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ORIGINAL ARTICLE

Executive Dysfunction Early Postnatal Biomarkers among Children Born Extremely Preterm

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Abstract

We evaluated the relationship between blood levels of inflammatory and neurotrophic proteins during the first postnatal month in 692 children born before the 28th week of gestation and executive function limitations among those 10-year olds who had an IQ ≥ 70. The measures of dysfunction were Z-scores ≤ -1 on the Differential Ability Scales–II working memory (WM) assessment) ($N = 164$), the NEPSY-II (A Developmental NEuroPSYchological Assessment-II) Inhibition-Inhibition assessment) ($N = 350$), the NEPSY-II Inhibition-Switching assessment) ($N = 345$), as well as a Z-score ≤ -1 on all three assessments (identified as the executive dysfunction composite ($N = 104$). Increased risks of the executive dysfunction composite associated with high concentrations of inflammatory proteins (IL-8, TNF-α, and ICAM-1) were modulated by high concentrations of neurotrophic proteins. This pattern of modulation by neurotrophins of increased risk associated with inflammation was also seen for the working memory limitation, but only with high concentrations of IL-8 and TNF-α, and the switching limitation, but only with high concentrations of ICAM-1. We infer that among children born extremely preterm, risks of executive function limitations might be explained by perinatal systemic inflammation in the absence of adequate neurotrophic capability.

Keywords Infant, premature/blood · Neurodevelopment · Inflammation · Neurotrophic factors · Executive function

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
EP	Extremely preterm
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-1β
IL-6	Interleukin-6
IL-6R	Interleukin-6 Receptor
IL-8	Interleukin-8
KBIT-2	Kaufman Brief Intelligence Test– 2
MMP-9	Matrix Metalloproteinase-9
MPO	Myeloperoxidase
NEPSY-II	A Developmental NEuroPSYchological Assessment
NT-4	Neurotrophin-4
PIGF	Placenta Growth Factor
RANTES	Regulated upon Activation, Normal T cell Expressed, and Secreted

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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
DAS-II	Differential Ability Scales–II
WM	Working memory
NEPSY-II	A Developmental NEuroPSYchological Assessment-II
EDC	Executive Dysfunction Composite

Introduction

Children born very preterm are often at higher risk of executive function limitations than their term-born peers (Farooqi et al. 2016; Taylor and Clark 2016), especially if they have early-identified structural abnormalities of the brain (Kalpakidou et al. 2014). Because inflammation appears to increase the risk of brain damage in the very preterm newborn (Dammann and Leviton 2014), we reasoned that perhaps systemic inflammation provides information about the risk of executive dysfunctions (EDs). Similarly, because growth factors with neurotrophic and/or angiogenic properties have the potential to minimize the occurrence of brain damage (Chew and DeBoy 2016; Krityakiarana et al. 2016), we also reasoned that high concentrations of such proteins might ameliorate the risks of executive dysfunctions associated with systemic inflammation.

The three-factor structure of executive function, which consists of working memory (WM), ability to inhibit responses, and ability to shift/switch tasks, was based on findings among adults, and applies to older children, and adolescents (Friedman and Miyake 2017). To avoid any controversy about what we consider executive function, we considered separately the three components when evaluating the relationship between the concentrations of neonatal biomarkers and execution dysfunctions. We also created an ED composite consisting of low scores on each of the three assessments.

Here we report our findings of the relationships between low scores on assessments of executive functions (WM, inhibition, and switching) and elevated blood concentrations of inflammation-related proteins and proteins with angiogenic and neurotrophic properties. We restricted our analyses to children with IQ ≥ 70 so that the outcome we studied was executive functions limitations in the context of normal intelligence.

Methods

Participants (Table 1a)

The ELGAN (Extremely Low Gestational Age Newborn) 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. (O'Shea et al. 2009) of the 1506 infants born before the 28th week and recruited before the end of the first postnatal day, 966 children were actively recruited for follow-up at age 10 (because of the availability of circulating protein markers from the first month of life). 857 (89%) returned for a neurocognitive assessment at age 10 years. The sample for this study consists of the 692 of these 857 children who had a DAS-II mean IQ ≥ 70 and were administered the DAS-II WM assessment and NEPSY-II Inhibition-Inhibition and Inhibition-Switching subtests.

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 admission log (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 a standard data set (Yudkin et al. 1987).

Procedures for the Assessments at Age 10 Years

A subset of the families ($n = 966$) who participated in the follow up at two years of age were contacted by mail and then by phone to invite them to participate in the 10-year follow up. This subset was selected because we had measurements of inflammation-related proteins in neonatal blood. 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 measures reported here were administered in 3 to 4 h, including breaks.

General Cognitive Ability

General cognitive ability (or IQ) was assessed with the School-Age DAS-II Verbal and Nonverbal Reasoning scales (Elliott 2007). We classified children based on the mean of their verbal and non-verbal components into two

Table 1 Sample

a. sample description

	Yes	No
Enrolled at birth	1506	
Survived to age 10 years	1198	308
Proteins measured in ≥2 blood spots collected in first postnatal month	1192	6
Returned for an assessment at age 10 years	857	335
DAS-II IQ ≥ 70	713	144
Had all 3 executive function assessments.	692	21

b. Children classified by whether or not they had Z-scores ≤ −1 on each of three assessments of executive functions.

Working Memory	Inhibition Inhibition	Inhibition Switching	N	Description
≤ −1	≤ −1	≤ −1	104	All 3 (Exec Dysfunction Composite) (EDC)
≤ −1	≤ −1		14	
≤ −1		≤ −1	28	
≤ −1			18	Isolated Working Memory (WM)
	≤ −1	≤ −1	138	
	≤ −1		94	Isolated Inhibition Inhibition (INI)
		≤ −1	78	Isolated Inhibition Switching (INS)
			221	Referent (no Z-score ≤ −1)
Summary				
All ≤ −1			164	All Working Memory
	All ≤ −1		350	All Inhibition Inhibition
		All ≤ −1	345	All Inhibition Switching
Total N			692	

groups: <70 and ≥ 70. Only those in the higher IQ group are included in this report.

Indicators of Executive Function

Executive functions were assessed with both the DAS-II (Elliott 2007) and the NEPSY-II (Korkman et al. 1998). The DAS Recall of Digits Backward and Recall of Sequential Order measured verbal WM, while the NEPSY-II Inhibition-Inhibition and Inhibition-Switching measured simple inhibition and inhibition in the context of set shifting, respectively. We created an executive dysfunction composite (EDC), which we defined by a Z-score ≤ −1 on all three assessments (i.e., the DAS-II WM, and the NEPSY inhibition and switching components) among children with DAS-II mean IQ ≥ 70.

Blood Spot Collection, Storage, and Protein 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. After 10 years of storage at −24 °C, median protein abundance in dried blood spots decreases only 7%.(Bjorkesten et al. 2017) Details about the elution of proteins from the blood spots are provided elsewhere.(Fichorova et al. 2015).

All protein measurements were made by Dr. Fichorova's Genital Tract Biology Laboratory at the Brigham and Women's Hospital in Boston MA. This laboratory is accredited by The College of American Pathologists. The following proteins were measured with the Meso Scale Discovery (MSD) electrochemiluminescence multiplex platform and Sector Imager 2400, which has high analytic(Fichorova et al. 2008) and clinical validity(Hecht et al. 2011; Leviton et al. 2011a; McElrath et al. 2011; Leviton et al. 2012b; Bose et al. 2013): C-Reactive Protein (CRP), serum amyloid A (SAA), myeloperoxidase (MPO), Interleukin-1 β (IL-1β), Interleukin-6 (IL-6), Interleukin-6 Receptor (IL-6R), Tumor Necrosis Factor-α (TNF-α), Tumor Necrosis Factor 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), matrix metalloproteinase-9 (MMP-9), Thyroid Stimulating Hormone (TSH), Erythropoietin (EPO),Vascular Endothelial Growth Factor (VEGF), Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1, also known as sFLT-1), Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2; KDR), and IGF Binding Protein-1 (IGFBP-1).

The Laboratory optimized an ELISA assay for measuring IGF-1 in the dry blood spots using Duoset assay reagents. In brief, each dry blood spot elution was incubated with an acidic solution in a ratio of 1:20 (R&D Systems Catalog Buffer 08, pH 2.15) for 10 min at room temperature, followed by pH neutralization with 1:50 1.2 N NaOH (Fisher) in 0.5 M HEPES Buffer (Invitrogen). The plates were incubated with capture antibody overnight, blocked with a 5% Tween 20 reagent (R& D Systems) for 1 h, washed and incubated with 100 μl standards and neutralized samples for 2 h, followed by automated wash in 0.05% Tween20/PBS, 100 μl biotinylated goat anti-human IGF-1 antibody for 2 h, automated wash, streptavidin-HRP solution for 20 min, automated wash, substrate reagent for 20 min, and neutralization with stop solution. The plates were read at 450 nm filter and reference filter 570 nm using Victor2 reader (Perkin Elmer, Boston, MA). The inter-assay coefficient of variation assessed by a quality control sample run on each plate was 17%. The pg/ml values intrapolated from the standard curve of each assay were normalized to mg of total protein measured by the Pierce BCA assay (Fisher Scientific, Pittsburgh, PA).

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

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 individual protein were normalized to mg total protein.

Data Analyses

In the ELGAN Study cohort, 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 score on the Kaufman Brief Intelligence Test, Second Edition™ (KBIT-2™), or was eligible for government-provided medical care insurance (Medicaid) (Leviton et al. 2016a). Thus, confounding by social class is not an issue. Similarly, other potential confounders such as sex were also not associated with systemic inflammation.

We evaluated the following generalized null hypotheses:

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 low scores (i.e., Z-score ≤ -1) on the three NEPSY assessments of working memory, the inhibition-inhibition, inhibition-switching, and were not at greater risk of the EDC (defined as a Z-score ≤ -1 on all three assessments) than children who had a lower concentration of each protein on each of five days during the first postnatal month (Appendix Tables 1–4, which are summarized in Table 2).

2. Early and late epochs: 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) and during the second two postnatal weeks (when specimens were obtained on days 21 and 28) were not at greater risk of low scores on the working memory, inhibition-inhibition, and the inhibition-switching assessments, and of the EDC than children who had lower concentrations (Appendix Table 5 and summarized in Table 3).

3. Early and late epochs: 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-neurotroph) on at least two days during the first and second epochs were not at higher or lower risk of low scores on the working memory, inhibition-inhibition, and the inhibition-switching assessments, and of the EDC than children who had lower concentrations (Appendix Tables 6–13) and summarized in Table 4).

The concentrations of inflammation-related proteins in the ELGAN Study varied with gestational age, and with the postnatal day of collection (Leviton et al. 2011d; Leviton et al. 2012a). In addition, 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. Consequently, we divided our sample into 30 groups defined by three gestational age categories (23–24, 25–26, 27 weeks), five postnatal days of blood collection (1, 7, 14, 21 and 28), and two measurement sets (2009–2010, 2015). Because we were interested in the contribution of low and high concentrations, and the concentrations of most proteins did not follow a normal distribution, the distribution of each protein's

Table 2 Executive dysfunctions whose risks were statistically-significant when the concentrations of the proteins listed on the left were in the top quartile. Individual days

	Day 1				Day 7				Day 14				Day 21				Day 28			
	W	I	S	E	W	I	S	E	W	I	S	E	W	I	S	E	W	I	S	E
CRP							Λ						Λ		Λ					
SAA									Λ											
MPO																				
IL-1β																				
IL-6		Λ		Λ																Λ
IL-6R										V		V						V	V	V
TNF-α	Λ		Λ						Λ											
TNF-R1																				
TNF-R2															Λ	Λ				
IL-8	Λ		Λ	Λ									Λ	Λ	Λ	Λ	Λ		Λ	
RANTES											V						V			
ICAM-1							Λ				Λ					Λ				
VCAM-1										V										
MMP-9																				V
TSH																				
EPO			Λ	Λ																
NT-4										V		V								
BDNF										V						V				
bFGF				V											V	V			V	
IGF-1														Λ						
IGFBP-1																			V	
VEGF																				
VEGF-R1																		Λ		
VEGF-R2															Λ					
PIGF																				
Ang-1										V	V	V			V	V				
Ang-2																				

This table summarizes Supplement Tables 1, 2, 3, and 4. Λ indicates increased risk of a Z-score < −1 on the executive dysfunction assessment(s) identified at the top of each column, while V indicates decreased risk. W indicates a DAS-II Working Memory Z-score ≤ −1, I indicates a NEPSY-II Inhibition-inhibition Z- score ≤ −1, S indicates a NEPSY-II Inhibition-Switching Z- score ≤ −1, and E indicates the executive dysfunction composite defined as a Z-score ≤ −1 on all three assessments

concentration was divided into four quartiles among children in each of the 30 groups.

In the ELGAN Study population, both low gestational age (Leviton et al. 2011d) and fetal growth restriction (Landis and Koch 1977) were associated with protein concentrations, as well as with low IQ (Joseph et al. 2016). Consequently, we adjusted for gestational age category (23–24, 25–26, 27 weeks) and birth weight Z-score < −1. We created logistic regression models of the risk of our indicators of executive dysfunctions that enabled us to calculate odds ratios and 95% confidence intervals associated with protein concentrations in the top quartile relative to the risk among children with lower concentrations. We selected variables as confounders if identified in the literature or if in our data they were associated with both the exposure and the outcome with probabilities ≤ .25 (Dales and Ury 1978). The final logistic regression models adjusted for gestational age category (23–24, 25–26, and 27 weeks) and birth weight Z-score < −1.

In separate multinomial logistic regression models, we evaluated the risk of each executive dysfunction 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 angiogenic, neurotrophic, or anti-inflammatory protein appears to have protective effects is a statistically significant increased risk of the executive dysfunction among children whose blood had a top quartile concentration of the inflammation-related

Table 3 Executive dysfunctions whose risks were statistically-significant when the concentrations of the proteins listed on the left were in the top quartile

	Early epoch				Late epoch			
Proteins	W	I	S	E	W	I	S	E
CRP		Λ						
SAA								
MPO								
IL-1β								
IL-6								
IL-6R								
TNF-α	Λ		Λ	Λ				
TNF-R1								
TNF-R2								
IL-8	Λ			Λ	Λ	Λ		Λ
RANTES								
ICAM-1					Λ			
VCAM-1								
MMP-9								
TSH			Λ					
EPO	Λ							
NT-4								
BDNF							V	V
bFGF			V					
IGF-1								
IGFBP-1								
VEGF								
VEGF-R1			V			Λ	Λ	
VEGF-R2								
PIGF								
Ang-1		V		V				
Ang-2								

This table summarizes Appendix Table 5, which deals with elevated concentrations on two days during the early epoch (first two postnatal weeks) and during the late epoch (third and fourth two postnatal weeks). Λ indicates increased risk of a Z-score < -1 on the assessment(s) identified at the top of each column, while V indicates decreased risk. W indicates a DAS-II Working Memory Z-score ≤ -1, I indicates a NEPSY-II Inhibition-inhibition Z- score ≤ -1, S indicates a NEPSY-II Inhibition-Switching Z- score ≤ -1, and E indicates the executive dysfunction composite defined as a Z-score ≤ -1 on all three assessments

protein and a lower concentration of the angiogenic, neurotrophic, or anti-inflammatory protein, while the risk was not increased among children whose blood had top quartile concentrations of both proteins.

Enrollment and consent procedures for this follow up study were approved by the institutional review board of every participating institution.

Results

Sample Description (Table 1b)

Of the 692 children who at age 10 years had an IQ ≥ 70, and an assessment of WM, inhibition and switching, 164 had a WM Z-score ≤ -1, while 350 had an inhibition Z-score ≤ -1, and 345 a switching Z-score ≤ -1. Only 104 children had a Z-score ≤ -1 on all three assessments, which we identify as the Executive Dysfunction Composite (EDC). Only 221 children had a Z-score > -1 on all three assessments.

Tables 2, 3, and 4

Detailed results are included in the tables in the Supplement, and the details of these tables are summarized in Tables 2, 3, and 4. Table 2 summarizes Supplement Tables 1–4, while Table 3 summarizes Supplement Table 5. Table 4 summarizes Supplement Tables 6 through 13.

Table 4 Summary of Appendix Tables 6–13, which address two proteins at a time for the early epoch (4a) and separately for the late epoch (4b)

	TNF-α				IL-8				ICAM-1			
Proteins	W	I	S	E	W	I	S	E	W	I	S	E
a. early epoch												
IL-6R	V			V	V			V			V	V
MMP-9	V	Λ			V		Λ	V				
RANTES	V			V	V			V			V	V
EPO	Λ		Λ		Λ				Λ			
NT-4	V			V	V			V				
BDNF	V		V	V				V			V	V
bFGF			Λ	V				V			V	V
IGF-1	V			V	V			V			V	
VEGF	V			V	V	V		V	V		V	V
VEGF-R1	V	V	V	V			V	V			V	V
VEGF-R2	V		V	V				V			V	
PIGF	V		V	V	V			V			V	V
Ang-1	V		V	V	V	V		V			V	V
Ang-2												
b. late epoch												
IL-6R									V			V
MMP-9						V		V				
RANTES					V			V	V		V	
EPO					V	V		V	V		V	V
NT-4						V	V		V			V
BDNF					V				V			
bFGF							V	V				
IGF-1						V	V				V	V
VEGF					V	V						
VEGF-R1					V		V	V		Λ	V	
VEGF-R2					Λ	V						
PIGF					V			V	V			V
Ang-1									V			
Ang-2						V			V		V	V

The inflammation-related protein is identified at the top of each set of columns, and the neurotrophic proteins are identified on the left. Λ indicates increased risk of a Z-score < -1 on the executive dysfunction assessment(s) identified just below the inflammation-related protein, while V indicates decreased risk. W indicates a DAS-II Working Memory Z-score ≤ -1, I indicates a NEPSY-II Inhibition-inhibition Z- score ≤ -1, S indicates a NEPSY-II Inhibition-Switching Z- score ≤ -1, and E indicates the executive dysfunction composite defined as a Z-score ≤ -1 on all three assessments

Individual Days (Table 2)

For each protein, we evaluated the relationship between elevated concentrations in specimens collected on each of five occasions and four indicators of executive dysfunction, for a total of 20 significance tests for each protein. We found associations between a high concentration of CRP, IL-6, TNF-α, and IL-8 on at least two days and one or more indicators of executive dysfunction. High concentrations of IL-6R, bFGF, BDNF, RANTES, and Ang-1 on at least two days were associated with a decreased risk of one or more executive function limitations.

Multiple Days in the Early and Late Epochs (Table 3)

Early Epoch

We classify the first two postnatal weeks as the early epoch and the second two postnatal weeks as the late epoch, and classify children as having increased concentrations during an epoch if the top quartile concentrations were evident on two (or more) days during that epoch. In the early epoch, the risks of low WM Z-scores were increased among children who had elevated concentrations of TNF-α, IL-8, and EPO. For example, the odds ratio for a low WM score was 2.2 (95% confidence interval:

1.4, 3.3) among children who had elevated concentrations of TNF-α, and (OR: 1.6; (1.01, 2.6)) among children who had elevated concentrations of IL-8 on two days during the early epoch. Increased risks of low Z-scores on the inhibition assessment were associated with top-quartile concentrations of CRP (OR: 1.5 (1.02, 2.3)), while low Z-scores on the switching component were associated with top-quartile concentrations of TNF-α (OR: 1.6; (1.03, 2.3)) and TSH (OR: 1.5; (1.02, 2.2)). Increased risks of the EDC were associated with top quartile concentrations of TNF-α (OR: 1.8; (1.1, 2.9)) and IL-8 (OR: (1.9; (1.1, 3.2)) during the early epoch.

Reduced risks of low Z-scores on the inhibition assessment were associated with high concentrations of Ang-1 during the early epoch (OR: 0.6; (0.4, 0.9)), while reduced risks of the EDC were associated with top quartile concentrations of Ang-1 (OR: 0.4; (0.2, 0.8)).

Late Epoch

The risks of low WM Z-scores were increased among children who had elevated concentrations of IL-8 (OR: 2.1; (1.1, 3.8)) and ICAM-1 (OR: 1.9; (1.03, 3.3)) on two days during the late epoch. Increased risks of low Z-scores on the inhibition assessment were associated with top-quartile concentrations of IL-8 (OR: 2.0; (1.1, 3.8)) and VEGF-R1 (OR: 2.6; (1.3, 3.8)), while increased risks of low Z-scores on the switching assessment were associated with top-quartile concentrations of VEGF-R1 only (OR: 1.9; (1.03, 3.4)), and increased risks of the EDC were associated with top-quartile concentrations of IL-8 only (OR: 2.0; (1.03, 3.9)).

Top quartile concentrations of BDNF during the late epoch were associated with reduced risks of low Z-scores on the switching assessment (OR: 0.6; (0.3, 0.96)), as well as reduced risks of the EDC (OR: 0.4; (0.2, 0.9)).

Sets of Inflammation and Neurotrophic Proteins (Table 4)

Early Epoch

The increased risks of low scores on the WM and inhibition assessments and of the EDC associated with top quartile concentrations of TNF-α, and separately, associated with top quartile concentrations of IL-8, were moderated by top quartile concentrations of most neurotrophic proteins, whereas low scores on the switching assessment were not. The increased risks of low scores on the switching assessment and of the EDC associated with high concentrations of ICAM-1 were also moderated by top quartile concentrations of most neurotrophic proteins, whereas this was not the case for low scores on the WM and inhibition assessments.

The risk of the EDC (defined as a Z-score ≤ -1 on the working memory, inhibition, and switching assessments) associated with a top quartile concentration of TNF-α was lower when the high concentration of TNF-α was accompanied by a top quartile concentrations of each of 11 of the 14 proteins that had neurotrophic, angiogenic, or anti-inflammatory properties. Similarly, the risk of the EDC associated with a top quartile concentration of IL-8 was lower when the high concentration of TNF-α was accompanied by a top quartile concentrations of 12 of these 14 proteins, and the risk of the EDC associated with a top quartile concentration of ICAM-1 was lower when the high concentration of ICAM-1 was accompanied by a top quartile concentrations of 8 of these 14 proteins. With 95% confidence intervals ($p < 0.05$), 2 of these 42 ORs calculated would be statistically significant by chance alone. In contrast, we found that 31 ORs were statistically-significant, about 15 times more than expected.

Late Epoch

The pattern of moderation by neurotrophins of increased risks associated with high concentrations of TNF-α during the early epoch is not seen in the late epoch. In contrast, low scores on all three assessments and EDC associated with high concentrations of IL-8 appear to be moderated by high concentrations of neurotrophic proteins. Similar, but less prominent examples of this modulation were associated with high concentrations of ICAM-1 and risks of low scores on assessments of WM, switching, and the EDC.

Discussion

What we Found

Our main findings are that extremely low gestational age newborns who had sustained/recurrent systemic inflammation during the first postnatal month are at increased risk of impairments on assessments of WM, inhibition, the ability to switch tasks (and responses), and the EDC, and that these increased risks appear to be ameliorated by (presumably concomitant) abundant amounts of proteins with neurotrophic and angiogenic properties.

What Others Found in Humans. How our Findings Are Similar or Different

We have not found any other report that assessed the contribution of neonatal systemic inflammation to the occurrence of executive dysfunctions at school age

among children born extremely or very preterm. Consequently, we advise caution in drawing inferences from our findings.

Executive Dysfunctions

Categorization of Executive Dysfunctions

No sharp break in assessment scores (or discontinuity) separates those with an executive dysfunction from their peers (Branum-Martin et al. 2013). The preference of epidemiologists to study categorical entities rather than continua is exemplified by the establishment of cut-offs for continuous measures of function/dysfunction (e.g., hypertension, diabetes mellitus, glaucoma). In keeping with this preference, we dichotomized Z-scores on the WM, inhibition, and switching assessments.

Executive Dysfunctions in General or Does the Blood Profile Associated with One Dysfunction Differ from that of Other Limitations?

Each executive function limitation is most often characterized by increased risks associated with systemic inflammation, which is attenuated in the presence of high concentrations of neurotrophic proteins. The differences among the protein profiles of the three limitations are relatively minor in comparison to their similarities. This might reflect the tendency for the three dysfunctions to co-occur. On the other hand, the variations on a theme might also reflect some differences that if not unique, at least reveal biologic differences.

Categorization of Exposure

Top-Quartile Concentrations

In the absence of information about what level of inflammation is needed to result in brain damage or increased risk of executive dysfunctions, we have assumed that relatively high levels are needed. Consequently, we have compared children whose concentrations of inflammation-related proteins are in the top quartile to children who had lower concentrations. This has allowed us to identify inflammation as an antecedent of ventriculomegaly when the newborn was in the intensive care nursery (Leviton et al. 2011b; Leviton et al. 2018b), and low Bayley Scales of Infant Development-II (O'Shea et al. 2012), an attention problem (Allred et al. 2017), cerebral palsy (Kuban et al. 2014), and microcephaly (Leviton et al. 2011c) at age 2 years, and low IQ (Kuban et al. 2017), attention deficit hyperactive disorder at age 10 years (Allred et al. 2017), and learning limitations (Leviton et al. 2018a).

High Concentrations on Multiple Days

Transient inflammation is less likely to damage the newborn EP brain than recurrent or sustained inflammation (Dammann and Leviton 2014). In addition, systemic inflammation tends to be sustained over the first postnatal weeks in our ELGAN Study population. These observations prompted us to examine not only single-day elevated concentrations, but also elevated concentrations on multiple days.

Inflammation Is Associated with Increased Risk of Executive Dysfunctions

When we did not consider neurotrophins, we found that the risks of executive dysfunctions were associated with single-day high concentrations of IL-8 (Table 2: days 1 and 21), and only meager associations with top quartile, single-day concentrations of IL-6, TNF-α, TNF-R2, and ICAM-1. Recurrent or sustained high concentrations of TNF-α during the first two weeks, however, were associated with increased risks of executive dysfunction, while recurrent or sustained high concentrations of IL-8 during the first two weeks, and separately during the second two weeks were also associated with increased risks of executive dysfunction (Table 3). This documentation of the highest risks of damage/dysfunction when the inflammation is sustained or recurrent is in keeping with our experience with other disorders.(Dammann and Leviton 2014; Kuban et al. 2015; Leviton et al. 2016b; Kuban et al. 2017) We now have additional evidence that in some cases, the inflammation can be sustained for a month.(Dammann et al. 2016).

Neurotrophins Protect

We found strong evidence that elevated concentrations of neurotrophins modulate the risk of executive dysfunctions (Table 4). Indeed, the inference from the discrepancy between Tables 3 and 4 suggests that the potential contribution of inflammation to dysfunctions is best evaluated in light of the potentially modulating effects of neurotrophic, angiogenic, and anti-inflammatory influences.

Failure of Neurotrophins to Protect

In isolated instances, increased risk occurred when the concentrations of both the inflammatory and neurotrophic proteins were in the top quartile. This pattern has several

interpretations. One is that high concentrations of both proteins are needed to cause damage. Another is that as a consequence of damage, both proteins are released into the circulation. The third interpretation borrows from both of these possibilities and postulates that the putative damage promoter does indeed promote damage, and in doing so also promotes the synthesis and/or release of the putative protector/repair-enhancer (Xing and Lo 2017). Because we are unable to distinguish which of these three possibilities applies to our findings, we identify this pattern as the "damage cause and/or consequence" pattern.

Co-Occurrence of Inflammation and Neurotrophic Proteins

The term "help-me signaling" has been applied to the release by damaged or diseased neurons of inflammation and neurotrophic proteins that are able to recruit cells to assist in repair/regeneration (Xing and Lo 2017). The co-occurrence of elevated concentrations of inflammation and neurotrophic proteins has been documented in ELGANS (Leviton et al. 2017). Others have emphasized the cross-talk between neurotrophins and inflammation (da Silva Meirelles et al. 2017).

Developmental Regulation

In general, compared to older children and adults, newborns tend to have diminished inflammatory and protective immune responses to inflammatory/infectious stimuli (Dammann and Leviton 2014). This can be especially pronounced among those born preterm (Kan et al. 2016).

Despite differences in gestational age, newborns with the same postconceptional age have similar profiles of gene expression (Zasada et al. 2014). Nevertheless, among extremely low gestational age newborns, the lower the gestational age, the higher the concentrations of inflammation-related cytokines in the blood (Leviton et al. 2011d), while the availability of neurotrophins increases with gestational age (van Tilborg et al. 2016). The result is that the more "mature" the newborn (along the developmental regulation schedule), the more s/he should be able to resolve inflammation (Dammann and Leviton 2014), and promote brain growth and well-being (Murase 2014). With these thoughts in mind, we offer the possibility that the high concentrations of the proteins we measured are surrogates for proteins we did not measure, and for developmental processes we did not identify.

Limitations and Strengths of this Study

Perhaps our main weakness is the limited number of children in some analyses. With 445 children who provided two specimens for the late epoch (Appendix Table 3), only 28 children are expected to have top-quartile concentrations of both proteins (445 x .25 x .25). As half of all children had a low score on the switching assessment (345/692), we would expect only 14 of these 28 children to have a switching impairment. On occasion, however, the cell representing the top-quartile concentrations of both proteins might be empty or have only one child, by chance alone, resulting in our inability to estimate the confidence interval. So long as, such empty cells are accompanied by statistically-significantly high risks for the cell represented by the top quartile concentration of the inflammation-related protein and a lower concentration of the neurotrophic protein, they can be viewed as support for the ability of neurotrophic proteins to modulate the increased risks associated with inflammation.

The interrelatedness of early and late systemic inflammation (Dammann et al. 2016), limits our ability to tease apart the contributions of each epoch to the occurrence of each dysfunction. We are also limited by the relatively small number of proteins measured. Inflammation is a broad and complex phenomenon (Zak and Aderem 2009), and not one fully assessed by our methods. In addition, the proteins we measured might not be in the causal and/or repair chains, but merely surrogates for other proteins in their broad group. 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 (Becher et al. 2017). Similarly, we view each neurotrophin as a surrogate for other proteins that possess neurotrophic characteristics (Oliveira et al. 2013).

We relied on blood specimens obtained for clinical indications. As their cardio-pulmonary function and blood gas exchange stabilized, 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.

What we measured was peripheral blood. The "periphery as a window to the brain" concept assumes that what is measured in the blood conveys valuable information about what is happening in the brain (Fernandes et al. 2015). Our study is further limited if this assumption is not true.

With more than 600 children and our exposure defined by a protein concentration in the top quartile, and identification of a WM limitation in 24% of children, we have power to appreciate risk ratios of 1.8 as statistically significant. Other strengths are the selection of infants based on gestational age (and not birth weight) (Arnold et al. 1991), prospective collection of all data, modest attrition, and finally, protein data of high quality (Fichorova et al. 2008), and high content validity (Fichorova et al. 2011; Hecht et al. 2011; Leviton et al. 2011d; McElrath et al. 2011).

Conclusions

1. In this sample of children born extremely preterm, systemic inflammation conveys information about heightened risks of executive dysfunctions, while high concentrations of neurotrophic proteins appear to modulate these increased risks.

2. Protein-concentration profiles in blood obtained during the first postnatal month discriminate minimally among the executive dysfunctions we assessed.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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