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Systemic inflammation on postnatal days 21 and 28 and indicators of brain dysfunction 2 years later among children born before the 28th week of gestation

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ABSTRACT

Background: Systemic inflammation during the first two postnatal weeks in extremely preterm newborns (<28 weeks gestation) has been associated with an increased risk of neurodevelopmental dysfunctions. Little is known, however, about the relationship between systemic inflammation during the third and fourth postnatal weeks and subsequent development.
Methods: We measured the concentrations of 16 inflammation-related proteins in blood spots collected on postnatal days 21 (N = 749) and 28 (N = 697) from infants born before the 28th week of gestation and assessed at age 2 years. We then sought the developmental correlates of top quartile concentrations for gestational age and day the specimen was collected. Odds ratios and 95% confidence intervals were calculated from regular or multinomial logistic regression models (as appropriate).
Results: Top quartile concentrations of CRP, IL-1β, IL-6, IL-6R, TNF-R2, IL-8, ICAM-1, and TSH on both days 21 and 28 were associated with ventriculomegaly (when in the NICU) and microcephaly at age 2 years. Top quartile concentrations of CRP, SAA, IL-6, TNF-R2, IL-8, and ICAM-1 were associated with mental development index (MDI) of the Bayley-II < 55, while top quartile concentrations of CRP, TNF-α (inversely), IL-8, and ICAM-1 were associated with psychomotor development index (PDI) < 55
Conclusion: Extremely preterm newborns who had systemic inflammation during the third and fourth postnatal weeks were at increased risk of ventriculomegaly during the months after birth, and of microcephaly, and low Bayley Scale scores at 2 years old.

1. Introduction

The ELGAN study of extremely low gestational age newborns (ELGANs) (i.e., born before the 28th week of gestation) measured blood concentrations of inflammation-related proteins on postnatal days 1, 7, and 14. In this study, concentrations in the top quartile on two separate occasions a week apart were associated with increased risks of ventriculomegaly during the intensive care nursery stay [1], and 2 years later with cerebral palsy [2], low Bayley Scales of Infant Development — II [3], an attention problem [4], and microcephaly [5].

These findings support the view that "intermittent or sustained systemic inflammation" contributes to brain damage in ELGANs [6]. The name "intermittent/sustained systemic inflammation" conveys the uncertainty that an elevated concentration on two separate days one week apart reflects sustained inflammation, two separate episodes of

Abbreviations: ELGAN, extremely low gestational age newborn; NICU, Neonatal Intensive Care Unit; BSID-II, Bayley Scales of Infant Development — Second Edition; MDI, mental development index of the BSID-II; PDI, psychomotor development index of the BSID-II; CRP, C-Reactive Protein; SAA, Serum Amyloid A; MPO, Myeloperoxidase; IL-1β, Interleukin-1 β; IL-6, Interleukin-6; IL-6R, Interleukin-6 Receptor; TNF-α, Tumor Necrosis Factor-α; TNF-R2, Tumor Necrosis Factor Receptor-2; IL-8, Interleukin-8 (CXCL8); RANTES, Regulated upon Activation, Normal T-cell Expressed, and Secreted (CCL5); ICAM-1, Intercellular Adhesion Molecule-1 (CD54); MMP-9, Matrix Metalloproteinase-9; VEGF, Vascular Endothelial Growth Factor; VEGF-R2, Vascular Endothelial Growth Factor Receptor-2 (KDR); TSH, thyroid-stimulating hormone; EPO, Erythropoietin.

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inflammation, or flare-ups of a low-level ongoing process. We could not distinguish among these possibilities because we did not have measurements of specimens collected at shorter intervals.

Recently, however, we were able to measure the concentrations of proteins in blood specimens collected from the same ELGAN subjects on postnatal days 21 and 28. This allowed us to consider that an elevated concentration on 4 days separated from each other by one week or more is unlikely to represent an intermittent process and more likely to reflect ongoing (sustained) inflammation.

Here we explore how well systemic inflammation at the end of the third and fourth weeks after birth of very preterm newborns conveys information about the risk of indicators of brain damage in the intensive care nursery and two years later.

2. Methods

2.1. Participants

During the years 2002–2004, women who gave birth before 28 weeks gestation at one of 14 participating hospitals in 5 states in the U.S. were invited to enroll. The individual institutional review boards approved the enrollment and consent.

Mothers were approached for consent either upon antenatal admission or shortly after delivery. A total of 1506 infants born to 1249 mothers were enrolled. The sample for the analyses of ventriculomegaly when the child was in the intensive care nursery is larger than the sample for the analyses that evaluated head circumference and function at age 2 years (Table 1).

2.2. Newborn variable

Gestational age was estimated based on date of embryo retrieval, intrauterine insemination, or fetal ultrasound before the 14th week (62%). When any of these were not available, the estimate was based on fetal ultrasound at week 14 or later (29%), last menstrual period (7%), or the gestational age recorded in the log of the Neonatal Intensive Care Unit (NICU) (1%).

2.3. Protocol ultrasound scans

Routine scans were performed by technicians at all of the hospitals using digitized high frequency transducers (7.5 and 10 MHz). Ultrasound studies always included the six standard quasi-coronal views and five sagittal views using the anterior fontanel as the sonographic window [7]. The three sets of protocol scans were defined by the postnatal day on which they were obtained (1st through 4th day; 5th through 14th day, and 15th day through the 40th week).

After creation of a manual and data collection form, observer variability minimization efforts included conference calls discussing aspects of images prone to different interpretations [8]. Templates of multiple levels of ventriculomegaly were included in the manual.

All ultrasound scans were read by two independent readers who were not provided clinical information. Each set of scans was first read by one study sonologist at the institution of the infant's birth. The images, usually as electronic images on a CD imbedded in the software eFilm Workstation™ (Merge Healthcare/Merge eMed, Milwaukee, WI) were sent to a sonologist at another ELGAN study institution for a second reading. The eFilm program allowed the second reader to see what the first reader saw, and provided options to adjust and enhance the studies similar to the original reader, including the ability to zoom and alter gains. When the two readers differed in their recognition of moderate/severe ventriculomegaly, the films were sent to a third (tie-breaking) reader who did not know what the readers reported.

Table 1
Sample description.

	Yes	No
Enrolled	1506	
Had head cranial ultrasound in the NICU	1455	55
Had day-1 proteins measured	1109	346
Had day-7 proteins measured	1140	315
Had day-14 proteins measured	1031	424
Had day-21 proteins measured	938	517
Had day-28 proteins measured	878	577
Survived to 24 months	1200	255
Had 24 month developmental assessment	1102	98
Had day-1 proteins measured	973	129
Had day-7 proteins measured	986	116
Had day-14 proteins measured	890	212
Had day-21 proteins measured	809	293
Had day-28 proteins measured	750	351

2.4. 24-month developmental assessment

Families were invited to bring their child for a developmental assessment close to the time when s/he would be 24 months corrected age. The full evaluation included a neurological examination, the Bayley Scales of Infant Development, Second Edition, the Gross Motor Function Classification System, and the Modified-Checklist for Autism in Toddlers.

Fully 91% of surviving children returned for the developmental assessment. Of these children, 75% had their exam within the range of 23.5–27.9 months, 14% were assessed before 23.5 months, and 12% were assessed after 27.9 months.

2.5. Head circumference

The head circumference was measured as the largest possible occipital–frontal circumference and rounded to the closest 0.1 cm. All head circumferences are presented as Z-scores because newborns were assessed at different approximations of 24 months corrected age (range: 16–44 months corrected age, with 68% assessed at 23–25 months corrected age). Z-scores were based on standards in the CDC data set [9].

2.6. Bayley Scales of Infant Development — Second Edition (BSID-II) [10]

Certified examiners administered and scored the BSID-II. Only 2% of examiners indicated at the time of the examination that they had more than a limited amount of information about the child. Before testing examiners were told the child's chronologic age. After completion of testing they were told the gestational age so that the mental development index (MDI) and psychomotor development index (PDI) could be age-adjusted as appropriate.

The child was classified as non-testable on a scale if her/his impairments prohibited standardized administration, or more than 2 items were judged to be 'not applicable.' Children considered non-testable were assigned their scores on scales 4 and/or 5 of the Vineland Adaptive Behavioral Composite which, like the BSID-II scales, have means of 100 and standard deviations of 14 [11].

2.7. Blood spot collection 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. Details about the elution of proteins from the blood spots are provided elsewhere [12].

Each sample was analyzed in duplicate using the Meso Scale Discovery electrochemiluminescence multiplex platform and Sector Imager