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Citation	Leviton, Alan, Raina N. Fichorova, T. Michael O'Shea, Karl Kuban, Nigel Paneth, Olaf Dammann, and Elizabeth N. Allred. 2013. "Two-hit model of brain damage in the very preterm newborn: small for gestational age and postnatal systemic inflammation." Pediatric research 73 (3): 362-370. doi:10.1038/pr.2012.188. http://dx.doi.org/10.1038/pr.2012.188.
Published Version	doi:10.1038/pr.2012.188
Citable link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:11876998
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Pediatr Res. Author manuscript; available in PMC 2013 September 01.

Published in final edited form as:
Pediatr Res. 2013 March ; 73(3): 362–370. doi:10.1038/pr.2012.188.

Two-hit model of brain damage in the very preterm newborn: small for gestational age and postnatal systemic inflammation

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Abstract

Background—We sought to disentangle the contributions of perinatal systemic inflammation and small for gestational age (SGA) to the occurrence of low Bayley Mental Development Indices (MDIs) at age 2 years.

Method—We measured the concentration of 25 inflammation-related proteins in blood obtained during the first 2 postnatal weeks from 805 infants who were born before the 28th week of gestation and who had MDI measurements at age 2 years and were able to walk independently.

Results—SGA newborns who did not have systemic inflammation (a concentration of an inflammation-related protein in the top quartile for gestational age on 2 days a week apart) were at greater risk of an MDI < 55, but not 55–69, than their peers who had neither SGA nor systemic inflammation. SGA infants who had elevated blood concentrations of IL-1beta, TNF-alpha, or IL-8 during the first two postnatal weeks were at even higher risk of an MDI < 55 than their SGA peers without systemic inflammation and of their non-SGA peers with systemic inflammation.

Conclusion—SGA appears to place very preterm newborns at increased risk of a very low MDI. Systemic inflammation adds considerably to the increased risk.

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[*]Please see Acknowledgments section.

Disclosure: The authors have no conflicts of interest.

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Introduction

Small for gestational age (SGA) occurs more commonly among very preterm newborns than among their peers born closer to term (1). SGA preterm infants are more likely than their more appropriately grown peers to have structural abnormalities of the brain (2). They are also more likely to have multiple expressions of developmental delay/limitations, including low scores on cognition assessments (3, 4). These abnormalities are compatible with early direct insults to the brain or disturbances to developmental processes.

Systemic inflammation also places preterm infants at increased risk of functional limitations and indicators of reduced brain volume (5–13).

In a two-hit model of brain damage, SGA appeared to sensitize neonatal rats to a subsequent inflammatory stimulus, resulting in more brain damage than seen in control rats following the same inflammatory stimulus (14). We are not aware of any report of an assessment of such a two-hit model in extremely low gestational age human newborns. Such an assessment might help elucidate how much of the brain damage in SGA preterm newborns can be attributed to growth-restriction, and how much to subsequent inflammation.

Results

Sample description

Of the 805 infants born before the 28th week of gestation who had inflammation-related proteins measured on two days in perinatal blood spots, a Bayley Scales of Infant Development assessment at age 2 years and a Gross Motor Function Classification System score < 1 at 2 years, 11% (N=91) had an MDI < 55, which is approximately 85-fold the expected 0.13% for a score more than 3 standard deviations below the expected mean (Table 1). Another 85 children had an MDI in the 55–69 range, which places their MDI more than 2 standard deviations below the expected mean, but above the 3 standard deviations criterion. The sample consists of these 176 children plus their 629 peers with higher MDI scores who also were able to walk and had proteins measured in perinatal blood spots.

Potential confounders: delivery and placenta characteristics

We sought characteristics and exposures that might distort an assessment of the relationships between each of the antecedents of interest (*i.e.,* SGA and postnatal systemic inflammation) and the outcomes of interest, an MDI < 55 and an MDI between 55 and 69, in light of the contribution of the other antecedents. Women who had severe preeclampsia were more likely than others to give birth to an SGA infant (55% vs 8% for pre-labor rupture of membranes and 7% for preterm labor) and to infants with a very low MDI (17% vs 14% for pre-labor rupture of membranes and 9% for preterm labor) (Table 2). Infants born to these women were not at increased risk of having persistent or recurring elevated blood concentrations of inflammation-related proteins. Delivery for a fetal indication was also associated with both an MDI in the 55–69 range and persistent/recurrent elevated concentrations of IL-1beta, IL-8, and ICAM-1. Receipt of magnesium for seizure prophylaxis (a correlate of preeclampsia) was associated with both SGA and an MDI < 55, but not with elevated concentrations of inflammation-associated proteins. Infants whose placenta harbored an organism or had histologic inflammation of the membranes or umbilical cord were less likely than others to have been SGA, but were not at increased risk of an MDI <55. Those whose placenta and umbilical cord had histologic inflammation were more likely than others to have elevated concentrations of inflammation-related proteins.

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Potential confounders: characteristics of the newborn

Boys were less likely than girls to be SGA (10% vs 19%) and more likely to have an MDI < 55 and an MDI between 55 and 69 (Table 3). The lower the gestational age, the higher the rate of very low MDI. SGA infants were more likely than others to have had elevated concentrations of IL-6, TNF-alpha, IL-8, and ICAM-1 on two separate days during the first two weeks following birth. The smaller the head circumference at birth, the higher the rate of very low MDI, and of elevated blood concentrations of inflammation-related proteins on two separate days.

Potential confounders: postnatal characteristics

Infants who had hyperoxemia, hypercarbia, and acidemia on two of the first three postnatal days were more likely than others to be SGA, but not appreciably more likely to have a very low MDI, although newborns with hypercarbia or acidemia were at increased risk of an MDI in the 55–69 range (Table 4). Newborns who had early postnatal acidemia were more likely than others to be SGA, and have prominent systemic inflammation. Bacteremia was associated with both an MDI <55 and with systemic inflammation, but not with SGA. Infants who were ventilated on days 7, 14 and 21 were more likely than others to have SGA, low MDIs, and systemic inflammation. MDI in the 55–69 range was minimally associated with both late bacteremia and ventilation.

Influence of the co-occurrence of SGA and inflammatory stimuli on low MDI

The risk of an MDI < 55 was highest in SGA infants who had bacteremia during the second, third, or fourth weeks, while the risk of an MDI between 55 and 69 was elevated among SGA infants who had bacteremia during the first postnatal week (Table 5). The risk of an MDI < 55 was also elevated in SGA infants who were ventilated on postnatal day 7 or after. In contrast, however, ventilation did not appear to have a disproportionate effect on SGA infants.

Odds ratios of a very low or moderately low MDI among children classified by whether or not they were SGA and by protein concentration elevations

Because we constructed a separate model for each protein, Table 6 displays the results obtained with 25 separate models.

MDI < 55

Compared to children who had neither SGA, nor an elevated concentration of the individual protein on two separate days separated by approximately one week (fourth data column), those with SGA, but without repeatedly high concentrations of an individual protein, had a risk of an MDI < 55 in the range of 1.7 to 3.8, with most odds ratios significant at p <.01 (third data column) (Table 6). In essence, these infants were at increased risk, regardless of the protein assessed. Their increased risk is associated with SGA and not with systemic inflammation. The odds ratios vary for different proteins because the risk of a very low MDI associated with SGA in these models is influenced by the effect of elevated protein concentrations on the risk among other infants. In contrast, those SGA babies who had repeatedly high concentrations of SAA, IL-1β, IL-6, TNF-α, IL-8, MIP-1β, ICAM-1, E-SEL, and/or IGFBP-1 were at much higher risk of a very low MDI (first data column). Most of the odds ratios for these children were double those of their SGA peers who did not have repeatedly elevated concentrations of these proteins (third data column). The risks of a very low MDI were significantly increased in infants who did not have SGA, but did have recurrent/persistent elevated concentrations of CRP, SAA, IL-8, ICAM-1, E-SEL, and VEGF-R2 (second data column). These odd ratios were not as high as those seen in among SGA infants who had systemic inflammation.

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In separate analyses (not shown), only bacteremia, and not the indication for delivery or duration of ventilation contributed to the risk of an MDI <55, without appreciably reducing the risks associated with SGA and indicators of systemic inflammation. The odds ratios for bacteremia were close to 2.0 and were statistically significant at $p < .01$. No interaction was seen between SGA and either bacteremia, delivery indication, or duration of ventilation.

MDI = 55–69

SGA infants who did not have repeatedly high concentrations of an individual protein, were not at appreciably increased risk of an MDI in the 55 to 69 range (next to last data column). Among SGA infants who had repeatedly high concentrations of individual proteins, SAA was the only protein to achieve statistical significance (third data column from the right). Although repeatedly high concentrations of several proteins were associated with increased risk of an MDI in the 55–69 range among SGA infants, only MIP-1beta had a statistically significantly elevated odds ratio (fourth data column from the right).

Discussion

Our main findings are that extremely preterm SGA newborns were at increased risk of a very low MDI (<55). When they had systemic inflammation in addition to SGA, their risks were considerably higher than the risks associated with systemic inflammation in infants who were not SGA at birth or infants without inflammation or SGA. In essence, SGA alone, or phenomena associated with SGA, appear to place SGA newborns at increased risk, and this risk is prominently heightened if they were also exposed to prolonged or repetitive systemic inflammation during the first two weeks after birth.

Limitations and strengths

The weaknesses of our study are those of all observational studies. We are unable to distinguish between causation and association as explanations for what we found.

Our study has several strengths. First, we selected infants based on gestational age, not birth weight, in order to minimize confounding due to factors related to fetal growth restriction (15). Second, we collected all of our data prospectively. Third, examiners were not aware of the medical histories of the children they examined, thereby minimizing “diagnostic suspicion bias” (16). Fourth, we have minimized observer variability as best we can in the assessments of neurodevelopmental functions (17). Fifth, attrition in the first two years was modest. Sixth, our protein data are of high quality (18), and have high content validity (19–22).

Maternal and fetal indications for delivery, SGA, and inflammation

Although preeclampsia might not be an inflammatory disorder primarily, many characteristics and associations link it to inflammatory phenomena (23). Thus, the first hit might have been exposure to maternal inflammation (24).

SGA infants tended to have higher blood concentrations of IL-6, TNF-alpha, IL-8, and ICAM-1 on repeated occasions than others. Nevertheless, although 55% of infants born to preeclamptic women were SGA, they were not more likely than others to have repeated elevations of blood concentrations of inflammation-associated proteins. In contrast, infants delivered for fetal indications tended to have prominently elevated concentrations of IL-1beta, IL-8, and ICAM-1, even though only 38% of these newborns were SGA. Perhaps the magnesium given to preeclamptic women dampened the inflammatory responses of their SGA newborns (25).

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SGA, postnatal systemic inflammation, and very low MDI

The odds ratios of an MDI < 55 associated with persistent/recurrent elevated concentrations of SAA, IL-1beta, IL-6, TNF-alpha, IL-8, ICAM-1, E-Selectin, and IGFBP-1 were considerably higher among SGA children than among their peers. These observations are compatible with two possibilities. One is that processes associated with SGA, and processes associated with systemic inflammation each contribute independently to risk of a very low MDI. The other is that processes associated with SGA sensitize the brain to the adverse effects of postnatal inflammation.

Two-hit models of brain damage

"Morbidities associated with preterm delivery appear to be additive to those associated with fetal growth restriction so SGA, preterm infants may be at great risk for poor neurodevelopmental outcome" (26). This quote exemplifies the concept of the greater the number of risk factors, the higher the risk. On the other hand, the heightened risk might not be additive.

This two-hit model is exemplified by the greater extent of brain damage following intracerebral lipopolysaccharide (endotoxin) among growth-restricted rats than among rats that were not growth-restricted (14). In this model, one hit (the inflammatory stimulus, lipopolysaccharide) is followed by damage, but a previous hit (a process associated with SGA, or perhaps multiple processes), allows the second hit to result in greater damage than if the first hit had not occurred. No abnormalities were seen in the brains of the growth-restricted rat pups not exposed to the inflammatory stimulus. Thus, this model qualifies for the concept of sensitization, which like preconditioning requires that the first exposure alone not produce damage (27). This is in contrast to some two-hit models that have the first hit produce some damage, and the second hit enhance or amplify the damage (28–32).

Why do two-hit models apply to an MDI < 55, but not to an MDI between 55 and 69?

Our two-hit models apply to an MDI more than three standard deviations below the mean, but not to the less severe reduction of an MDI between two and three standard deviations below the mean. We are not sure why what we see prominently for an extreme outcome is not seen less extremely for a less extreme outcome. One possibility is that the set of risk factors for MDI < 55 differs from those for MDI 55–69. Another possibility is that what we see are the consequences of a threshold effect.

SGA, inflammation, and adult diseases

The presumed late consequences of SGA, including adult-onset diabetes, obesity, hypertension, and atherosclerotic disease are associated with inflammation (33). We did not see evidence of inflammation on the first postnatal day (21). Perhaps what we saw just a few weeks later is the earliest indication of an intense inflammatory proclivity that accounts for some of the adult diseases associated with SGA.

Epigenetic mechanisms

Epigenetic mechanisms, which can influence phenotypic plasticity without altering genomic DNA, have been invoked to explain a wide variety of SGA consequences, including adult diseases, (33)., as well as inflammatory processes associated with neonatal brain damage (34). If processes associated with growth restriction *in utero* account for some of the brain's sensitivity to postnatal inflammation, then therapies that interfere with epigenetic processes might protect the brain (34).

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Choice of potential confounders

Our search for potential confounders of the relationships among SGA, systemic inflammation, and low MDI was especially difficult because some of them might be in the causal chain leading from SGA to low MDI. For example, the majority of SGA infants were born to women who had severe preeclampsia or were delivered for fetal indications. Also, magnesium was given for seizure prophylaxis, almost exclusively to women with severe preeclampsia. Indication for delivery and duration of ventilation neither added independent information about the risk of an MDI < 55, nor altered the odds ratios associated with SGA or any indicator of systemic inflammation. On the other hand, bacteremia contributed supplemental information about the risk of a very low MDI without altering contributions of SGA and indicators of systemic inflammation. These observations support our decision to present analyses without adjusting for these variables.

We did adjust for gestational age, because this is probably our best indicator of unidentified correlates of maturity (35). We also adjusted for sex because SGA usually predicts low MDI differently in each sex (36).

Implications

If our findings are replicated, they will be an example in humans of a two-hit model of brain damage. Two implications follow from our observations. First, our findings might be simply explained as an example of an additive model of increased risk, although consideration should be given to the possibility that the consequences of SGA and systemic inflammation are more than additive. Second, effective intervention to improve outcome among SGA infants might be possible even following delivery.

Conclusions

Very preterm SGA newborns appear to be at increased risk of severely limited mental development, especially if they experienced early postnatal systemic inflammation.

METHODS

The ELGAN study was designed to identify characteristics and exposures that increase the risk of structural and functional neurologic disorders in ELGANs (the acronym for Extremely Low Gestational Age Newborns). During the years 2002–2004, women delivering before 28 weeks gestation at one of 14 participating institutions were asked to enroll in the study. Each institution's review board approved enrollment and consent procedures and documents. A full description of the methods is provided elsewhere (37). Here we focus on those most relevant to these analyses.

The sample for this report consists of the 805 newborns for whom we had information about protein concentrations on at least two of the three protocol days (days 1, 7, and 14), who had a developmental assessment at age 2 years post-term equivalent, and did not have a motor handicap that might interfere in assessments of cognition (Table 1).

After delivery, a trained research nurse interviewed each mother in her native language using a structured data collection form and following procedures defined in a manual. Shortly after the mother's discharge, the research nurse reviewed the maternal chart using a second structured data collection form. The medical record was relied on for events following admission.

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Definitions of pregnancy disorders that led to preterm delivery

The clinical circumstances that led to each maternal admission and ultimately to each preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the medical record (38). Each mother/infant pair was assigned to the category that described the primary reason for the preterm delivery. Preterm labor was defined as progressive cervical dilation with regular contractions and intact membranes. The diagnosis of preterm, premature rupture of fetal membranes (pPROM) was defined as the presence of vaginal pooling with either documented nitrazine positive testing or ferning prior to regular uterine activity. Preeclampsia was defined as new onset hypertension and proteinuria of sufficient severity to warrant delivery for either a maternal or fetal indication. A diagnosis of cervical insufficiency was made when a woman presented with cervical dilation of greater than two centimeters, but no membrane rupture or perceived uterine activity. Placental abruption was defined as appreciable vaginal bleeding (either documented in the medical record or a post-partum hematocrit <24%) and a clinical diagnosis of placental abruption in the absence of cervical change. Presentations under the category of fetal indication included severe intrauterine growth restriction based on antepartum ultrasound examination, non-reassuring fetal testing, oligohydramnious, and Doppler abnormalities of umbilical cord blood flow.

Placenta bacteriology and morphology

Delivered placentas were placed in a sterile exam basin and transported to a sampling room, where they were biopsied under sterile conditions and flash frozen. Eighty-two percent of the samples were obtained within 1 hour of delivery. At a later time, the samples were allowed to thaw at room temperature, a portion approximately 1 cm squared was removed and weighed, then diluted 1:10 with sterile phosphate buffered saline (PBS), and homogenized. Aliquots were plated on selective and non-selective media, including pre-reduced Brucella-base agar with 5% sheep blood enriched with hemin and vitamin K1, tryptic soy agar with 5% sheep blood, chocolate agar, and A-7 agar. After incubation, the various colony types were enumerated, isolated and identified by established criteria.

For histologic evaluation, representative sections were taken from all abnormal areas as well as routine sections from the umbilical cord and a membrane roll, and full thickness sections from the center and a paracentral zone of the placental disc. After training to minimize observer variability, study pathologists examined the slides for histologic characteristics listed on a standardized data form they helped create (15,16). Briefly, infarcts and inter-villous fibrin, fetal stem vessel thrombosis, and decidual hemorrhage and fibrin deposition consistent with abruption were coded as present or absent. Chorionic villi were scored for syncytial knots (none, occasional, or increased).

At the chorionic plate of the disc, grade 3 acute inflammation was defined as neutrophils up to amnionic epithelium and stage 3 was defined as >20 neutrophils/20×). Grade 3 inflammation of the external membranes, as well as of the chorion/decidua required numerous large or confluent foci of neutrophils.

Inflammation in the umbilical cord was graded from 0–5. Grade 3 required neutrophils in perivascular Wharton's jelly, grade 4 required panvasculitis and umbilical cord vasculitis extending deep into Wharton's jelly, and grade 5 required a 'Halo lesion' (ring of precipitate in Wharton's jelly encircling each vessel). Neutrophilic infiltration into fetal stem vessels in the chorionic plate required that neutrophils appeared to have migrated towards the amnionic cavity.

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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 sequentially on a fetal ultrasound at 14 or more weeks (29%), LMP without fetal ultrasound (7%), and gestational age recorded in the log of the neonatal intensive care unit (1%).

A newborn was considered to be SGA if the birth weight was in the lowest decile of the birth weight distribution in an external standard (19). This is equivalent to a birth weight more than 1.28 standard deviations below the mean in a referent population.

Documented early bacteremia required recovery of an organism from blood drawn during first postnatal week, and late bacteremia as recovery of an organism from blood drawn during the second, third, or fourth week. Presumed bacteremia was defined as culture-negative, but the clinician ordered antibiotics for more than 72 hours.

The lowest and highest arterial blood measurements of PaO_2, PCO_2, and pH on postnatal days 1, 2, and 3 were routinely recorded. We identified ELGANs whose blood gas measurements were in the extreme quartile for gestational age on at least two of the three days.

Information about mode of ventilation was collected for every day during the first 7 days and weekly thereafter until 28 days. ELGANs were classified into three mutually exclusive groups: those with consistently low FiO_2 (an $FiO_2 < 0.23$ every day between postnatal days 3 and 7 and receiving $FiO_2 \leq 0.25$ on day 14), those with pulmonary deterioration (PD: an $FiO_2 < 0.23$ on any day between days 3 and 7 and receiving $FiO_2 > 0.25$ on day 14), and those with early and persistent pulmonary dysfunction (EPPD: an $FiO_2 \geq 0.23$ on all days between 3 and 7 days of life and receiving $FiO_2 > 0.25$ on day 14) 5.

24-month developmental assessment

Fully 91% of surviving children returned for a developmental assessment close to the time when s/he would be 24-months corrected age. Of these children, 77% had their exam within the range of 23.5–27.9 months. Most others were examined before 23.5 months.

Certified examiners administered and scored the Bayley Scales of Infant Development Second Edition (39). We chose as our main outcome a Mental Development Index (MDI) < 55 because it is three standard deviations below the expected mean and therefore constitutes a severe impairment, and because the predictive ability of an MDI < 55 is higher than that of a score below 70, which is two standard deviations below the expected mean (40). Because some MDI test items require intact motor function, we excluded all infants with significantly impaired gross motor function, defined as an inability to walk independently (a Gross Motor Function Classification System level ≥ 1), regardless of whether or not they were given a cerebral palsy diagnosis.

Blood spot collection

After blood was collected for clinical indications, drops were blotted on filter paper on the first postnatal day (range: 1–3 days), the 7th postnatal day (range: 5–8 days), and the 14th postnatal day (range: 12–15 days). Dried blood spots were stored at −70°C in sealed bags with desiccant until processed. All references below to protein concentrations refer to the 3 samples obtained on or about days 1, 7 and 14

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Protein measurement

Details about elution of proteins from blood spots and measurement of the proteins with the Meso Scale Discovery (MSD) electrochemiluminescence system are provided elsewhere (22). Inter-assay variations are invariably less than 20%. Measurements of each protein were normalized to milligrams of total protein.

The Laboratory of Genital Tract Biology of the Department of Obstetrics, Gynecology and Reproductive Biology at Brigham and Women's Hospital, Boston measured the following 25 proteins: IL-1beta (Interleukin-1beta), IL-6 (Interleukin-6), IL-6R (interleukin-6 receptor), TNF-alpha (tumor necrosis factor-alpha), TNF-R1 (tumor necrosis factor-alpha-receptor1), TNF-R2 (tumor necrosis factor-alpha-receptor2), IL-8 (CXCL8) (interleukin-8), MCP-1 (CCL2) (monocyte chemotactic protein-1), MCP-4 (CCL13) (monocyte chemoattractant protein-4) (CCL13), MIP-1beta (CCL4) (Macrophage Inflammatory Protein-1beta) (CCL4), RANTES (CCL5) (regulated upon activation, normal T-cell expressed, and [presumably] secreted), I-TAC (CXCL11) (Interferon-inducible T cell Alpha-Chemoattractant), ICAM-1 (CD54) (intercellular adhesion molecule-1), ICAM-3 (CD50) (intercellular adhesion molecule-3), VCAM-1 (CD106) (vascular cell adhesion molecule-1), E-SEL (CD62E) (E-selectin) (CD62E), MMP-1 (matrix metalloproteinase-1), MMP-9 (matrix metalloproteinase-9), CRP (C-Reactive Protein), SAA (serum amyloid A), MPO (myeloperoxidase). VEGF (vascular endothelial growth factor), VEGF-R1 (vascular endothelial growth factor-receptor1), VEGF-R2 (vascular endothelial growth factor-receptor2), and IGFBP-1 (Insulin Growth Factor Binding Protein-1).

In previous analyses in this sample, protein elevations in the top quartile (for gestational age and postnatal day) on two separate days provided considerably more discriminating risk information than did elevations on just one day (10–12). Thus, our indicator of postnatal systemic inflammation is a concentration of an inflammation-related protein in the top quartile that persisted or recurred.

Data analysis

We evaluated the following hypotheses about the risk of a low MDI, defined as either a very low MDI (*i.e.,* <55) or a moderately low MDI (*i.e.,* between 55 and 69). First, compared to ELGANs who were neither SGA nor had recurrent/persistent post-natal systemic inflammation, those who had both characteristics were more likely to have a low MDI two years later. Second, among ELGANS who were SGA, those who had recurrent/persistent early post-natal systemic inflammation were much more likely to have a low MDI two years later. Third, among ELGANS who had recurrent/persistent early post-natal systemic inflammation, those who were SGA, were more likely to have a low MDI two years later.

Because our outcomes of interest (an MDI < 55 and an MDI 55–69) are mutually exclusive and each is appropriately compared to the same referent group (MDI ≥ 70), we created multinomial logistic regression models. This allowed us to calculate odds ratios and 99% confidence intervals in separate models for each protein that included variables for SGA, persistent/recurrent protein concentrations in the top quartile on two days a week apart, as well as variables for gestational age (23–24, 25–26, and 27 weeks) and sex.

Acknowledgments

Statement of financial support: This study was supported by a cooperative agreements with the National Institute of Neurological Disorders and Stroke (grants 5U01NS040069-05 and 2R01NS040069 - 06A2), and a center grant award from the National Institute of Child Health and Human Development (grant 5P30HD018655-28).

The authors gratefully acknowledge the contributions of their subjects, and their subjects' families, as well as the following ELGAN Study colleagues for all their contributions:

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Bhavesh L. Shah, Baystate Medical Center, Springfield MA; Camilia Martin, Beth Israel Deaconess Medical Center, Boston MA; Linda Van Marter, Brigham & Women's Hospital, Boston MA; Robert Insoft, Massachusetts General Hospital, Boston, MA; Francis Bednarek, U Mass Memorial Health Center, Worcester, MA; Olaf Dammann, John Fiascone, Cynthia Cole, Tufts Medical Center, Boston MA; Richard A. Ehrenkranz, Yale-New Haven Children's Hospital, New Haven CT; Stephen C. Engelke, University Health Systems of Eastern Carolina, Greenville NC; Carl Bose, University of North Carolina, Chapel Hill NC; Mariel Poortenga, DeVos Children's Hospital, Grand Rapids MI; Padima Karna, Sparrow Hospital, Lansing MI; Michael D. Schreiber, University of Chicago Hospital, Chicago IL; Daniel Batton, William Beaumont Hospital, Royal Oak MI; Greg Pavlov, Frontier Science and Technology Research Foundation, Amherst, NY; Deborah Hirtz, National Institute of Neurological Disorders and Stroke, Bethesda, MD.

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Table 1

Sample description

	Yes
Enrolled	1506
Survived to 2 years	1200
Bayley Scales of Infant Development assessment at age 2 years	1018
Gross Motor Function Classification System < 1 at 2 years	921
Proteins measured in blood collected on 2 or 3 days	805
Mental Development Index (MDI) ≥ 70	629
MDI ≥ 55, < 70	85
MDI < 55	91

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Table 2

The distribution of intrauterine growth restriction and postnatal systemic inflammation in categories of delivery and placenta characteristics. These are row percents.

Characteristics of the delivery		SGA[a]	MDI[b]		Protein concentration in top quartile on 2 days[c]					N
			< 55	55–69	IL-1β	IL-6	TNF-α	IL-8	ICAM-1	
Antenatal corticosteroid course	Complete	15	12	11	17	17	18	15	17	517
	Partial	15	10	11	15	13	15	15	19	204
	None	11	8	8	12	14	19	20	24	84
Pregnancy complication	Preterm labor	7	9	10	15	14	16	16	16	366
	pPROM[d]	8	14	8	21	19	18	16	16	178
	Preeclampsia	55	17	12	13	17	12	9	23	102
	Abruption	7	5	11	10	9	19	10	11	88
	Cervical insufficiency	3	15	10	13	21	26	21	28	39
	Fetal indication	38	16	22	28	22	28	34	38	32
Magnesium	None	11	10	13	16	14	18	16	20	254
	Tocolysis	9	11	9	18	17	18	16	15	443
	Seizure prophylaxis	47	16	13	11	14	14	13	24	108
Cesarean delivery	Yes	19	10	10	15	16	16	15	17	538
	No	6	13	12	18	16	19	17	20	267
Fever[e]	Yes	5	14	14	16	16	20	18	27	44
	No	15	11	10	16	16	17	15	17	738
# of bacterial species isolated	0	15	11	11	14	13	14	16	16	388
	1	17	9	10	20	23	19	15	20	182
	2+	8	12	10	18	16	23	15	15	176
Membrane inflammation[f]	Yes	5	9	11	27	20	27	23	25	148
	No	16	11	11	14	15	15	14	15	584
Umbilical cord inflammation[g]	Yes	7	11	11	26	19	30	23	23	125
	No	16	10	11	15	16	14	13	16	591
Maximum number of infants		116	91	85	130	127	138	125	144	805
Maximum number of placentas		108	83	81	121	121	129	116	127	746

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[a] Birth weight below the 10th percentile for gestational age

[b] Bayley Scales of Infant Development Mental Developmental Index when Gross Motor Function Classification System < 1

[c] Protein concentrations in the top quartile on 2 separate days a week apart

[d] Preterm premature rupture or membranes

[e] Within the interval from before delivery to 48 hours post delivery

[f] Membrane inflammation is defined as inflammation of the chorionic plate (stage 3, and severity 3) or of the chorion/decidua (moderate or severe)

[g] Inflammation of the umbilical cord grade 3 or higher, which requires neutrophils in perivascular Wharton's jelly

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Table 3

The distribution of intrauterine growth restriction and postnatal systemic inflammation in categories of the newborn's characteristic listed on the left. These are row percents.

Characteristics of the infant		SGA[a]	MDI[b]		Protein concentration in top quartile on 2 days[c]					N
			< 55	55–69	IL-1β	IL-6	TNF-α	IL-8	ICAM-1	
Sex	Male	10	15	13	15	17	15	16	18	416
	Female	19	7	8	17	14	19	15	18	389
Type of gestation	Singleton	15	12	10	16	15	16	17	19	527
	Multiple	13	9	12	17	16	16	17	14	278
Gestational age (weeks)	23–24	8	15	10	13	17	16	15	17	149
	25–26	19	12	10	17	15	17	15	19	379
	27	11	8	12	17	16	18	17	17	277
Birth weight (g)	≤ 750	40	13	13	15	19	21	20	21	284
	750–1000	0	12	10	19	15	17	15	20	362
	> 1000	0	6	9	12	12	9	8	9	159
SGA	Yes	100	18	11	18	27	23	22	27	116
	No	0	10	10	16	14	16	14	16	689
Head circumference Z-score[d]	< −2	77	17	11	23	25	38	28	25	64
	≥ −2, < −1	30	15	14	20	19	21	19	23	186
	≥ −1	1	9	9	15	14	14	13	16	529
Maximum number of infants		116	91	85	130	127	138	125	144	805

[a] Birth weight below the 10th percentile for gestational age

[b] Bayley Scales of Infant Development Mental Developmental Index when Gross Motor Function Classification System < 1

[c] Protein concentrations in the top quartile on 2 separate days a week apart

[d] Yudkin standard

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Table 4

The distribution of intrauterine growth restriction and postnatal systemic inflammation in categories of the newborn's early postnatal characteristics. These are row percents.

Postnatal factors		SGA[a]	MDI[b]		Protein concentration in top quartile on 2 days[c]					N
			< 55	55–69	IL-1β	IL-6	TNF-α	IL-8	ICAM-1	
Lowest quartile P_aO_2[d]	Yes	18	14	10	17	23	23	23	25	133
	No	14	11	10	16	16	17	14	17	540
Highest quartile P_aO_2[d]	Yes	20	15	9	15	17	24	19	24	138
	No	14	10	11	17	17	17	15	18	535
Lowest quartile PCO_2[d]	Yes	17	16	8	19	23	20	16	25	143
	No	15	9	11	15	15	17	16	17	530
Highest quartile PCO_2[d]	Yes	23	13	16	24	21	28	24	22	154
	No	13	11	8	14	16	15	14	18	519
Lowest quartile pH[d]	Yes	25	12	14	28	26	32	29	30	139
	No	13	11	9	13	15	14	12	17	534
Early bacteremia	None/suspected	13	10	9	17	16	15	13	16	478
	Presumed	17	12	11	14	16	19	19	17	278
	Definite	15	16	15	19	21	35	19	26	48
Late bacteremia	None/suspected	12	10	9	16	14	15	14	15	481
	Presumed	21	6	12	13	14	16	14	14	121
	Culture positive	16	17	12	19	24	22	20	22	202
Mechanical/high frequency ventilation, day 7	Yes	19	14	12	18	18	21	20	21	469
	No	8	7	9	14	14	12	10	11	336
Mechanical/high frequency ventilation, day 14	Yes	19	13	12	16	19	20	19	22	460
	No	8	8	9	16	13	14	11	11	344
Mechanical/high frequency ventilation, day 21	Yes	20	13	11	17	18	21	20	21	446
	No	7	9	10	16	14	16	10	12	355
Respiratory group classification	EPPD[e]	19	11	12	17	17	22	20	21	320
	PD[f]	14	13	10	16	17	15	13	15	304

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Postnatal factors		SGA[a]	MDI[b]		Protein concentration in top quartile on 2 days[c]					N
			< 55	55–69	IL-1β	IL-6	TNF-α	IL-8	ICAM-1	
	Low FiO_2	17	7	8	15	13	11	10	13	159
Maximum number of infants		116	91	85	130	127	138	125	144	805

[a] Birth weight below the 10th percentile for gestational age

[b] Bayley Scales of Infant Development Mental Developmental Index when Gross Motor Function Classification System < 1

[c] Protein concentrations in the top quartile on 2 separate days a week apart

[d] Extreme quartile for gestational age on two of the first three postnatal days

[e] Early and persistent pulmonary dysfunction

[f] Pulmonary deterioration

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Table 5

Percent of children who had both the row and column characteristic who had an MDI < 55 (left 2 data columns) or an MDI 55–69 (right 2 data columns). These are cell specific percents.

Postnatal factors		MDI < 55[a]		MDI 55–69[a]	
		SGA[b]		SGA[b]	
		Yes	No	Yes	No
Bacteremia during the first Week	None/suspected	19	8	6	10
	Presumed	17	12	15	10
	Culture positive	14	17	29	12
Bacteremia during weeks 2–4	None/suspected	12	9	15	9
	Presumed	12	6	12	13
	Culture positive	34	14	3	14
Mechanical/high frequency ventilation, day 7	Yes	22	12	12	12
	No	4	8	7	9
Mechanical/high frequency ventilation, day 14	Yes	20	12	13	12
	No	14	8	7	9
Mechanical/high frequency ventilation, day 21	Yes	21	11	10	11
	No	8	9	16	10

[a] Bayley Scales of Infant Development Mental Developmental Index when Gross Motor Function Classification System < 1

[b] Birth weight below the 10th percentile for gestational age

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Table 6

Odds ratio (and 99% confidence interval) of an MDI < 55 or an MDI 55–69 vs those with and MDI ≥ 70 among children who had the characteristics listed at the top of each column. The logistic regression models are adjusted for gestational age (23–24, 25–26, 27 weeks) and sex. The sample consisted of children who had a GMFCS < 1 and had proteins measured on 2 separate days (maximum N=805). The referent group consists of children who were not growth restricted at birth and who did not have two days of elevated concentrations of the protein listed on the left. Bold indicates odds ratios significantly > 1.0 ($p < 0.01$).

	MDI[a] < 55				**MDI[a] 55–69**			
	Protein concentration in top quartile on 2 separate days a week apart							
	Yes		**No**		**Yes**		**No**	
	SGA[b]				**SGA[b]**			
Protein	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
CRP	2.2 (0.5, 9.5)	**3.2 (1.5, 6.7)**	**3.8 (1.6, 8.9)**	1.0	1.6 (0.4, 7.1)	1.6 (0.7, 3.7)	1.5 (0.5, 4.2)	1.0
SAA	**6.6 (1.6, 27)**	**3.3 (1.7, 7.1)**	**2.7 (1.1, 6.5)**	1.0	1.7 (0.2, 13)	**2.2 (1.01, 5.0)**	1.6 (0.6, 4.2)	1.0
MPO	2.6 (0.5, 14)	1.1 (0.5, 2.6)	**2.6 (1.2, 5.9)**	1.0	0.7 (0, 9.8)	0.7 (0.3, 1.8)	1.5 (0.6, 3.7)	1.0
IL-1β	**4.7 (1.2, 19)**	1.7 (0.7, 3.8)	**2.4 (1.02, 5.7)**	1.0	1.4 (0.2, 10)	1.0 (0.4, 2.6)	1.4 (0.6, 3.7)	1.0
IL-6	**6.9 (2.3, 21)**	1.9 (0.9, 4.4)	1.7 (0.6, 4.6)	1.0	2.2 (0.5, 9.6)	1.1 (0.4, 2.7)	1.3 (0.5, 3.4)	1.0
IL-6R	2.3 (0.4, 13)	1.3 (0.6, 2.8)	**2.7 (1.2, 6.2)**	1.0	1.8 (0.3, 10)	0.8 (0.3, 1.9)	1.3 (0.5, 3.3)	1.0
TNF-α	**6.9 (2.0, 24)**	2.0 (0.9, 4.6)	2.1 (0.9, 4.3)	1.0	3.5 (0.8, 14)	1.5 (0.7, 3.5)	1.1 (0.4, 3.2)	1.0
TNF-R1	1.2 (0.2, 8.9)	1.1 (0.5, 2.6)	**3.0 (1.3, 6.7)**	1.0	2.6 (0.6, 10)	0.6 (0.2, 1.7)	1.0 (0.4, 2.9)	1.0
TNF-R2	2.5 (0.4, 14)	1.8 (0.8, 4.0)	**2.9 (1.3, 6.6)**	1.0	3.3 (0.7, 15)	1.9 (0.9, 4.1)	1.3 (0.5, 3.6)	1.0
IL-8 (CXCL8)	**5.7 (1.7, 19)**	**2.4 (1.1, 5.5)**	2.3 (0.9, 5.6)	1.0	1.2 (0.2, 8.7)	2.1 (0.9, 4.8)	1.7 (0.7, 4.5)	1.0
MCP-1 (CCL2)	2.8 (0.8, 10)	1.3 (0.6, 3.1)	**2.6 (1.1, 6.3)**	1.0	1.3 (0.2, 6.6)	1.0 (0.4, 2.5)	1.5 (0.6, 3.9)	1.0
MCP-4 (CCL13)	1.9 (0.4, 8.2)	0.8 (0.3, 2.0)	**2.6 (1.1, 6.1)**	1.0	1.1 (0.2, 5.8)	0.6 (0.2, 1.5)	1.4 (0.5, 3.6)	1.0
MIP-1β (CCL4)	**6.3 (1.4, 29)**	2.0 (0.95, 4.3)	**2.6 (1.1, 6.1)**	1.0	**5.3 (1.2, 24)**	1.5 (0.7, 3.2)	1.1 (0.4, 3.1)	1.0
RANTES (CCL5)	5.6 (0.9, 36)	1.3 (0.6, 2.8)	**2.5 (1.1, 5.5)**	1.0	1.2 (0.1, 20)	0.7 (0.3, 1.7)	1.3 (0.5, 3.3)	1.0
I-TAC (CXCL11)	2.3 (0.4, 13)	1.0 (0.4, 2.4)	**2.5 (1.1, 5.7)**	1.0	2.8 (0.7, 11)	0.4 (0.1, 1.3)	1.0 (0.3, 2.7)	1.0
ICAM-1 (CD54)	**4.1 (1.2, 14)**	**2.6 (1.2, 5.6)**	**2.8 (1.1, 6.8)**	1.0	1.4 (0.3, 7.1)	1.5 (0.6, 3.4)	1.6 (0.6, 4.2)	1.0
ICAM-3 (CD50)	1.7 (0.2, 13)	1.6 (0.8, 3.3)	**3.1 (1.4, 6.9)**	1.0	1.2 (0.2, 8.9)	0.6 (0.2, 1.6)	1.3 (0.5, 3.4)	1.0
VCAM-1 (CD106)	3.3 (0.7, 16)	1.4 (0.7, 3.1)	**2.7 (1.2, 6.2)**	1.0	2.0 (0.4, 11)	0.8 (0.3, 1.8)	1.2 (0.5, 3.2)	1.0
E-SEL (CD62E)	**4.4 (1.2, 17)**	**2.2 (1.03, 4.7)**	**2.7 (1.1, 6.5)**	1.0	3.1 (0.8, 12)	1.5 (0.7, 3.4)	1.2 (0.4, 3.4)	1.0

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	MDI[a] < 55				MDI[a] 55–69			
	Protein concentration in top quartile on 2 separate days a week apart							
	Yes		No		Yes		No	
	SGA[b]				SGA[b]			
Protein	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
MMP-1	2.7 (0.5, 16)	0.7 (0.3, 1.8)	**2.3 (1.04, 5.3)**	1.0	2.3 (0.4, 14)	0.6 (0.3, 1.5)	1.2 (0.4, 3.0)	1.0
MMP-9	1.3 (0.1, 21)	0.8 (0.3, 2.1)	**2.6 (1.2, 5.6)**	1.0	1.2 (0.1, 19)	0.7 (0.3, 1.8)	1.4 (0.6, 3.3)	1.0
VEGF	1.3 (0.1, 21)	1.5 (0.7, 3.0)	**3.0 (1.3, 6.7)**	1.0	0.8 (0.1, 12)	0.4 (0.2, 1.1)	1.3 (0.5, 3.2)	1.0
VEGF-R1	2.1 (0.5, 8.2)	0.6 (0.2, 1.8)	**2.5 (1.1, 5.9)**	1.0	2.7 (0.8, 8.9)	0.8 (0.3, 2.0)	0.9 (0.3, 2.8)	1.0
VEGF-R2	2.2 (0.4, 12)	**2.4 (1.2, 5.0)**	**3.4 (1.5, 7.8)**	1.0	2.2 (0.5, 9.9)	1.0 (0.4, 2.4)	1.2 (0.5, 3.4)	1.0
IGFBP-1	**4.9 (1.7, 15)**	2.0 (0.9, 4.8)	2.0 (0.8, 5.3)	1.0	1.9 (0.5, 7.3)	0.9 (0.3, 2.6)	1.2 (0.4, 3.5)	1.0

[a]Bayley Scales of Infant Development Mental Developmental Index when Gross Motor Function Classification System < 1

[b]Birth weight below the 10th percentile for gestational age.