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Original Article

The Breadth and Type of Systemic Inflammation and the Risk of Adverse Neurological Outcomes in Extremely Low Gestation Newborns

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ABSTRACT

BACKGROUND: We hypothesized that the risk of brain damage in extremely preterm neonates increases with the breadth and type of systemic inflammation, indexed by the number of elevated inflammation-related proteins and the number of functional categories of inflammation-related proteins exhibiting an elevated concentration. **METHODS:** In blood from 881 infants born before 28 weeks gestation, we measured the concentrations of 25 inflammation-related proteins, representing six functional categories (cytokines, chemokines, growth factors, adhesion molecules, metalloproteinases, and liver-produced acute phase reactant proteins) on postnatal days 1, 7, and 14. We evaluated associations between the number and type of proteins whose concentrations were elevated on two separate occasions a week apart and the diagnoses of ventriculomegaly as a neonate, and at 2 years, microcephaly, impaired early cognitive functioning, cerebral palsy, and autism risk as assessed with the Modified Checklist for Autism in Toddlers screen, and in a subset of these children from 12 of 14 sites (n = 826), an attention problem identified with the Child Behavior Checklist. **RESULTS:** The risk of abnormal brain structure and function overall was increased among children who had recurrent and/or persistent elevations of the 25 proteins. The risk for most outcomes did not rise until at least four proteins in at least two functional categories were elevated. When we focused our analysis on 10 proteins previously found to be associated consistently with neurological outcomes, we found the risk of low Mental Development Index on the Bayley Scales of Infant Development-II, microcephaly, and a Child Behavior Checklist-defined attention problem increased with higher numbers of these recurrently and/or persistently elevated proteins. **INTERPRETATION:** Increasing breadth of early neonatal inflammation, indexed by the number of protein elevations or the number of protein functional classes elevated, is associated with increasing risk of disorders of brain structure and function among infants born extremely preterm.

Keywords: extremely preterm infants, inflammation-related proteins, adverse neurological outcomes, early life predictors of outcome

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Introduction

Inflammation is an antecedent of brain damage in very preterm newborns. Persistent and/or recurrent elevations of inflammation-related proteins in the blood of neonates in the first 2 postnatal weeks[1,2] predict diffuse white matter damage in the first months of life,[3] and at 2 years of age, microcephaly,[4] very low Mental Development Index (MDI) on the Bayley Scales of Infant Development-II,[1] attention problem identified with the Child Behavior Checklist (CBCL),[5] and cerebral palsy.[2]

Previously, we demonstrated that the inflammation signal was associated with adverse neurological findings based on the strength of the protein signal (whether the concentration of inflammation-related protein was in the highest quartile) and the duration of the signal (whether the elevation was present on more than one measurement over the course of at least 1 week).[1-4,6] If inflammation is associated with increased risk of perinatal brain damage, then the risk might also increase in proportion to the breadth of inflammation, defined by the number of elevated blood levels of inflammation-related proteins and the number of functional categories represented by these proteins. In this study, we evaluated the extent to which the breadth of neonatal systemic inflammation and perinatal brain damage are related in a dose-response or threshold manner.

Methods

The Extremely Low Gestational Age Newborns (ELGANs) study was designed to identify characteristics and exposures that increase the risk of structural and functional neurological disorders in ELGANs.[7] During the years 2002-2004, women delivering before 28 weeks gestation at any of 14 participating institutions were asked to enroll in the study. At each site, enrollment and consent processes were approved by the institutional review board.

Mothers were approached for consent either on antenatal admission or shortly after delivery, depending on clinical circumstance and institutional preference. A total of 1249 mothers of 1506 infants consented. Approximately, 260 women were either missed or did not consent to participate.

Of the 1200 ELGANs who survived to age 2 years, 1102 (92%) returned for follow-up. Although 939 (85%) of those who were followed had one or more protein measures, only 881 (80%) of the 1102 had protein measures on ≥2 days in the first 2 postnatal weeks, and these children are the subjects of most parts of this report. Because not all children had an Modified Checklist for Autism in Toddlers (M-CHAT) or a CBCL, the samples for these assessments are smaller (M-CHAT, N = 786; CBCL attention problems, N = 826). Among children who survived to 2 years, those who had protein measures on ≥2 days did not differ from those who did not in mothers' marital or insurance status or in their own gestation at birth, birth weight, birth weight *z* score, or sex.

Gestation 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 fourteenth week (62%). When these were not available, reliance was placed sequentially on a fetal ultrasound at ≥14 weeks (29%), last menstrual period (7%), and gestation recorded in the log of the neonatal intensive care unit (1%).

24-month neurological assessment

Procedures to standardize the neurological examination and minimize examiner variability are presented elsewhere.[8] The topographic diagnosis of cerebral palsy (quadriparesis, diparesis, or hemiparesis) was based on an algorithm using these data.[9]

24-month developmental assessment

Fully 85% of the 1200 surviving children returned for a developmental and neurological assessment, including head circumference measurement, at about 24 months corrected age. Of these children, 88% had their examination within the range of 23-28 months. Nine percent were examined between 16 and 23 months, and 3% were examined between 28 and 44 months.

Certified examiners administered and scored the Bayley Scales of Infant Development-II,[10] which yields a mental scale and a motor scale. For both scales, the standard deviation is 15; thus scores <55 are >3 standard deviations lesser than the mean.

At the time of the 24-month developmental assessment, the parent or other caregiver completed the CBCL for ages 1.5-5 years, a 99-item survey of child behavior problems.[11] The scoring system identifies attention problems according to each child's percentile based on a normative sample. Children were considered to have an attention problem if their *T* score was at or above the ninety-third percentile.[1-5]

The M-CHAT asks the parent or other caregiver to report on 23 behaviors. A child screened positive if two of six "critical" items (Items 2, 7, 9, 13, 14, 15) or three of any of the 23 total items were abnormal. The M-CHAT results obtained from children with severe motor, visual, or hearing disability, the presence of which may independently lead to a positive screen, were excluded from this analysis.[1-5]

Blood protein measurements

Drops of whole blood were collected on (Schleicher & Schuell 903) filter paper on the first postnatal day (range, 1-3 days), the seventh postnatal day (range, 5-8 days), and the fourteenth postnatal day (range, 12-15 days). Twenty-five proteins 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, MD), 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 proteins are explained elsewhere.[1,2,12]

We measured the following 25 inflammation-regulating proteins, categorized as cytokines: interleukin (IL)-1, IL-6, and its receptor (IL-6R), tumor necrosis factor (TNF)-alpha and its receptors (TNF-alpha R1,2); adhesion molecules: intercellular adhesion molecule (ICAM)-1, ICAM-3, vascular cell adhesion molecule, and E-selectin (E-SEL); growth factors: vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1,2) and insulin-like growth factor binding protein-1; chemokines: IL-8, monocyte chemotactic protein-1, monocyte chemoattractant protein-4, macrophage inflammatory protein-1beta, regulated upon activation, normal T-cell expressed, and (presumably) secreted, and interferon-inducible T cell alpha-chemoattractant; metalloproteinases: matrix metalloproteinase (MMP)-1, MMP-9; liver produced: serum amyloid A (SAA), C-reactive protein (CRP); and neutrophil activation: myeloperoxidase. We also examined a subset of 10 proteins (CRP, SAA, IL-1β, IL-6, TNF-α, IL-8, ICAM-1, E-SEL, MMP-9, VEGF) that we have found to be associated consistently with structural and functional neurological outcomes.[1,2,4,5]

Statistics

We evaluated two null hypotheses. The first postulates that the risks of indicators of perinatal brain damage do not vary with the number of persistently and/or recurrently elevated inflammation-related proteins in the blood. One set of analyses of the relationship between number of protein elevations and risk of adverse outcomes was based on data on all 25 proteins. Another set of analyses used data only for the subset of 10 proteins associated with structural and functional neurological outcomes.[1,2,4,5] The second hypothesis postulates that the risks of indicators of brain damage do not vary with specific categories or the number of