Among Children Born Extremely Preterm a Higher Level of Circulating Neurotrophins Is Associated with Lower Risk of Cognitive Impairment at School Age

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Objectives To test the hypothesis that higher blood levels of neurotrophic proteins (proteins that support neuronal survival and function) in the first 2 weeks of life are associated with a lower risk of cognitive impairment at 10 years.

Study design We evaluated 812 10-year-old children with neonatal blood specimens enrolled in the multicenter prospective Extremely Low Gestational Age Newborn Study, assessing 22 blood proteins collected on 3 days over the first 2 weeks of life. Using latent profile analysis, we derived a cognitive function level based on standardized cognitive and executive function tests. We defined high exposure as the top quartile neurotrophic protein blood level on ≥2 days either for ≥4 proteins or for a specific cluster of neurotrophic proteins (defined by latent class analysis). Multinomial logistic regression analyzed associations between high exposures and cognitive impairment.

Results Controlling for the effects of inflammatory proteins, persistently elevated blood levels of ≥4 neurotrophic proteins were associated with reduced risk of moderate (OR, 0.35; 95% CI, 0.18-0.67) and severe cognitive impairment (OR, 0.22; 95% CI, 0.09-0.53). Children with a cluster of elevated proteins including angiopoietin 1, brain-derived neurotrophic factor, and regulated upon activation, normal T-cell expressed, and secreted had a reduced risk of adverse cognitive outcomes (OR range, 0.31-0.6). The risk for moderate to severe cognitive impairment was least with 0-1 inflammatory and >4 neurotrophic proteins.

Conclusions Persisting elevations of circulating neurotrophic proteins during the first 2 weeks of life are associated with lowered risk of impaired cognition at 10 years of age, controlling for increases in inflammatory proteins. *(J Pediatr 2018;■■:■■-■■).*

Advances in neonatal intensive care have increased the survival of extremely preterm children born at <28 weeks of gestation.[1] Increased survival rates have not been accompanied by similar improvements in neurodevelopmental outcomes, and one-quarter of survivors have cognitive impairment.[2,3] Reduction of the risk of cognitive impairment depends on an improved understanding of its etiology.

The Extremely Low Gestational Age Newborn (ELGAN) Study was designed to test the hypothesis that perinatal inflammation is associated with persisting brain structural and functional disorders. In the ELGAN cohort of about 1000 children born at <28 weeks of gestation, neonatal elevations of specific inflammation-associated protein biomarkers in blood robustly predicted cognitive impairment at 2 years of age.[4,5] These indicators of neonatal systemic inflammation also were associated with impaired cognition at 10 years of age.[6]

ANG	Angiopoietin
BDNF	Brain-derived neurotrophic factor
CRP	C-reactive protein
EF	Executive function
ELGAN	Extremely Low Gestational Age Newborn
IRG	Inflammatory risk group
LCA	Latent class analysis
NRG	Neurotrophin group
RANTES	Regulated upon activation, normal T-cell expressed, and secreted
SAA	Serum amyloid A

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In the ELGAN Study, blood samples were taken in the first 2 weeks of life and we measured levels of neurotrophic proteins, including growth factors, neurotrophins, and angiotrophins that might influence developmental outcomes.[7,8] These proteins support the growth, survival, and differentiation of developing neurons. Lower levels of these proteins may signal less resilience against inflammation-associated injury and higher levels may prevent damage.[9] Inflammation-related proteins are associated with cognitive impairment, and neurotrophic proteins may be associated with better cognitive outcomes, but elevations within these 2 protein families that operate in opposite directions frequently occur simultaneously. We, therefore, tested the hypothesis that higher blood levels of neurotrophic proteins measured in the first 2 weeks of life would be associated with a lesser risk of cognitive impairment at 10 years of age, controlling for the presence of inflammation-associated proteins.

Methods

The ELGAN Study is a multicenter, observational study of the risk of structural and functional neurologic disorders in extremely preterm infants. From 2002 to 2004, women delivering at <28 weeks of gestation were asked for consent to enroll their child into the study. This analysis includes 812 children (a subset of the surviving 1200 study participants) who had ≥2 sets of neonatal blood samples for proteins and were evaluated at 10 years of age (**Figure 1**; available at www.jpeds.com). This study was approved by the institutional review boards of all participating institutions and informed consent was obtained from all participants.

Cognitive Assessment and Derivation of Levels of Function

Child assessments included the full-scale IQ from the School-Age Differential Ability Scales–II Verbal and Nonverbal Reasoning scales[10] and executive function (EF) (from the School-Age Differential Ability Scales–II Verbal and Nonverbal Reasoning scales and the Developmental NEeuroPSYchological Assessment, 2nd Edition[11]).

Even though the development and maturation of neural circuits underlying aspects of IQ and EF differ from each other,[12] both can be reliably measured by 10 years of age.[13] As previously reported, we evaluated cognitive outcomes using latent class analysis (LCA) classifications, which represented both IQ and EF abilities, to provide a better predictor of adaptive outcomes, such as academic success, than IQ alone.[2,14] Children were classified into their most likely latent class for analysis. With LCA, we identified 4 subgroups of children in our cohort corresponding with overall cognitive functioning that was normal (34% of cohort; normal mean IQ and EF scores), low-normal (41%; mean IQ and EF scores ranging from 0.5 to 1.5 SDs below norm), moderately impaired (17%; mean IQ and EF measures from 1.5 to 2.5 SDs below norm), and severely impaired (8%; mean IQ and EF measures from 2.5 to 4.0 SDs below norm).[2]

Assessment of Inflammation and Neutrophic Proteins

Blood Protein Measurements. Drops of whole blood were collected on postnatal days 1 (range, 1-3 days), 7 (range, 5-8 days), and 14 (range, 12-15 days).[15,16] Protein concentration quartiles were normalized for gestational age and day of collection.[17] Because single day elevations of proteins are not as strongly associated with cognitive outcomes as are persistent elevations,[6] we defined protein concentration elevation as being in the highest quartile on ≥2 of 3 measures obtained.

Identification of Clusters of Neurotrophic and Inflammation-Associated Proteins with LCA. The specific neurotrophic proteins evaluated are listed in **Table I** (available at www.jpeds.com). Given that the blood levels of neurotrophic proteins under study might correlate with one another, we conducted separate LCA analyses on each postnatal day, fitting models with 2-5 classes and choosing an appropriate model based on fit statistics, entropy, interpretability, and consistency of results across days (**Table II**; available at www.jpeds.com). Analyses consistently identified 3 subgroups of children with similar patterns of neurotrophin elevations. Based on these analyses, we categorized children into 3 distinct subgroups (**Table III**; available at www.jpeds.com). The neurotrophic-related protein group (NRG) 1 had ≤2 elevated proteins; NRG2 had elevations of ≥3 proteins including ≥2 of the following 3 neurotrophic proteins: regulated upon activation, normal T-cell expressed, and secreted (RANTES), brain-derived neurotrophic factor (BDNF), and angiopoietin 1 (Ang-1); and NRG3 had low levels on ≥2 of 3 NRG2 proteins, RANTES, BDNF, and Ang-1, but elevated levels of ≥3 of the other neurotrophic proteins (**Table III**).

The analyses also included values for 8 inflammation-related proteins obtained at the same 3 time points as the neurotrophic proteins. These inflammatory-related proteins have been associated with structural and functional neurological outcomes in previous ELGAN Study analyses **Table I**.[4,18,19] As with the neurotrophic proteins, we conducted LCA on postnatal days 1, 7, and 14 and found that a 3-class solution was most consistent across all 3 days (**Table II**). Based on these analyses, we identified 3 distinct subgroups of children: inflammatory group (IRG) 2 had ≥3 elevated proteins that included elevation of either C-reactive protein (CRP) or serum amyloid A (SAA). IRG3 had normal CRP and SAA but had elevations of ≥3 other inflammatory proteins (**Table III**).

We a priori operationally defined neurotrophic protein exposure in 2 ways. First, we considered the number of sustained elevated inflammatory and sustained elevated neurotrophic proteins as measures of the breadth of inflammatory or neurotrophic exposure (0-1 proteins [referent group], 2-3 proteins, >4 proteins). Second, we considered the at-risk subgroups of children based on a pattern of elevated proteins derived from LCA.

Statistical Analyses

We tested the hypothesis that elevation of neurotrophic proteins in the first 2 weeks of life is associated with a decreased

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