because of the availability of the concentrations of 27 proteins with inflammation-related and/or neurotrophic/angiogenic properties in blood specimens obtained during the first postnatal month from children born before the 28$^{th}$ week of gestation and assessments of their educational achievements at age 10 years with the Wechsler Individual Achievement Test, Third Edition (WIAT-III).

## 2. Methods

### 2.1 Participants

The ELGAN study is a multi-center prospective, observational study of the risk of structural and functional neurologic disorders in extremely preterm infants.(41) A total of 1506 infants born before the 28th week of gestation were enrolled during the years 2002–2004 and 1198 survived to age 10 years. At age 10 years, 966 of these were recruited for an age-appropriate assessment of cognition, executive function, behaviors, and achievement, 889 (92%) returned for follow up, and 874 were administered the neurocognitive tests (Table 1). Because children who had early systemic inflammation are at heightened risk of cognitive impairment,(42) and children who are cognitively impaired do not do well on academic achievement tests, we wanted to restrict our search for any relationship between early systemic inflammation and learning limitations not attributed to global limitation to children who were not cognitively impaired. Thus, the sample for the analyses presented here is restricted to the 666 children who had a DAS-II verbal IQ ≥ 70 and non-verbal IQ ≥ 70. Enrollment and consent procedures for this follow up study were approved by the institutional review boards of all participating institutions.

### 2.2 Procedures

All families who participated in the previous follow up were contacted by mail and then by phone to invite them to participate in the 10-year follow up. Lost to follow-up families were searched for on state vaccination registries, and other openly-available websites. Facebook was also used where approved by the local institution's IRB.

Families willing to participate were scheduled for one visit during which all of the tests reported here were administered in 3 to 4 hours, including breaks. The assessments were selected to provide the most comprehensive information about neurocognitive and academic function in one testing session. While the child was tested, the parent or primary caregiver completed questionnaires regarding the child's educational, medical, and neurological status and behavior, and completed the Kaufman Brief Intelligence Test – 2 (KBIT-2) nonverbal subscale.(43)

**2.2.1 General cognitive ability—**General cognitive ability (or IQ) was assessed with the School-Age Differential Ability Scales–II (DAS-II) Verbal and Nonverbal Reasoning scales.(44) We required that children have scores of 70 or higher on both scales to be included in our sample for these analyses.

**2.2.2 Academic Function—**The Wechsler Individual Achievement Test-III (WIAT-III) provides grade- and age-adjusted standard scores for the Word Reading and Numeric Operations subtests.(45) We defined each learning limitation as a Z-score ≤ −1 (*i.e.*, below the 16th centile, which is equivalent to a score ≤ 85) on a grade-based WIAT-III achievement test.(46) Thus, we identified four mutually-exclusive groups, reading limitation only (Word Reading Z-score ≤ −1, Numerical Operations Z-score > −1), math limitation only (Numerical Operations Z-score ≤ −1, Word Reading Z-score > −1), both reading and math

limitations (Word Reading Z-score ≤ −1, Numerical Operations Z-score ≤ −1), and neither limitation (Word Reading Z-score > −1, Numerical Operations Z-score > −1) (Table 1).

Because the reading and math limitations occur more commonly than would be expected if they were independent of each other,(9, 47–51) we considered it prudent to view children with the combination as possibly having protein profiles that differed from those of children with either isolated limitation. Thus, we have three outcomes of interest, isolated reading limitation (*i.e.*, not accompanied by a math limitation), isolated math limitation (*i.e.*, not accompanied by a reading limitation), and the combination of reading and math limitations. Children with these entities were compared to children who had no reading or math limitation.

**2.2.3 Systemic inflammation ascertainment procedures—**Drops of blood were collected on filter paper on the first postnatal day (range: 1–3 days), the 7$^{th}$ postnatal day (range: 5–8 days), the 14$^{th}$ postnatal day (range: 12–15 days), the 21$^{st}$ postnatal day (range: 19–23 days), and the 28$^{th}$ postnatal day (range: 26–29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at −70°C in sealed bags with a desiccant until processed. Details about the elution of proteins from the blood spots are provided elsewhere.(52)

Because some spots are thicker than others, we have normalized our measurements to mg of total protein in the eluent. 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.

## 2.3 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, Myloeperoxidase (MPO) Interleukin-1 β (IL-1β), 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) was used to measure angiopoietin-1 (Ang-1), Ang-2, placenta growth factor (PIGF), Neurotrophin-4 (NT-4), Brain Derived Neurotrophic Factor (BDNF), and basic Fibroblast Growth Factor (bFGF). ELISA (R&D Systems) was used to measure IGF-1.

One set of measurements was made in 2009–2010, and the second in 2015, and, while the distributions of each were similar, they were not identical. In addition, the protein concentrations varied with gestational age, and with the postnatal day of collection.(53, 54) Consequently, we stratify our sample into 30 groups defined by two measurement sets (2009–2010, 2015), three gestational age categories (23–24, 25–26, 27 weeks), and five postnatal days of blood collection (1, 7, 14, 21 and 28). Because we were interested in the contribution of high concentrations, and the concentrations of most proteins did not follow a normal distribution, the distribution of each protein's concentration was dichotomized into the highest quartile and the lower three quartiles among children in each of the 30 groups.

### 2.4 Data analyses

Because we did not evaluate achievement scores in a full-term comparison group, we relied on comparisons to the WIAT-III normative sample.(45) We calculated Z-scores to allow for the differences in school grade at the time of the assessment. In our sample, the distribution of grade-adjusted scores on the WIAT-III Word Reading and Numerical Operations subtests had a larger number of children at the low end of the distribution than expected, prompting us to focus on those who had scores one or more standard deviations below the expected mean (Z-score $\leq -1$).

We tested the null hypotheses that among children who have both DAS-II Verbal and Non-verbal IQ scores $\geq 70$, elevated protein concentrations (defined as in the top quartile) are not associated with risk of three learning limitations, reading only (Word Reading Z-scores $\leq -1$ with Numerical Operations Z-scores $> -1$), math only (Numerical Operations Z-scores $\leq -1$ with Word Reading Z-scores $> -1$), and both reading and math limitations (Z-scores $\leq -1$ on both Word Reading and Numerical Operations assessments). Thus, in essence, we tested two hypotheses for each dysfunction. One is that elevated concentrations of inflammation-related proteins are associated with increased risk, while the other is that elevated concentrations of angio-neurotrophic proteins are associated with reduced risk.

**1. Individual days: single proteins—**We evaluated the hypothesis that children who had a top quartile concentration of each protein on each day were not at greater risk of a reading limitation, and separately of a mathematics limitation, than children who had a lower concentration of each protein on each of five days during the first postnatal month (Appendix Tables Reading-A, Math-A, and Reading-and-Math-A, which are summarized in Table 2).

**2. Early epoch: single proteins—**We evaluated the hypotheses that children who had a top quartile concentration of each protein on at least two days during the first two postnatal weeks (when specimens were obtained on days 1, 7, and 14) were not at greater risk of a reading limitation, and separately of a mathematics limitation, than children who had lower concentrations (Appendix Tables Reading-B, Math-B, and Reading-and-Math-B, which are also summarized in Table 2).

**3. Late epoch: single proteins—**Similarly, we compared children who had top quartile concentrations of each protein on both days 21 and 28 (the late epoch) to their peers with

lower concentrations of each protein (Appendix Tables Reading-B, Math-B, and Reading-and-Math-B, which are also summarized in Table 2).

**4. Early epoch: Combinations of inflammation-related and angio-neurotrophic proteins—**We evaluated the hypotheses that children who had top quartile concentrations of one or both of two proteins (one an inflammation-related protein and the other an angio-neurotrophic protein) on at least two days during the first two postnatal weeks (i.e., the early epoch) were not at higher or lower risk of each learning limitation than children who had lower concentrations of both proteins (Appendix Tables Reading-C, Math-C, and Reading-and-Math-C, and summarized in Table 3).

**5. Late epoch: Combinations of inflammation-related and angio-neurotrophic proteins—**Similarly, we evaluated the hypotheses that children who had top quartile concentrations of one or both of two proteins (one an inflammation-related protein and the other an angio-neurotrophic protein) on two days a week apart during the third and fourth postnatal weeks (i.e., the late epoch) were not at higher or lower risk of each learning limitation than children who had lower concentrations of both proteins (Appendix Tables Reading-D, Math-D, and Reading-and-Math-D, and summarized in Table 4).

We created multinomial logistic regression models that allowed us to calculate odds ratios and 95% confidence intervals of the risk of each LL among children who had a top quartile concentration of each protein to the risk among children who had a concentration below the $75^{th}$ centile. In separate multinomial logistic regression models, we evaluated the risk of each LL associated with top quartile concentrations of two proteins simultaneously relative to the risk among children who had lower concentrations of both proteins. The indicator that an angio-neurotrophic protein appears to have protective effects is a statistically significant increased risk of the LL 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.

In the larger sample, children who had top quartile concentrations of inflammation-related proteins were no more likely than their peers who had lower concentrations to have a mother who had limited educational achievement, a low KBIT-2, or was eligible for government-provided medical care insurance (Medicaid). Thus, confounding by social class is not an issue. Similarly, other potential confounders such as the child's sex were also not associated with systemic inflammation. On the other hand, low birth weight Z-score, indicative of fetal growth restriction, is associated in this sample with elevated concentrations of some proteins,(55, 56) and with low Word Reading scores.(57) Thus, low birth weight Z-score is a potential confounder. Low maternal education achievement (defined as a KBIT-2 < 85) is not associated with protein concentration,(56) and is therefore not a confounder. We have adjusted for this variable, however, because we consider it the most likely effect modifier.

## 3. Results

The details of individual learning limitations are provided in the appendix along with the relevant tables. Here, we present an overview and identify commonalities and differences among the three learning limitation groups (reading only, math only, both reading and math).

### 3.1 Sample (Table 1)

Of the 660 children for whom 2 blood specimens were available, who had both DAS-II verbal and non-verbal IQ scores of IQ ≥ 70, and had a WIAT-III assessment, 48 had a Z-score ≤ −1 on the Word Reading component only, 113 had a Z-score ≤ −1 on the Numerical Operations component only, and 69 had a Z-score ≤ −1 on both the Word Reading and Numerical Operations assessments.

### 3.2 Individual proteins on individual days and on 2 days of each epoch (Table 2)

Of all the cells in Table 2, only the one for top quartile concentrations of MPO on both days of the late epoch is identified with increased risks of both the reading only and math only limitations. Increased risks of the math only and the reading+math (identified in the table with a B for both) limitations are associated with top quartile concentrations of CRP on days 14 and 21, and TNF-R2 on day 21. Increased risks of math only limitations are associated with top quartile concentrations of TNF-R2 on days 14 and 21 and during the early epoch, and IL-8 on days 21 and 28 and during the late epoch. Increased risks of reading+math limitations are associated with top quartile concentrations of ICAM-1 on days 14 and 21 and during the late epoch.

ICAM-1 is the only inflammation-related protein whose top quartile concentration is associated with reduced risk of a learning limitation (math only on day 1). In contrast, high concentrations of multiple angio-neurotrophic proteins were associated with reduced risk of math only limitations (including BDNF on day 14, bFGF on day 1, and VEGF on day 7) and both reading+math limitations (including RANTES on day 28 and during the late epoch, NT-4 on day 7, BDNF on day 28, and Ang-1 on days 7 and 21). We created Table 2 to help us identify the inflammation-related and AN-trophic proteins that might be most suitable for examining in light of each other. Because we decided that Tables 3 and 4 should have only 3 inflammation-related proteins these tables do not include CRP, TNF-R2, SAA, MPO, IL-1β, IL-6, TNF-R1, TNF-R2, and VCAM-1. We did retain rows for IL-6R, RANTES, and MMP-9 because each has what appear to be inflammation-modulating capabilities in the ELGAN Study population.

### 3.3 Sets of proteins during the early epoch (Table 3)

The increased risk of both the math limitation associated with top quartile concentrations of TNF-α was modulated by top quartile concentrations of VEGF-R1 only. The increased risk of reading+math limitations together (but not of either individually) associated with top quartile concentrations of TNF-α was modulated by top quartile concentrations of IL-6R, MMP-9, bFGF, VEGF, VEGF-R1, VEGF-R2, and PIGF.

### 3.4 Sets of proteins during the late epoch (Table 4)

The increased risk of math only limitations associated with top quartile concentrations of TNF-α was modulated by top quartile concentrations of Ang-2, while the increased risk associated with top quartile concentrations of IL-8 was modulated by top quartile concentrations of RANTES, EPO, NT-4, VEGF-R2, and PIGF. The increased risk of both reading+math limitations together (but not of either individually) associated with top quartile concentrations of ICAM-1 was moderated by top quartile concentrations of IL-6R, MMP-9, EPO, NT-4, BDNF, IGF-1, PIGF, Ang-1, and Ang-2.

Because of the small number of children who had the reading limitation only, the modulation of increased risks associated with top quartile concentrations of IL-8 and ICAM-1 by angio-neurotrophic proteins never achieved statistical significance. In Table 4, we identify with a lower case “v” the many risk ratios of 2.0 or greater in Appendix Table 3b cells characterized by top quartile concentrations of IL-8 or ICAM-1 and lower concentrations of the angio-neurotrophic protein.

## 4. Discussion

### 4.1 Our strategy

Because systemic inflammation appears to increase the risk of perinatal brain damage,(13–16) and angio-neurotrophic proteins have the capacity to minimize brain damage,(21, 58–64) we evaluated the contribution of high (which we have operationally-defined as top-quartile) concentrations of inflammation-related proteins in light of the presence or absence of high concentrations of angio-neurotrophic proteins. In essence, our ability to identify increased risk associated with an inflammation-related protein is expected to be diminished in the setting of relative abundance of an angio-neurotrophic protein. Similarly, an angio-neurotrophic protein cannot be so identified in a setting that lacks the potential for increased risk.

### 4.2 What we found

Our main findings are that in the extremely preterm newborn, high blood concentrations of inflammation-related proteins are associated with increased risk of mathematics limitations and to a lesser extent, reading limitations, and that angio-neurotrophic proteins appear to modulate the increased risks of learning limitations potentially attributable to systemic inflammation. Some of our findings suggest that reading and math limitations have distinguishable protein profiles. For example, angio-neurotrophic proteins appears able to modulate the increased risks of reading limitations and combined reading and math limitations associated with high concentrations of IL-8 and ICAM-1 during the late epoch, while (with the single exception of Ang-2) isolated math limitations do not share this profile. Many of these same angio-neurotrophic proteins (i.e., VEGF, VEGF-R1, VEGF-R2, Ang-1, and Ang-2) appear to modulate the risks of the combined reading and math limitations associated with high concentrations of TNF-α during the early epoch.

### 4.3 What others found. How our findings are similar or different

We are not aware of any report that assessed the contribution of systemic inflammation in the extremely preterm newborn to the occurrence of reading and math limitations at school age. However, children who survived sepsis-associated encephalopathy perform less well after than they did previously.(65) Prolonged neonatal medical treatment, especially with mechanical ventilation, which appears to increase the risk of systemic inflammation,(66) has been associated with specific impairments in mathematic tasks.(67) Also, studies that provoked inflammation in adult rodents,(68–70) and reports about adults with systemic inflammation,(71, 72) support the view that concurrent or very recent inflammation impairs learning.(73)

### 4.4 Why focus on top-quartile concentrations

In this sample, the concentrations of most proteins do not follow a normal distribution.(53) This prompted us to avoid calculating means and standard deviations. Rather, we evaluated the risks of indicators of brain damage among four quartiles of protein concentrations.(74, 75) Rarely did the risks increase with increasing protein concentration quartile. Rather, the highest risks tended to be found among children whose protein concentrations were in the top quartile.

### 4.5 Why focus on top-quartile concentrations on multiple days

Systemic inflammation on two occasions a week apart during the first two postnatal weeks was associated in this sample with ventriculomegaly when the newborn was in the intensive care nursery,(74) and at age 2 years with low Bayley Scales of Infant Development-II,(76) an attention problem,(77) cerebral palsy,(78) and microcephaly.(75) When reporting these findings we were not sure if the systemic inflammation was intermittent or sustained.(13) We now have more confidence that some of the systemic inflammation is sustained.(56)

The main drivers of postnatal systemic inflammation in this sample are fetal growth restriction,(79) mechanical ventilation,(66) bacteremia,(80) and necrotizing enterocolitis.(81) Perhaps efforts to reduce the duration of mechanical ventilation and minimize presumed barotrauma will result in reduced brain damage.(82)

### 4.6 Categorization of outcome

The dimensional view of reading abilities holds that no sharp break (or discontinuity) separates those with a reading limitation and their peers who are better readers.(83) The same can be said for mathematics abilities.

Epidemiologists, fully aware that measures of a dysfunction represent a continuum, prefer to establish a cut-off for their efforts to identify the antecedents associated with disease entities defined by dichotomizing a continuous measure of function/dysfunction (*e.g.*, hypertension, diabetes mellitus, glaucoma).(84–86) In keeping with this approach, we dichotomized Z-scores at $\leq -1$ on the WIAT-III Word Reading and the Numerical Operations subtests.

### 4.7 Co-occurrence of reading and math limitations (comorbidity)

Reading and math limitations tend to occur together more commonly than can be explained by chance.(47, 48) Multiple explanations have been offered for the relatively high rate of the co-occurrence of mathematics and verbal/spelling/reading limitations, with most current explanations invoking shared circuits/networks,(9, 49–51)

We report here that all three learning limitations we examined are characterized by increased risks associated with systemic inflammation, and reduction of these risks when angio-neurotrophic proteins are abundant. The prominence of late epoch high concentrations of ICAM-1 with reading limitations, but not with math limitations raise the possibility that these two disorders differ in the inflammation pathways that influence their risk of occurrence.

### 4.8 Surrogates and developmental regulation

We view each protein as a surrogate for all proteins in its class. For example, although we found inflammatory signals for TNF-α, IL-8, and ICAM-1, we prefer not to focus on these specific proteins, but rather on broader inflammatory processes.(87, 88) Similarly, we view each angio-neurotrophic protein as a surrogate for many other proteins that possess neurotrophic and angiogenic characteristics.

The concept that each protein is only a surrogate for others is based, in part, on our limited understanding of developmental regulation. Not all inflammation-related proteins have the same pattern of developmental regulation,(89, 90) nor do all angio-neurotrophic proteins have the same developmental regulation schedule.(91) In general, however, the more “mature” the newborn (i.e., the further along the developmental regulation schedule) the more s/he should be able to resolve inflammation, and promote brain growth, repair, and well-being.(92)

### 4.9 Limitations and strengths of this study

Perhaps our main weakness is the relatively small number of children with a reading only limitation. Because of this, odds ratios in the 3 to 8 range were not statistically significant.

We are also limited by the interrelatedness of early and late systemic inflammation, which limits our ability to tease apart the contributions of each to the occurrence of each learning achievement limitation. We are also limited by the relatively small number of proteins measured. Inflammation is a broad and complex phenomenon,(93, 94) and we have assessed only 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 as the number of children for whom we had specimens from both of the late-epoch days tended to be about 2/3 of the number for whom we had specimens from two of the three early-epoch days.

Not only did the clinical indications for specimens increase the potential for bias, but it also diminished power. Only 305 children provided specimens for both days 21 and 28 (*i.e.*, the

late-epoch). This can be expected to provide 20 children in the cell for top-quartile concentrations of both proteins (305 × .25 × .25). With only 8% of children having the combined reading and math limitation, only 1 or 2 of the 20 children in this cell would have this learning limitation. Among the consequences of a small number of subjects are very wide confidence intervals, occasional empty cells, and of course, very limited power to detect a difference between the odds ratio associated with high concentrations of both the inflammation-related protein and the angio-neurotrophic protein (left upper cell) and the odds ratio associated with high concentrations of the inflammation-related and low concentrations of the angio-neurotrophic protein (left lower cell).

This study has sufficient power to identify an odds ratio of 1.9 for the math limitation in the individual analyses and 2.0 in the two-protein analyses. Other strengths are the selection of infants based on gestational age, not birth weight,(95) prospective collection of all data, modest attrition, and finally, protein data of high quality,(19,20) and high content validity. (53, 96–98)

## 5. Conclusions

In this cohort of children born extremely preterm and at high-risk group of learning problems, high concentrations of inflammation-related proteins during the first postnatal month are associated with increased risks of reading and math limitations, which appear to be modulated by high concentrations of angio-neurotrophic proteins.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

The authors express their gratitude to the children and their families who participated in this study. They also gratefully acknowledge the contributions of the ELGAN Study Investigators, listed below.

Boston Children’s Hospital, Boston MA

Janice Ware, Taryn Coster, Brandi Henson, Rachel Wilson, Kirsten McGhee, Patricia Lee, Aimee Asgarian, Anjali Sadhwani

Tufts Medical Center, Boston MA

Ellen Perrin, Emily Neger, Kathryn Mattern, Jenifer Walkowiak, Susan Barron

University of Massachusetts Medical School, Worcester MA

Jean Frazier, Lauren Venuti, Beth Powers, Ann Foley, Brian Dessureau, Molly Wood, Jill Damon-Minow

Yale University School of Medicine, New Haven, CT

Richard Ehrenkranz, Jennifer Benjamin, Elaine Romano, Kathy Tsatsanis, Katarzyna Chawarska, Sophy Kim, Susan Dieterich, Karen Bearrs

Wake Forest University Baptist Medical Center, Winston-Salem NC

T. Michael O’Shea, Nancy Peters, Patricia Brown, Emily Ansusinha, Ellen Waldrep, Jackie Friedman, Gail Hounshell, Debbie Allred

University Health Systems of Eastern Carolina, Greenville, NC

Stephen C. Engelke, Nancy Darden-Saad, Gary Stainback

North Carolina Children's Hospital, Chapel Hill, NC

Diane Warner, Janice Wereszczak, Janice Bernhardt, Joni McKeeman, Echo Meyer

Helen DeVos Children's Hospital, Grand Rapids, MI

Steve Pastyrnak, Wendy Burdo-Hartman, Julie Rathbun, Sarah Nota, Teri Crumb

Sparrow Hospital, Lansing, MI

Madeleine Lenski, Deborah Weiland, Megan Lloyd

University of Chicago Medical Center, Chicago, IL

Scott Hunter, Michael Msall, Rugile Ramoskaite, Suzanne Wiggins, Krissy Washington, Ryan Martin, Barbara Prendergast, Megan Scott

William Beaumont Hospital, Royal Oak, MI

Judith Klarr, Beth Kring, Jennifer DeRidder, Kelly Vogt

**Financial Support:** This study was supported by grants from the National Institute of Neurological Disorders and Stroke (5U01NS040069-05; 2R01NS040069-06A2), The National Eye Institute (1-R01-EY021820-01), the National Institute of Child Health and Human Development (5P30HD018655-34), and the Office of the National Institutes of Health Director (1UG3OD023348-01).

## Abbreviations

| | |
|---|---|
| **Ang-1** | Angiopoietin-1 |
| **Ang-2** | Angiopoietin-2 |
| **BDNF** | Brain-Derived Neurotrophic Factor |
| **bFGF** | basic Fibroblast Growth Factor |
| **CRP** | C-Reactive Protein |
| **DAS-II** | Differential Ability Scales–II |
| **EPO** | Erythropoietin |
| **ICAM-1** | Intercellular Adhesion Molecule-1 |
| **IGF-1** | Insulin-like growth factor-1 |
| **IGFBP-1** | Insulin-like growth factor binding protein-1 |
| **IL-1β** | Interleukin-1 β |
| **IL-6** | Interleukin-6 |
| **IL-6R** | Interleukin-6 Receptor |
| **IL-8** | Interleukin-8 |

| | |
|---|---|
| **KBIT-2** | Kaufman Brief Intelligence Test – 2 |
| **LL** | Learning limitation |
| **MMP-9** | Matrix Metalloproteinase-9 |
| **MPO** | Myeloperoxidase |
| **NT-4** | Neurotrophin-4 |
| **PIGF** | Placenta Growth Factor |
| **RANTES** | Regulated upon Activation, Normal T-cell Expressed, and Secreted |
| **SAA** | Serum Amyloid A |
| **TNF-R1** | Tumor Necrosis Factor-α Receptor-1 |
| **TNF-R2** | Tumor Necrosis Factor-α Receptor-2 |
| **TNF-α** | Tumor Necrosis Factor-α |
| **TSH** | Thyroid-Stimulating Hormone |
| **VCAM-1** | Vascular Cell Adhesion Molecule-1 |
| **VEGF** | Vascular endothelial growth factor |
| **VEGF-R1** | Vascular endothelial growth factor Receptor-1 |
| **VEGF-R2** | Vascular endothelial growth factor Receptor-2 |
| **WIAT-III** | Wechsler Individual Achievement Test-III |

## References


1. Levandowski ML, Hess AR, Grassi-Oliveira R, de Almeida RM. Plasma interleukin-6 and executive function in crack cocaine-dependent women. Neurosci Lett. 2016; 628:85–90. Epub 2016/06/15. [PubMed: 27297769]
2. Guarini A, Sansavini A, Fabbri C, Savini S, Alessandroni R, Faldella G, et al. Long-term effects of preterm birth on language and literacy at eight years. Journal of child language. 2010; 37(4):865–85. Epub 2009/08/25. [PubMed: 19698208]
3. Simms V, Cragg L, Gilmore C, Marlow N, Johnson S. Mathematics difficulties in children born very preterm: current research and future directions. Arch Dis Child Fetal Neonatal Ed. 2013; 98(5):F457–63. Epub 2013/06/14. [PubMed: 23759519]
4. Simms V, Gilmore C, Cragg L, Clayton S, Marlow N, Johnson S. Nature and origins of mathematics difficulties in very preterm children: a different etiology than developmental dyscalculia. Pediatr Res. 2015; 77(2):389–95. Epub 2014/11/20. [PubMed: 25406898]
5. Tatsuoka C, McGowan B, Yamada T, Espy KA, Minich N, Taylor HG. Effects of Extreme Prematurity on Numerical Skills and Executive Function in Kindergarten Children: An Application of Partially Ordered Classification Modeling. Learning and individual differences. 2016; 49:332–40. Epub 2016/11/08. [PubMed: 27818602]
6. Taylor HG, Espy KA, Anderson PJ. Mathematics deficiencies in children with very low birth weight or very preterm birth. Developmental disabilities research reviews. 2009; 15(1):52–9. Epub 2009/02/13. [PubMed: 19213016]

7. Clark CA, Nelson JM, Garza J, Sheffield TD, Wiebe SA, Espy KA. Gaining control: changing relations between executive control and processing speed and their relevance for mathematics achievement over course of the preschool period. Frontiers in psychology. 2014; 5:107. Epub 2014/03/07. [PubMed: 24596563]

8. Scott FE, Mechelli A, Allin MP, Walshe M, Rifkin L, Murray RM, et al. Very preterm adolescents show gender-dependent alteration of the structural brain correlates of spelling abilities. Neuropsychologia. 2011; 49(9):2685–93. Epub 2011/06/10. [PubMed: 21651922]

9. Ashkenazi S, Black JM, Abrams DA, Hoeft F, Menon V. Neurobiological underpinnings of math and reading learning disabilities. Journal of learning disabilities. 2013; 46(6):549–69. Epub 2013/04/11. [PubMed: 23572008]

10. Feldman HM, Lee ES, Yeatman JD, Yeom KW. Language and reading skills in school-aged children and adolescents born preterm are associated with white matter properties on diffusion tensor imaging. Neuropsychologia. 2012; 50(14):3348–62. Epub 2012/10/24. [PubMed: 23088817]

11. Travis KE, Leitner Y, Feldman HM, Ben-Shachar M. Cerebellar white matter pathways are associated with reading skills in children and adolescents. Human brain mapping. 2015; 36(4): 1536–53. Epub 2014/12/17. [PubMed: 25504986]

12. Martin A, Schurz M, Kronbichler M, Richlan F. Reading in the brain of children and adults: a meta-analysis of 40 functional magnetic resonance imaging studies. Human brain mapping. 2015; 36(5):1963–81. Epub 2015/01/30. [PubMed: 25628041]

13. Mann GE, Kahana M. The uncomfortable reality… We simply do not know if general anesthesia negatively impacts the neurocognitive development of our small children. International journal of pediatric otorhinolaryngology. 2015; 79(9):1379–81. Epub 2015/07/06. [PubMed: 26143125]

14. Hartkopf AD, Graf J, Simoes E, Keilmann L, Sickenberger N, Gass P, et al. Electronic-Based Patient-Reported Outcomes: Willingness, Needs, and Barriers in Adjuvant and Metastatic Breast Cancer Patients. JMIR cancer. 2017; 3(2):e11. Epub 2017/08/09. [PubMed: 28784595]

15. Korzeniewski SJ, Allred E, Logan JW, Fichorova RN, Engelke S, Kuban KC, et al. Elevated endogenous erythropoietin concentrations are associated with increased risk of brain damage in extremely preterm neonates. PLoS ONE. 2015; 10(3):e0115083. Epub 2015/03/21. [PubMed: 25793991]

16. O'Shea TM, Joseph RM, Kuban KC, Allred EN, Ware J, Coster T, et al. Elevated blood levels of inflammation-related proteins are associated with an attention problem at age 24 mo in extremely preterm infants. Pediatr Res. 2014; 75(6):781–7. Epub 2014/03/13. [PubMed: 24614800]

17. Reuss ML, Paneth N, Susser M. Does the loss of placental hormones contribute to neurodevelopmental disabilities in preterm infants? Dev Med Child Neurol. 1994; 36(8):743–7. [PubMed: 7519571]

18. Su YW, Zhou XF, Foster BK, Grills BL, Xu J, Xian CJ. Roles of neurotrophins in skeletal tissue formation and healing. Journal of cellular physiology. 2018; 233(3):2133–45. Epub 2017/04/04. [PubMed: 28370021]

19. Kermani P, Rafii D, Jin DK, Whitlock P, Schaffer W, Chiang A, et al. Neurotrophins promote revascularization by local recruitment of TrkB+ endothelial cells and systemic mobilization of hematopoietic progenitors. J Clin Invest. 2005; 115(3):653–63. Epub 2005/03/15. [PubMed: 15765148]

20. Usui T, Naruo A, Okada M, Hayabe Y, Yamawaki H. Brain-derived neurotrophic factor promotes angiogenic tube formation through generation of oxidative stress in human vascular endothelial cells. Acta Physiol (Oxf). 2014; 211(2):385–94. Epub 2014/03/13. [PubMed: 24612679]

21. Larpthaveesarp A, Ferriero DM, Gonzalez FF. Growth factors for the treatment of ischemic brain injury (growth factor treatment). Brain sciences. 2015; 5(2):165–77. Epub 2015/05/06. [PubMed: 25942688]

22. Wagenaar N, de Theije CGM, de Vries LS, Groenendaal F, Benders M, Nijboer CHA. Promoting neuroregeneration after perinatal arterial ischemic stroke: Neurotrophic factors and mesenchymal stem cells. Pediatr Res. 2017 Epub 2017/09/28.

23. Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci U S A. 2002; 99(18):11946–50. Epub 2002/08/16. [PubMed: 12181492]
24. Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, et al. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J Clin Invest. 2003; 111(12):1843–51. Epub 2003/06/19. [PubMed: 12813020]
25. Madri JA. Modeling the neurovascular niche: implications for recovery from CNS injury. Journal of physiology and pharmacology. 2009; 60(Suppl 4):95–104. Epub 2010/01/30.
26. Meng Z, Li M, He Q, Jiang S, Zhang X, Xiao J, et al. Ectopic expression of human angiopoietin-1 promotes functional recovery and neurogenesis after focal cerebral ischemia. Neuroscience. 2014; 267:135–46. Epub 2014/03/13. [PubMed: 24607344]
27. Rosa AI, Goncalves J, Cortes L, Bernardino L, Malva JO, Agasse F. The angiogenic factor angiopoietin-1 is a proneurogenic peptide on subventricular zone stem/progenitor cells. J Neurosci. 2010; 30(13):4573–84. Epub 2010/04/02. [PubMed: 20357108]
28. Marteau L, Pacary E, Valable S, Bernaudin M, Guillemot F, Petit E. Angiopoietin-2 regulates cortical neurogenesis in the developing telencephalon. Cereb Cortex. 2011; 21(7):1695–702. Epub 2010/12/04. [PubMed: 21127017]
29. Liu XS, Chopp M, Zhang RL, Hozeska-Solgot A, Gregg SC, Buller B, et al. Angiopoietin 2 mediates the differentiation and migration of neural progenitor cells in the subventricular zone after stroke. J Biol Chem. 2009; 284(34):22680–9. Epub 2009/06/26. [PubMed: 19553662]
30. Wang Y, Tian Y, Wang D, Wei H, Zhao Z, Jiang R, et al. High Angiopoietin-1 levels predict a good functional outcome within 72 h of an aneurysmal subarachnoid hemorrhage: A prospective study from a single center. J Neurol Sci. 2015; 356(1–2):72–6. Epub 2015/07/26. [PubMed: 26208799]
31. Hansen TM, Moss AJ, Brindle NP. Vascular endothelial growth factor and angiopoietins in neurovascular regeneration and protection following stroke. Curr Neurovasc Res. 2008; 5(4):236–45. Epub 2008/11/11. [PubMed: 18991658]
32. Kosacka J, Nowicki M, Kacza J, Borlak J, Engele J, Spanel-Borowski K. Adipocyte-derived angiopoietin-1 supports neurite outgrowth and synaptogenesis of sensory neurons. J Neurosci Res. 2006; 83(7):1160–9. Epub 2006/02/24. [PubMed: 16493688]
33. Zacchigna S, Lambrechts D, Carmeliet P. Neurovascular signalling defects in neurodegeneration. Nat Rev Neurosci. 2008; 9(3):169–81. Epub 2008/02/07. [PubMed: 18253131]
34. Giampietro C, Deflorian G, Gallo S, Di Matteo A, Pradella D, Bonomi S, et al. The alternative splicing factor Nova2 regulates vascular development and lumen formation. Nature communications. 2015; 6:8479. Epub 2015/10/09.
35. Shin HK, Lee HR, Lee DH, Hong KW, Lee JH, Park SY, et al. Cilostazol enhances neovascularization in the mouse hippocampus after transient forebrain ischemia. J Neurosci Res. 2010; 88(10):2228–38. Epub 2010/02/23. [PubMed: 20175201]
36. Finkelstein Y, Milatovic D, Lazarovici P, Ophir A, Richter ED, Aschner M, et al. Peaceful use of disastrous neurotoxicants. Neurotoxicology. 2010; 31(5):608–20. Epub 2010/07/14. [PubMed: 20620165]
37. Nag S. Morphology and properties of brain endothelial cells. Methods Mol Biol. 2011; 686:3–47. Epub 2010/11/18. [PubMed: 21082365]
38. Saito T, Shibasaki K, Kurachi M, Puentes S, Mikuni M, Ishizaki Y. Cerebral capillary endothelial cells are covered by the VEGF-expressing foot processes of astrocytes. Neurosci Lett. 2011; 497(2):116–21. Epub 2011/05/04. [PubMed: 21536099]
39. Liu X, Zhang T, He S, Hong B, Chen Z, Peng D, et al. Elevated serum levels of FGF-2, NGF and IGF-1 in patients with manic episode of bipolar disorder. Psychiatry research. 2014; 218(1–2):54–60. Epub 2014/05/06. [PubMed: 24793757]
40. Lafuente JV, Ortuzar N, Bengoetxea H, Bulnes S, Argandona EG. Vascular endothelial growth factor and other angioglioneurins: key molecules in brain development and restoration. International review of neurobiology. 2012; 102:317–46. Epub 2012/07/04. [PubMed: 22748835]
41. O'Shea TM, Allred EN, Dammann O, Hirtz D, Kuban KC, Paneth N, et al. The ELGAN study of the brain and related disorders in extremely low gestational age newborns. Early Hum Dev. 2009; 85(11):719–25. Epub 2009/09/22. [PubMed: 19765918]

42. Kuban KC, Joseph RM, O'Shea TM, Heeren T, Fichorova RN, Douglass L, et al. Circulating Inflammatory-Associated Proteins in the First Month of Life and Cognitive Impairment at Age 10 Years in Children Born Extremely Preterm. J Pediatr. 2017; 180:116–23 e1. Epub 2016/10/30. [PubMed: 27788929]
43. Ritterband LM, Thorndike FP, Cox DJ, Kovatchev BP, Gonder-Frederick LA. A behavior change model for internet interventions. Annals of behavioral medicine: a publication of the Society of Behavioral Medicine. 2009; 38(1):18–27. Epub 2009/10/06. [PubMed: 19802647]
44. Hansen-Pupp I, Hovel H, Lofqvist C, Hellstrom-Westas L, Fellman V, Huppi PS, et al. Circulatory insulin-like growth factor-I and brain volumes in relation to neurodevelopmental outcome in very preterm infants. Pediatr Res. 2013; 74(5):564–9. Epub 2013/08/15. [PubMed: 23942554]
45. Fiks AG, Mayne S, Karavite DJ, DeBartolo E, Grundmeier RW. A shared e-decision support portal for pediatric asthma. The Journal of ambulatory care management. 2014; 37(2):120–6. Epub 2014/03/07. [PubMed: 24594560]
46. Costa DS, Miranda DM, Burnett AC, Doyle LW, Cheong JLY, Anderson PJ. Executive Function and Academic Outcomes in Children Who Were Extremely Preterm. Pediatrics. 2017; 140(3) Epub 2017/08/31.
47. Tannock R. Rethinking ADHD and LD in DSM-5: proposed changes in diagnostic criteria. Journal of learning disabilities. 2013; 46(1):5–25. Epub 2012/11/13. [PubMed: 23144062]
48. Landerl K, Moll K. Comorbidity of learning disorders: prevalence and familial transmission. Journal of child psychology and psychiatry, and allied disciplines. 2010; 51(3):287–94. Epub 2009/10/01.
49. Willcutt EG, Petrill SA, Wu S, Boada R, Defries JC, Olson RK, et al. Comorbidity between reading disability and math disability: concurrent psychopathology, functional impairment, and neuropsychological functioning. Journal of learning disabilities. 2013; 46(6):500–16. Epub 2013/03/02. [PubMed: 23449727]
50. Slot EM, van Viersen S, de Bree EH, Kroesbergen EH. Shared and Unique Risk Factors Underlying Mathematical Disability and Reading and Spelling Disability. Frontiers in psychology. 2016; 7:803. Epub 2016/07/05. [PubMed: 27375508]
51. Lopes-Silva JB, Moura R, Julio-Costa A, Wood G, Salles JF, Haase VG. What Is Specific and What Is Shared Between Numbers and Words? Frontiers in psychology. 2016; 7:22. Epub 2016/02/13. [PubMed: 26869946]
52. Blum DE, Eskola J, Bortz JJ, Fisher RS. Patient awareness of seizures. Neurology. 1996; 47(1): 260–4. [PubMed: 8710091]
53. Leviton A, Fichorova R, Yamamoto Y, Allred EN, Dammann O, Hecht J, et al. Inflammation-related proteins in the blood of extremely low gestational age newborns. The contribution of inflammation to the appearance of developmental regulation. Cytokine. 2011; 53(1):66–73. Epub 2010/10/12. [PubMed: 20934883]
54. Leviton A, Allred EN, Yamamoto H, Fichorova RN, Investigators. ftES. Relationships among the concentrations of 25 inflammation-associated proteins during the first postnatal weeks in the blood of infants born before the 28th week of gestation. Cytokine. 2012
55. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977; 33(1):159–74. Epub 1977/03/01. [PubMed: 843571]
56. Leviton A, Allred EN, Fichorova RN, Kuban KC, O'Shea TM, Dammann O. Antecedents of inflammation biomarkers in preterm newborns on days 21 and 28. Acta Paediatr. 2016; 105(3): 274–80. Epub 2015/11/27. [PubMed: 26610180]
57. Ackshoomoff N, Joseph RM, Taylor HG, Allred EN, Heeren T, O'Shea TM, et al. Academic Achievement Deficits and their Neuropsychological Correlates in Children Born Extremely Preterm. Journal of Developmental and Behavioral Pediatrics. in press.
58. Raff MC, Lillien LE. Differentiation of a bipotential glial progenitor cell: what controls the timing and the choice of developmental pathway? Journal of cell science Supplement. 1988; 10:77–83. Epub 1988/01/01. [PubMed: 3077944]
59. Barres BA, Hart IK, Coles HSR, Burne JF, Voyvodic JT, Richardson WD, et al. Cell death and control of cell survival in the oligodendrocyte lineage. Cell. 1992; 70:31–64. [PubMed: 1623522]

60. Bögler O, Wren D, Barnett S, Land H, Noble M. Cooperation between two growth factors promotes extended self renewal and inhibits differentiation of oligodendrocyte type-2 astrocyte progenitor cells. Proc Natl Acad Sci USA. 1990; 87:6368–72. [PubMed: 2201028]

61. Oppenheim RW, Yin QW, Prevette D, Yan Q. Brain-derived neurotrophic factor rescues developing avian motoneurons from cell death. Nature. 1992; 360(6406):755–7. Epub 1992/12/24. [PubMed: 1465146]

62. Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. Nature. 1992; 360(6406): 757–9. Epub 1992/12/24. [PubMed: 1465147]

63. Yan Q, Elliott J, Snider WD. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. Nature. 1992; 360(6406):753–5. Epub 1992/12/24. [PubMed: 1281520]

64. Unsicker K. Neurotrophic molecules in the treatment of neurodegenerative disease with focus on the retina: status and perspectives. Cell Tissue Res. 2013; 353(2):205–18. Epub 2013/03/07. [PubMed: 23463189]

65. Kaur J, Singhi P, Singhi S, Malhi P, Saini AG. Neurodevelopmental and Behavioral Outcomes in Children With Sepsis-Associated Encephalopathy Admitted to Pediatric Intensive Care Unit: A Prospective Case Control Study. J Child Neurol. 2016; 31(6):683–90. Epub 2015/10/27. [PubMed: 26500243]

66. Bose CL, Laughon MM, Allred EN, O'Shea TM, Van Marter LJ, Ehrenkranz RA, et al. Systemic inflammation associated with mechanical ventilation among extremely preterm infants. Cytokine. 2013; 61(1):315–22. Epub 2012/11/15. [PubMed: 23148992]

67. Jaekel J, Bartmann P, Schneider W, Wolke D. Neurodevelopmental pathways to preterm children's specific and general mathematic abilities. Early Hum Dev. 2014; 90(10):639–44. Epub 2014/09/01. [PubMed: 25173650]

68. Rogers, EM. Diffusion of innovations. NY: The Free Press; 1995.

69. Chesnokova V, Pechnick RN, Wawrowsky K. Chronic peripheral inflammation, hippocampal neurogenesis, and behavior. Brain Behav Immun. 2016; 58:1–8. Epub 2016/01/24. [PubMed: 26802985]

70. Ryan SM, Nolan YM. Neuroinflammation negatively affects adult hippocampal neurogenesis and cognition: can exercise compensate? Neurosci Biobehav Rev. 2016; 61:121–31. Epub 2015/12/24. [PubMed: 26695382]

71. Hong S, Banks WA. Role of the immune system in HIV-associated neuroinflammation and neurocognitive implications. Brain Behav Immun. 2015; 45:1–12. Epub 2014/12/03. [PubMed: 25449672]

72. Wardill HR, Mander KA, Van Sebille YZ, Gibson RJ, Logan RM, Bowen JM, et al. Cytokine-mediated blood brain barrier disruption as a conduit for cancer/chemotherapy-associated neurotoxicity and cognitive dysfunction. International journal of cancer Journal international du cancer. 2016; 139(12):2635–45. Epub 2016/07/02. [PubMed: 27367824]

73. Donzis EJ, Tronson NC. Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences. Neurobiology of learning and memory. 2014; 115:68–77. Epub 2014/08/26. [PubMed: 25151944]

74. Leviton A, Kuban K, O'Shea TM, Paneth N, Fichorova R, Allred EN, et al. The Relationship between early concentrations of 25 blood proteins and cerebral white matter injury in preterm newborns: The ELGAN Study. J Pediatr. 2011; 158(6):897–903 e5. Epub 2011/01/18. [PubMed: 21238986]

75. Leviton A, Kuban KC, Allred EN, Fichorova RN, O'Shea TM, Paneth N, et al. Early postnatal blood concentrations of inflammation-related proteins and microcephaly two years later in infants born before the 28th post-menstrual week. Early Hum Dev. 2011; 87:325–30. Epub 18 February 2011. [PubMed: 21334149]

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

77. O'Donnell KJ, Glover V, Barker ED, O'Connor TG. The persisting effect of maternal mood in pregnancy on childhood psychopathology. Development and psychopathology. 2014; 26(2):393–403. Epub 2014/03/14. [PubMed: 24621564]

78. Faupel-Badger JM, Wang Y, Staff AC, Karumanchi SA, Stanczyk FZ, Pollak M, et al. Maternal and cord steroid sex hormones, angiogenic factors, and insulin-like growth factor axis in African-American preeclamptic and uncomplicated pregnancies. Cancer Causes Control. 2012; 23(5):779–84. Epub 2012/03/16. [PubMed: 22418778]

79. McElrath TF, Allred EN, Van Marter L, Fichorova RN, Leviton A. Perinatal systemic inflammatory responses of growth-restricted preterm newborns. Acta Paediatr. 2013; 102(10):e439–42. Epub 2013/07/04. [PubMed: 23819682]

80. Leviton A, O'Shea TM, Bednarek FJ, Allred EN, Fichorova RN, Dammann O. Systemic responses of preterm newborns with presumed or documented bacteraemia. Acta Paediatr. 2012; 101(4):355–9. Epub 2011/11/17. [PubMed: 22085230]

81. Martin CR, Bellomy M, Allred EN, Fichorova RN, Leviton A. Systemic inflammation associated with severe intestinal injury in extremely low gestational age newborns. Fetal Pediatr Pathol. 2013; 32(3):222–34. Epub 2012/09/26. [PubMed: 23002960]

82. Barton SK, Tolcos M, Miller SL, Christoph-Roehr C, Schmolzer GM, Moss TJ, et al. Ventilation-Induced Brain Injury in Preterm Neonates: A Review of Potential Therapies. Neonatology. 2016; 110(2):155–62. Epub 2016/04/23. [PubMed: 27105430]

83. Branum-Martin L, Fletcher JM, Stuebing KK. Classification and identification of reading and math disabilities: the special case of comorbidity. Journal of learning disabilities. 2013; 46(6):490–9. Epub 2012/12/13. [PubMed: 23232442]

84. Lovasi GS, Underhill LJ, Jack D, Richards C, Weiss C, Rundle A. At Odds: Concerns Raised by Using Odds Ratios for Continuous or Common Dichotomous Outcomes in Research on Physical Activity and Obesity. The open epidemiology journal. 2012; 5:13–7. Epub 2012/09/25. [PubMed: 23002407]

85. Ragland DR. Dichotomizing continuous outcome variables: dependence of the magnitude of association and statistical power on the cutpoint. Epidemiology. 1992; 3(5):434–40. Epub 1992/09/01. [PubMed: 1391136]

86. Wartenberg D, Northridge M. Defining exposure in case-control studies: a new approach. Am J Epidemiol. 1991; 133(10):1058–71. Epub 1991/05/15. [PubMed: 2035506]

87. Lai JS, Yount S, Beaumont JL, Cella D, Toia J, Goldman S. A patient-centered symptom monitoring and reporting system for children and young adults with cancer (SyMon-SAYS). Pediatr Blood Cancer. 2015; 62(10):1813–8. Epub 2015/04/10. [PubMed: 25856587]

88. Zhao Y, Forst CV, Sayegh CE, Wang IM, Yang X, Zhang B. Molecular and genetic inflammation networks in major human diseases. Molecular bioSystems. 2016; 12(8):2318–41. Epub 2016/06/16. [PubMed: 27303926]

89. Clapp DW. Developmental regulation of the immune system. Semin Perinatol. 2006; 30(2):69–72. Epub 2006/05/30. [PubMed: 16731279]

90. Zasada M, Kwinta P, Durlak W, Bik-Multanowski M, Madetko-Talowska A, Pietrzyk JJ. Development and maturation of the immune system in preterm neonates: results from a whole genome expression study. BioMed research international. 2014; 2014:498318. Epub 2014/07/02. [PubMed: 24982884]

91. Oliveira SL, Pillat MM, Cheffer A, Lameu C, Schwindt TT, Ulrich H. Functions of neurotrophins and growth factors in neurogenesis and brain repair. Cytometry Part A: the journal of the International Society for Analytical Cytology. 2013; 83(1):76–89. Epub 2012/10/10. [PubMed: 23044513]

92. van Tilborg E, Heijnen CJ, Benders MJ, van Bel F, Fleiss B, Gressens P, et al. Impaired oligodendrocyte maturation in preterm infants: Potential therapeutic targets. Prog Neurobiol. 2016; 136:28–49. Epub 2015/12/15. [PubMed: 26655283]

93. VanderWeele TJ, Shpitser I. On the definition of a confounder. Annals of statistics. 2013; 41(1): 196–220. Epub 2013/02/01. [PubMed: 25544784]

94. Zak DE, Aderem A. Systems integration of innate and adaptive immunity. Vaccine. 2015; 33(40): 5241–8. Epub 2015/06/24. [PubMed: 26102534]

95. Arnold CC, Kramer MS, Hobbs CA, McLean FH, Usher RH. Very low birth weight: a problematic cohort for epidemiologic studies of very small or immature neonates. Am J Epidemiol. 1991; 134(6):604–13. [PubMed: 1951265]

96. Fichorova RN, Onderdonk AB, Yamamoto H, ML D, Dubois AM, Allred E, et al. Maternal microbe-specific modulation of inflammatory response in extremely low gestational age newborns. mBio. 2011; 2(1):e00280–10.

97. Hecht JL, Fichorova RN, Tang VF, Allred EN, McElrath TF, Leviton A. Relationship between neonatal blood protein profiles and placenta histologic characteristics in ELGANs. Pediatr Research. 2011; 69:68–73. Epub 2010 Oct 1.

98. McElrath TF, Fichorova RN, Allred EN, Hecht JL, Ismail MA, Yuan H, et al. Blood protein profiles of infants born before 28 weeks differ by pregnancy complication. Am J Obstet Gynecol. 2011; 204(5):418.e1–e12. Epub 2011/02/26. [PubMed: 21349490]

## Highlights

- Extremely preterm children are at increased risk of early systemic inflammation
- Those who have early systemic inflammation are at high risk of learning limitations
- Abundance of neurotrophins modulates this heightened risk

**Table 1**

Sample description

| | **Yes** |
|---|---|
| Enrolled | 1506 |
| Survived to age 10 years | 1198 |
| Recruited for assessment at age 10 years | 966 |
| Returned for an assessment at age 10 years | 889 |
| Proteins measured in blood collected on 2 separate days | 857 |
| DAS-II verbal IQ ≥ 70 and non-verbal IQ ≥ 70 | 666 |
| Word Reading and Numberical Operations Z-scores available | 660 |
| Word Reading Z-score ≤ −1 + Numerical Operations Z-score > −1 | 48 |
| Numerical Operations Z-score ≤ −1 + Word Reading Z-score > −1 | 113 |
| Word Reading Z-score ≤ −1 + Numerical Operations ≤ −1 | 69 |
| Word Reading Z-score > −1 + Numerical Operations > −1 | 430 |

**Table 2**

Statistically-significant odds ratios for Z-scores ≤ −1 at age 10 years on the reading (R) assessment only, the mathematics (M) assessment only, or the co-occurrence of low scores on both (B), associated with a concentration in the top quartile of the protein listed on the left, among children whose DAS-II verbal and nonverbal IQs were ≥ 70. This table summarizes Appendix Tables A (individual days) and B (epochs) for these entities. Upper-case regular typeface indicates statistically significantly increased odds ratios, while *lower-case italic* typeface indicates statistically significantly reduced odds ratios ($p < 0.05$). Adjustment has been made for birth weight Z-score < −1 and mother's K-BIT Z-score < −1.

| | **Protein in highest quartile on: individual day** | | | | | **Two days of epoch** | |
|---|---|---|---|---|---|---|---|
| | **Day 1** | **Day 7** | **Day 14** | **Day 21** | **Day 28** | **Early** | **Late** |
| **Proteins traditionally viewed as inflammation-associated** | | | | | | | |
| CRP | | | M, B | M, B | | | |
| SAA | | | M | | | | |
| MPO | | | | | M | | R, M |
| IL-1β | | | B | | | | |
| IL-6 | | | | | R | | |
| IL-6R | R | | | | | | |
| TNF-α | | | M | | | | |
| TNF-R1 | | | M | | | | |
| TNF-R2 | | | M | M, B | | M | |
| IL-8 | | | | M | M | | M |
| RANTES | | | | | *b* | | *b* |
| ICAM-1 | *m* | | B | B | | | B |
| VCAM-1 | | | | | | | |
| **Proteins traditionally viewed as neurotrophic and/or angiogenic** | | | | | | | |
| TSH | | | | | | | |
| EPO | | | | | | B | |
| NT-4 | | *b* | | | | | |
| BDNF | | | *m* | | *b* | | |
| bFGF | *m* | | | | | | |
| IGF-1 | | | | | | | |
| IGFBP-1 | M | R | | B | | | |

| | Protein in highest quartile on: individual day | | | | | Two days of epoch | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Early | Late |
| VEGF | | *m* | | | | | |
| VEGF-R1 | | | | | | | |
| VEGF-R2 | | | M | | | | |
| PIGF | | | | | | | |
| Ang-1 | | *b* | | *b* | | | |
| Ang-2 | | | | | | | |

**Table 3**

This is a summary of all 3 Appendix Tables C (which evaluate the risk of a Z-score ≤ −1 on the WIAT-III academic achievement assessment in light of top quartile concentrations of two proteins at a time for the early epoch). An inflammation-related protein is identified at the top of each set of columns, and the angio-neurotrophic proteins are identified on the left. A “V” in the cell indicates that a top quartile concentration of the angio-neurotrophic protein (listed on the left) appears to modulate the increased risk of the learning limitation (identified at the top of each column) associated with a top quartile concentration of the inflammation-related protein that heads each set of columns.

| | **TNF-α** | | | **IL-8** | | | **ICAM-1** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Read** | **Math** | **Both** | **Read** | **Math** | **Both** | **Read** | **Math** | **Both** |
| IL-6R | | | V | | | | | | |
| MMP-9 | | | V | | | | | | |
| RANTES | | | | | | | | | |
| EPO | | | | | | | | | |
| NT-4 | | | | | | | | | |
| BDNF | | | | | | | | | |
| bFGF | | | V | | | | | V | |
| IGF-1 | | | | | | | | | |
| VEGF | | | V | | | | V | V | |
| VEGF-R1 | | V | V | | | | | V | |
| VEGF-R2 | | | V | | | | | | |
| PIGF | | | V | | | | | V | |
| Ang-1 | | | | | | | | V | |
| Ang-2 | | | | | | | | V | |

**Table 4**

This is a summary of all 3 Appendix Tables D (which evaluate the risk of a Z-score ≤ −1 on the WIAT-III academic achievement assessment in light of top quartile concentrations of two proteins at a time for the late epoch). The inflammation-related protein is identified at the top of each set of columns, and the angio-neurotrophic proteins are identified on the left. A "V" in the cell indicates that a top quartile concentration of the angio-neurotrophic proteins (listed on the left) appeared to modulate the increased risk of the learning limitation (identified at the top of each column) associated with a top quartile concentration of the inflammation-related protein that heads each set of columns. The small 'v's in other cells indicate the same pattern of modulated risk, but with too few children to achieve statistical significance.

| | **TNF-α** | | | **IL-8** | | | **ICAM-1** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Read** | **Math** | **Both** | **Read** | **Math** | **Both** | **Read** | **Math** | **Both** |
| IL-6R | | | | v | | | | | V |
| MMP-9 | | | | v | | | v | | V |
| RANTES | | | | v | V | | | | |
| EPO | | | | v | V | | | | V |
| NT-4 | | | | v | V | V | v | | V |
| BDNF | | | | v | | | | | V |
| bFGF | | | | v | | | | | |
| IGF-1 | | | | | | | v | | V |
| VEGF | | | | v | | | v | | |
| VEGF-R1 | | | | v | | | v | | |
| VEGF-R2 | | | | | V | | | | |
| PIGF | | | | v | V | | v | | V |
| Ang-1 | | | | | | | | | V |
| Ang-2 | | V | | | | V | v | | V |