Circulating Inflammatory-Associated Proteins in the First Month of Life and Cognitive Impairment at Age 10 Years in Children Born Extremely Preterm

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Objectives To evaluate whether in children born extremely preterm, indicators of sustained systemic inflammation in the first month of life are associated with cognitive impairment at school age.

Study design A total of 873 of 966 eligible children previously enrolled in the multicenter Extremely Low Gestational Age Newborn Study from 2002 to 2004 were evaluated at age 10 years. We analyzed the relationship between elevated blood concentrations of inflammation-associated proteins in the first 2 weeks ("early elevations"; n = 812) and the third and fourth week ("late elevations"; n = 532) of life with neurocognition.

Results Early elevations of C-reactive protein, tumor necrosis factor-α, interleukin (IL)-8, intercellular adhesion molecule (ICAM)-1, and erythropoietin were associated with IQ values >2 SD below the expected mean (ORs: 2.0-2.3) and with moderate to severe cognitive impairment on a composite measure of IQ and executive function (ORs: 2.1-3.6). Additionally, severe cognitive impairment was associated with late protein elevations of C-reactive protein (OR: 4.0; 95% CI 1.5, 10), IL-8 (OR: 5.0; 1.9, 13), ICAM-1 (OR: 6.5; 2.6, 16), vascular endothelial growth factor-receptor 2 (OR: 3.2; 1.2, 8.3), and thyroid-stimulating hormone (OR: 3.1; 1.3, 7.3). Moderate cognitive impairment was most strongly associated with elevations of IL-8, ICAM-1, and vascular endothelial growth factor-receptor 2. When 4 or more inflammatory proteins were elevated early, the risk of having an IQ <70 and having overall impaired cognitive ability was more than doubled (ORs: 2.1-2.4); the presence of 4 or more inflammatory protein elevated late was strongly linked to adverse cognitive outcomes (ORs: 2.9-4.8).

Conclusions Extremely preterm children who had sustained elevations of inflammation-related proteins in the first postnatal month are more likely than extremely preterm peers without such elevations to have cognitive impairment at 10 years. *(J Pediatr 2017;180:116-23).*

Advances in neonatal intensive care have increased the survival of extremely preterm children born before 28 weeks of gestation.[1] Little progress has been made, however, in the prevention of moderate to severe neurocognitive impairments that affect about 40% of extremely preterm survivors.[1-13] Improvement in outcomes of extremely preterm children requires a better understanding of the antecedents and causes of neurocognitive impairment in this population, which could lead to development of new technologies and approaches. In the initial phase of the Extremely Low Gestational Age Newborn (ELGAN) Study that evaluated more than 900 children born before 28 weeks ges-

BSID-II	Bayley Scales of Infant Development, Second Edition
CRP	C-reactive protein
DAS–II	Differential Ability Scales, Second Edition
ELGAN	Extremely low gestational age newborn
ICAM	Intercellular adhesion molecule
IL	Interleukin
LPA	Latent profile analysis
MDI	Mental development index
MMP	Matrix metalloproteinase
NEPSY-II	Neuropsychological Assessment, Second Edition
SAA	Serum amyloid A
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
VEGF-R2	VEGF-receptor 2

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tation, neonatal elevations of specific molecular biomarkers (ie, inflammation-associated proteins in blood), robustly predicted cognitive impairment at 2 years of age.[14,15] Moreover, concentrations of inflammation-related proteins in blood spot samples collected in the third and fourth weeks of life were associated with 2-year outcomes beyond associations with protein concentrations from the first 2 postnatal weeks alone.[16]

Cognitive assessments at 2 years among infants born with extremely low birth weight, however, have correlated only modestly with school-age cognitive abilities,[12,17] which better predict later academic achievement and vocational and social competence.[18] For a more definitive evaluation of the long-term impact of neonatal elevations of inflammation-related proteins, we assessed cognitive abilities during school age in the ELGAN Study cohort when assessment of cognitive ability is reliable.

We report here analyses that test the hypothesis that persistent, elevated concentrations of circulating inflammation-associated proteins in the first 2 postnatal weeks (early) are associated with an increased risk of cognitive deficits at 10 years of age in extremely preterm children. We also test the hypothesis that persistent, elevated concentrations of circulating inflammation-associated proteins in the third and fourth postnatal weeks are associated with risk of cognitive deficits at age 10 years beyond the risk conferred by the "early" elevations of inflammatory proteins.

Methods

The ELGAN Study is a multicenter observational study of the risk of structural and functional neurologic disorders in extremely preterm infants. During 2002-2004, women delivering before 28 weeks gestation were asked to enroll in the study. A total of 1249 mothers of 1506 infants consented to participate. At 10 years of age, 966 surviving children for whom we obtained neonatal blood specimens for measurement of inflammation-related proteins were targeted for recruitment. The families of 889 (92%) of these children returned for follow-up. The institutional review boards of all participating institutions approved enrollment and consent procedures for this follow-up study.

Of the 889 children evaluated, 11 did not accompany the parent or caregiver during the follow-up visit (hence, informed consent could not be obtained), and 5 children did not cooperate with the child assessment, leaving a final sample of 873 children. In the analyses that included only early protein elevations, of the 873 participants, we evaluated risk in the 812 for whom both early blood samples and 10-year outcome data were available. When we evaluated risk associated with late elevations (third and fourth postnatal week), we considered the risk above that attributable to the early elevations, and included only the 532 children for whom we possessed blood samples at both the early and late time intervals. Because of severe motor, visual, and cognitive disability, 29 children were assigned floor scores on all tests, and 11 were assigned floor scores on some tests.

Families willing to participate were scheduled for 1 visit, usually at the institution of birth. Child measures were selected to provide the most comprehensive assessment of cognitive and academic function obtainable in a single testing session. Evaluations were administered by certified child psychologists blinded to clinical information in a 3- to 4-hour session that included breaks. All psychologist examiners underwent a 1-day in-person training and verification of competency for administering the neurocognitive test battery.

Assessments

General cognitive ability (or IQ) was assessed with the school-age Differential Ability Scales, Second Edition (DAS-II)[19] and Verbal and Nonverbal Reasoning scales. Because DAS-II Verbal and Nonverbal IQ scores were strongly correlated within the sample, the mean of these 2 measures was used as an estimate of general cognitive ability.

Attention and executive function were assessed with the DAS-II and the Neuropsychological Assessment, Second Edition (NEPSY-II).[20] DAS-II Recall of Digits Backward and Recall of Sequential Order measured verbal working memory. NEPSY-II Auditory Attention and Auditory Response Set measured sustained auditory attention, set switching, and inhibition. NEPSY-II Inhibition-Inhibition and Inhibition-Switching tasks measured simple inhibition and inhibition in the context of set shifting, respectively. NEPSY-II Animal Sorting measured concept generation and mental flexibility. For purposes of these analyses, executive function was considered in conjunction with IQ using a latent profile analysis (LPA) construct.

We used LPA to classify children in our sample into subgroups based on similarities in their profiles of IQ and executive function scores. These analyses identified 4 subgroups in our cohort corresponding to overall cognitive functioning that was normal (34% of cohort, with mean IQ and executive function scores within normal range on all measures), low-normal (41%, with mean IQ and executive function scores ranging from 0.5 to 1 SDs below the norm), moderately impaired (17%, with mean IQ and executive function measures between 1.5 and 2.5 SDs below the norm), and severely impaired (8%, with mean IQ and executive function measures from 3 to 4 SDs below the norm).

Blood Protein Measurements

Drops of whole blood were collected on Schleicher and Schuell 903 (Schleicher and Schuell, Inc, Keene, New Hampshire) filter paper on the first postnatal day (range: 1-3 days) and the seventh (range: 5-8 days) and 14th (range: 12-15 days) postnatal days. Twenty-eight proteins taken from blood samples in the first 2 weeks of life and 16 proteins taken from blood sample in the third and fourth weeks of life were measured in the Laboratory of Genital Tract Biology, Brigham and Women's Hospital, using the Meso Scale Discovery multiplex platform and Sector Imager 2400 (Meso Scale Discovery, Gaithersburg, Maryland), which has been validated against enzyme-linked immunosorbent assay. Details about the procedure for processing the blood spots and for measuring protein concentrations and absolute value ranges for 28 inflammation-regulating proteins are explained elsewhere.[21]