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UMBILICAL CORD SERUM INTERLEUKIN-6, C-REACTIVE PROTEIN, AND MYELOPEROXIDASE CONCENTRATIONS AT BIRTH AND ASSOCIATION WITH NEONATAL MORBIDITIES AND LONG TERM NEURODEVELOPMENTAL OUTCOMES

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Abstract

OBJECTIVE—To determine if umbilical cord serum concentrations of interleukin-6 (IL-6), C-reactive protein (CRP), and myeloperoxidase (MPO), in pregnancies at risk for preterm birth (PTB), are associated with neonatal morbidities and/or altered neurodevelopmental outcomes in the children.

STUDY DESIGN—Umbilical cord serum samples were collected at birth from 400 newborns delivered within a multicenter randomized controlled trial of repeated versus single course of antenatal corticosteroids (ACs), in women at increased risk for PTB. Newborns were followed through discharge and were evaluated between 36 and 42 months corrected age with neurological examination and Bayley Scales of Infant Development. Umbilical cord serum concentrations of IL-6, CRP, and MPO were determined using enzyme-linked immunoassays. Multivariate logistic

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regression analyses explored the relationship between umbilical cord serum IL-6, CRP and MPO levels, adverse newborn outcomes and PTB < 32 weeks of gestational age (GA).

RESULTS—Univariate analysis revealed that umbilical cord IL-6 above the 75th percentile was associated with increased respiratory distress syndrome (RDS) and chronic lung disease (CLD), but not with necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), or neonatal sepsis; however, this association was not significant after adjusting for gestational age at delivery and treatment group. No significant associations between CRP or MPO, and RDS, CLD, NEC, sepsis or IVH were evident. Regression analysis revealed that CRP above the 75th percentile was associated with a decreased risk of CLD (O.R. 0.10, 95% C.I. 0.02–0.41). No associations between umbilical cord IL-6, CRP or MPO, and MDI < 70 or PDI < 70 were evident. Umbilical cord serum IL-6, CRP, and MPO, above the 75th percentile, were associated with more frequent PTB < 32 weeks GA.

CONCLUSION—Elevated umbilical cord serum concentration of CRP is associated with reduced risk for CLD even after adjusting for gestational age at delivery. Occurrence of levels > 75th percentile of IL-6, CRP, and MPO in umbilical cord serum were associated with PTB < 32 weeks GA. Elevated umbilical cord serum concentrations of IL-6, CRP, and MPO at birth were not associated with poor neurodevelopmental outcomes.

Keywords

Umbilical cord serum; cytokines; preterm birth; neonatal morbidity; neurodevelopmental infant outcome

INTRODUCTION

Subclinical intrauterine infection and/or inflammation is thought to account for 25–40% of preterm birth (PTB).[1–2] Fetuses exposed to intraamniotic infection and/or inflammation are at increased risk for pulmonary and neurological damage, and neonatal and infant morbidities, including respiratory distress syndrome (RDS)[3] bronchopulmonary dysplasia (BPD)[4–6] sepsis,[3,7,8] necrotizing enterocolitis (NEC)[7–9] intraventricular hemorrhage (IVH),[10–11] periventricular leukomalacia (PVL)[9–10,12–13] and cerebral palsy (CP).[14] The most advanced, and serious, stage of ascending intrauterine infection is fetal infection. Pregnancies with the fetal inflammatory response syndrome (FIRS), defined as elevated concentrations of interleukin-6 (IL-6) in fetal plasma[3] are at increased risk for severe neonatal morbidities (RDS, neonatal sepsis, pneumonia, BPD, IVH, PVL or NEC.[3] Neonates with funisitis, a histological hallmark of FIRS,[15] are at increased risk for neonatal sepsis,[16] BPD [6] and CP.[14]

Inflammatory mediators play a role in the mechanism of preterm parturition, and pulmonary and neurological injury. Cytokines mediate acute lung injury,[17] exacerbate ventilator-associated lung injury,[18] and modulate host defenses.[19] Increased cytokine concentrations are observed in tracheal aspirates[20–21] and serum[22–23] of infants with RDS who subsequently develop BPD. The process responsible for neurological damage that leads to some cases of PVL and CP begins *in utero*, and is related to exposure to intraamniotic inflammation.[24–25] Intrauterine infections instigate activation of the maternal and fetal

cytokine networks that leads to PTB and also white matter damage in the neonatal brain.[24–25] Increased cytokine concentrations in the amniotic fluid[4,10,14] and umbilical cord blood[12,26] of preterm infants have been associated with PVL[4,10,12,26] and CP.[14,26]

There is a paucity of data concerning the association between umbilical cord serum concentrations of IL-6, CRP, and MPO at birth, and neonatal morbidities, and long term neurodevelopmental morbidities in children of women identified during pregnancy to be at increased risk for PTB.

The purpose of this study was to examine whether umbilical cord serum concentrations at birth of IL-6, CRP, and MPO, in pregnancies that were at risk for PTB, are markers for neonatal morbidities, and/or poor neurodevelopmental outcomes, and for PTB < 32 weeks of GA.

MATERIAL AND METHODS

Study design

This is a secondary analysis utilizing data and umbilical cord serum samples collected during a randomized, double-masked, placebo-controlled, multicenter clinical trial of repeated versus a single course of antenatal corticosteroids (ACs), and data from long-term follow-up of the children. The primary trial was performed at 18 centers of the *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network. Recruitment took place from March 2000 to April 2003. The overall study population and methods for this trial have previously been described.[27] Follow-up examinations were performed between July 2002 and May 2006, and results of the long-term follow-up of children enrolled in the randomized trial have been previously described.[28] The original clinical trial and follow-up study of children were approved by the institutional review boards at all participating centers, and informed consent was obtained from all participants. The institutional review board of Wayne State University School of Medicine approved the secondary analysis described here.

Patient population and data collection

Pregnant women with intact membranes between 23 weeks and 0 days and 31 weeks 6 days GA who had received one course of AC 7 to 10 days earlier, and were at increased risk for spontaneous PTB were randomized to receive additional weekly courses of betamethasone or identical-appearing placebo. Exclusion criteria were insulin-dependent diabetes mellitus, systemic corticosteroid use during pregnancy, clinical diagnosis of chorioamnionitis, non-reassuring fetal status, preterm rupture of membranes, or a major fetal anomaly. The first 67 enrolled women received weekly repeat courses of betamethasone until 33 6/7 weeks' GA. Subsequently the number of repeat courses was limited to four. Maternal, clinical, and demographic data were collected at the time of randomization, delivery, and discharge.

Umbilical cord serum

A sample of mixed umbilical cord blood was collected from the umbilical cord at birth. After clot formation at room temperature, the sample was centrifuged for 10 minutes at 3400 rpm; the serum was divided into aliquots, frozen, and stored for future batch analysis.

Newborn data and outcomes

Newborns were followed until either discharge from the hospital, or up to 120 days, at which time relevant data from the nursery records were obtained. Cranial ultrasounds were performed by 14 days of age on all infants for the occurrence of IVH, PVL, and were read centrally by masked reviewers. RDS was defined as requiring oxygen from 6 to 24 hours of age, clinical features of RDS within 24 hours of age, respiratory support from 6 to 24 hours of age, and an abnormal X-ray within 24 hours of age. CLD was defined as the need for supplemental oxygen at 36 weeks' corrected age in infants born before 34 weeks GA. The diagnosis of sepsis required a clinically ill infant in whom systemic infection was suspected with a positive blood, cerebrospinal fluid, or catheterized/suprapubic urine culture, or in the absence of positive cultures, with clinical evidence of cardiovascular collapse, or an unequivocal X-ray confirming infection.

Evaluation of children

The cohort of enrolled women was contacted by the study personnel at least every 3 months. The children were to return for evaluation when they were between 24 and 35 months of age, corrected for gestational age at birth, for a detailed medical history, evaluation of developmental milestones, physical and neurological examination, and measurement of Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) scores from the Bayley Scales of Infant Development, second edition. Centrally trained and certified study personnel, who were unaware of treatment assignment, performed the follow-up examinations using a standardized protocol. Neurological examinations were performed by pediatricians or pediatric neurologists. The Bayley MDI and PDI scores were determined by trained study psychologists or psychometrists and calculated on the basis of corrected age. Children who returned for evaluation when they were between 36 and 42 months of corrected age were included and underwent both physical and Bayley assessments.

IL-6, CRP, and MPO Immunoassays

Highly sensitive enzyme-linked immunoassays were used to determine concentrations of IL-6 (R&D Systems [Minneapolis, MN]), CRP and MPO (ALPCO Diagnostics [Salem, NH]) in umbilical cord vein serum samples. Serum was incubated in duplicate in micro titer plates which had been pre-coated with antigen specific (IL-6, CRP, or MPO) monoclonal antibodies. The concentrations of IL-6, CRP or MPO in umbilical cord serum samples were determined by interpolation from individual standard curves composed of human IL-6, CRP or MPO. The calculated inter-assay and intra-assay coefficients of variation (CV) for IL-6, CRP and MPO immunoassays in our laboratory were 5.8%, 8.6% and 4.7% and 3.2%, 3.2% and 3.8%, respectively. The calculated lower limits of detection (sensitivity) for IL-6, CRP and MPO immunoassays were 0.14 pg/ml, 0.86 ng/ml, and 0.66 ng/ml respectively.

Statistical analyses

The associations between umbilical cord serum concentration of IL-6, CRP, and MPO with rates of individual neonatal morbidities, poor neurodevelopmental outcomes (Bayley MDI score < 70 or Bayley PDI score < 70) at 24 to 36 months, and PTB < 32 weeks GA were examined. Umbilical cord serum concentrations of IL-6, CRP and MPO were dichotomized into high (> 75th percentile) and not high (≤ 75th percentile) groups. For PTB < 32 weeks GA, singletons and twins with the highest analyte concentration were included in the analysis and the associations between high and not high analyte concentrations and PTB < 32 weeks GA were compared using the Chi-square test.

Multivariable logistic regression analysis was used to explore these associations while adjusting for possible confounders. The regression models included GA at delivery, treatment group, and analyte. *P*-values, and the unadjusted and adjusted ORs were generated using General Estimating Equations (GEE) Models.[29] GEE regression analysis was also used to examine differences between treatment groups in log transformed cytokine values. This model was used since it accounts for correlation between twins. A nominal two-sided *p* value of less than 0.05 was considered to indicate statistical significance. Analyses were performed with SAS statistical software (SAS Institute Inc, Cary NC).

RESULTS

There were 495 patients randomized in the trial, 252 in the repeat AC group and 243 in the placebo group. There were no significant differences between the two groups in demographic parameters.[27] There were 492 neonates with neonatal outcomes, 250 in the AC group and 242 in the placebo group. Four hundred and sixty-five children underwent Bayley examinations, 235 in the AC group and 230 in the placebo group.

Umbilical cord serum samples were collected at birth from 400 newborns (GA: 34.8 +/− 3.8 weeks). Umbilical cord serum concentrations of IL-6, CRP, and MPO were not significantly different between repeat AC (treatment group) and placebo groups (Table I), but there was a non-significant trend for higher concentrations of CRP in the AC group.

Univariate analysis showed that umbilical cord concentrations of IL-6, above the 75th percentile, were associated with RDS (p=0.02) and CLD (p=0.04), but not with IVH, necrotizing enterocolitis or neonatal sepsis. However, this association was no longer significant after adjustment for GA at delivery and treatment group in the regression analysis. In the univariate analysis there was no significant relationship between umbilical cord serum concentrations of CRP and MPO, above the 75th percentile, and development of neonatal RDS, CLD, sepsis, neonatal enterocolitis, and IVH. Logistic regression analysis for each analyte indicated that umbilical cord serum concentration of CRP, but not IL-6 or MPO, above the 75th percentile, was significantly associated with a decreased risk of CLD (O.R. 0.10, 95% C.I. 0.02–0.41) even after adjusting for GA at delivery and treatment group (Table II). Regression analysis demonstrated no association between umbilical cord concentrations of IL-6, CRP and MPO, above the 75th percentile and poor neurodevelopmental outcomes (MDI <70 or PDI < 70) (Table III). Umbilical cord serum

median concentrations of IL-6, CRP, and MPO, above the 75th percentile, were associated with more frequent PTB < 32 weeks (Table IV).

For the subgroup of pregnancies delivered before 32 weeks of gestation, logistic regression analysis for each analyte demonstrated that umbilical cord serum concentration of CRP, but not IL-6 or MPO, above the 75th percentile, was associated with decreased risks of RDS (O.R. 0.27, 95% C.I. 0.08–0.95) and CLD (O.R 0.06, 95% C.I. 0.01–0.80), and increased risk of NEC (O.R. 5.93, 95% C.I. 1.00–35), after adjusting for GA at delivery and treatment group (Table V). Regression analysis demonstrated no associations between umbilical cord serum concentrations of IL-6, CRP, and MPO, above the 75th percentile, and MDI < 70 or PDI < 70 in pregnancies that delivered at < 32 weeks of gestation (Table VI). Among neonates that delivered at > 32 weeks of gestation there were very few with morbidies (not shown), not enough for statistical comparison.

DISCUSSION

Principal Findings of the Study

The results of this study suggest that in women identified to be at increased risk for PTB because of a prior episode of preterm labor or other risk factors, elevated umbilical cord serum concentrations of IL-6, CRP, and MPO were not associated with development of neonatal RDS, sepsis, NEC, IVH, or poor neurodevelopmental outcomes (MDI <70 or PDI < 70). However, an elevated umbilical cord serum concentration of CRP, but not IL-6 or MPO, was associated with a decreased risk of CLD, after adjusting for GA at delivery and treatment group. An elevated umbilical cord serum concentration of IL-6, CRP, and MPO was associated with PTB < 32 weeks GA. In the subgroup of women who delivered at < 32 weeks of gestation, an elevated umbilical cord serum concentration of CRP, but not IL-6 or MPO, was associated with a decreased risk of RDS and CLD. Umbilical cord serum median concentrations of the 3 analytes were not significantly different between neonates born to women allocated to receive either repeat AC or placebo groups, but there was a non-significant trend for higher concentrations of CRP in the AC group.

Interpretation of the Findings

The lack of association between the umbilical cord blood concentrations of IL-6, CRP and MPO and the development of neonatal RDS, sepsis, NEC, IVH or poor neurodevelopmental outcomes was unexpected because previous studies indicate a relationship between the risk of some of these complications and elevated umbilical cord concentrations of acute phase reactants, cytokines, and chemokines. The same is the case for the relationship between an elevated umbilical cord serum CRP and a decreased risk of CLD.

The association between higher concentrations of CRP, IL-6 and MPO and delivery before 32 weeks of gestation is consistent with previous reports that the earlier gestational age at birth, the higher the risk of intraamniotic infection,[30] intraamniotic inflammation,[31] histologic chorioamnionitis,[32] funisitis, [16] and evidence of systemic FIRS.[33]

Interleukin 6

IL-6, a pleiotropic proinflammatory cytokine, is a major mediator of host response to inflammation and infection and an early marker of the acute phase response to infection or tissue injury. An elevated umbilical cord serum IL-6 has been used to define the FIRS.[3,34] Many investigators have examined the relationship between umbilical cord serum concentration of IL-6 and neonatal, and/or infant, morbidity. The results have not been consistent. While some reports found a significant association between elevated umbilical cord serum concentrations of IL-6 and BPD, [6] CLD, [35] sepsis [7–8,36–37] NEC, [7,9] PVL, [12] and IVH, [7,11] in pregnancies that either delivered after PPROM, [36] or had PTB (GA range < 36 weeks – <32 weeks),[6,8–9,12,35,37] or birthweight < 1501 grams,[11] other investigators found no association between elevated umbilical cord serum concentrations of IL-6 and IVH, [38] PVL, [38] PDI scores, [39] MDI < 85 and/or PDI < 85, [40] or CP, [40] in pregnancies complicated with either PTL [39] or PTB < 32 weeks GA. [38–40] The discrepancy in results has been attributed to differences in study design, inclusion criteria, availability of umbilical cord samples, characteristics of the study population, performance features of the assay, and lack of control for potential confounding factors.

We expected that infection and/or inflammation in the fetal compartment would be reflected by an elevated umbilical cord serum IL-6 concentration in pregnancies at risk for PTB and especially in those that delivered <32 weeks GA. The parent study did not include analyses of amniotic fluid or histology of the placenta; therefore, we were unable to examine if this relationship occurred in this study. Nonetheless, the significantly higher concentrations of umbilical cord serum IL-6, among patients who delivered <32 weeks of gestation suggest that this may have been the case.

There are several explanations for the lack of association between Il-6 and short and long term morbidity. First, this study was based in a unique population, not studied before; pregnancies at risk for PTB, who received a dose of AC and remained undelivered for 7–10 days. The maternal complications that confer the risks of PTB were heterogeneous, e.g. prior spontaneous PTB at 20–34 weeks GA, placenta previa, chronic abruption. It is possible that the frequency of infection/inflammation among these groups may be different and that this cohort was at low risk for infection by virtue of remaining pregnant long enough to be randomized to a second course of steroids. It is noteworthy that the mean gestational age at birth was 34.8 +/− 3.8 weeks. This is a relatively advanced GA at delivery, and the frequency and severity of intraamniotic infection/inflammation was low. This may explain in part the lack of association with morbidity. It is also important to note that the number of women who delivered before 32 weeks GA is small.

C-reactive protein

CRP is an acute-phase reactant produced by liver cells, in response to IL-6, synthesized during the course of tissue injury or infection. CRP concentrations have been used in neonatology to assess the risk of neonatal sepsis. CRP determinations are simple, well-established, inexpensive, rapid, and widely available in most clinical laboratories to determine whether or not patients have evidence of a plasma acute phase response to infection or tissue injury. More recently investigators have used a new highly sensitive assay

of CRP and observed that serum CRP concentration is a predictor of risk for the development of atherosclerosis and coronary artery disease.[41] Yoon et al[12,42] found associations between umbilical serum concentrations of CRP and neonatal sepsis,[42] and PVL[12] in pregnancies with PTB < 36 weeks GA. In contrast, we were unable to demonstrate an association between umbilical cord serum concentration of CRP and either neonatal sepsis or PVL, but we found an association between umbilical cord serum CRP and decreased risk of CLD. While neonatal cord concentrations of some cytokines were found to be significantly increased in neonates with CLD,[43] a recent study of neonatal blood collected from over 1000 infants, who weighed 401 to 1000 g at birth, found higher concentrations of some cytokines including IL-1β, IL-6, IL-8, and IL-10, as well as interferon γ, and lower concentrations of other cytokines such as IL-17 and tumor necrosis factor β associated with BPD or death.[44] These investigators did not find a relationship between CRP concentrations and the subsequent development of BPD. Based on the results of multivariate analyses, the authors propose that the risk of BPD may be due to an impairment in the transition from the innate immune response mediated by neutrophils to the adaptive immune response mediated by T lymphocytes. It is important to note that these conclusions were based not on results of umbilical cord blood collected at the time of birth, but rather on neonatal blood collected within 4 hours of birth and on days 3, 7, 14 and 21 of life. Therefore, blood samples used in this study were obtained at a different time point and we do not have longitudinal observations. It is unlikely that the mothers included in the study by Ambalavanan are similar to that of the current report.

Our findings of the relationship between CRP in the umbilical cord and RDS and CLD, but not NEC, are in variance with some observational and experimental data suggesting that markers of antenatal infection, and presence of antenatal infection, are associated with an increased risk of RDS and lung disease in the neonates.[45] Kramer *et al*[45] suggest that fetal exposure to inflammation has both detrimental and beneficial effect on the preterm lung. In a recent review of new data concerning lung disease resulting from complications of very preterm birth, Alan Jobe concludes that recent clinical research about the association between antenatal infection/inflammation and RDS or BPD remains incompletely understood.[46] Pathogenesis of CLD was first attributed to barotrauma and oxygen toxicity acting on immature lung tissue, but current data suggest that the main risk factors for CLD include gestational age at delivery, antenatal infection/inflammation, postnatal infection/inflammation, presence and duration of mechanical ventilation and supplemental oxygen.[47] Fetal exposure to inflammation contributes to the relatively low incidence of RDS in very preterm infants.[48–50] Chorioamnionitis decreased the incidence of BPD in unventilated infants, but increased the risk of BPD in ventilated preterm infants (3). In the ELGAN study which included over 1300 infants < 28 weeks GA, there was no correlation between chorioamnionitis and severity of early respiratory disease or BPD.[51] Inflammation limited to the placenta and chorion was associated with decreased RDS, while chorioamnionitis with fetal involvement increased the risk of RDS in early gestational age infants.[52] In a multicenter study, clinical chorioamnionitis was associated with an increased risk of IVH and early-onset sepsis, but not respiratory outcomes.[53] Populations of fetuses ascertained with different criteria, as well as the methods used for the diagnosis of infection and/or inflammation, represent overlapping but distinct populations that may have different

outcomes. For example, in the Alabama Preterm Birth study, histologic chorioamnionitis was associated with a decreased frequency of RDS,[54] while a positive umbilical cord blood culture for ureaplasma and/or mycoplasma had no effect on the frequency incidence of RDS.[54] Interestingly, the converse was true for BPD in the same patient cohort. BPD was more frequent in the infants with ureaplasma/mycoplasma-positive cord blood cultures,[55] but not for the overall population of infants with histological chorioamnionitis.[54] Increased concentration of CRP in umbilical cord serum may reflect presence of antenatal infection and/or inflammation, but in the current study umbilical cord blood cultures, amniotic fluid, or placentas were not available to ascertain the precise nature and frequency of infection and/or inflammation.

Animal studies suggest that the association between chorioamnionitis, RDS, and BPD are confounded by several factors, e.g. duration of fetal exposure, extent of fetal responses, severity of inflammation, and the organism inciting the inflammatory response, that are usually unknown in clinical research.[46] These and other factors that might modify responses (oxygen, ventilation) or suppress responses (antenatal steroids) that contributes to the frequency of a specific outcome in a sub-population of fetuses.[45] Simple explanations of a clinical or histological diagnosis of chorioamnionitis may mask populations of infants with either increased or decreased risks of outcomes such as RDS or BPD.[46]

Another potential explanation for the findings reported herein is the nature of the study population. The group of patients enrolled in this study is highly selective, because it includes women at risk for PTB who received one dose of steroids and remained undelivered and therefore, eligible for a second dose of steroids. This population is very different from the one that is the subject of observational studies in which patients admitted with preterm labor are observed, and there is not a preselection of patients who remain undelivered. Populations of fetuses with different markers of antenatal infection may have different RDS and CLD outcomes, partly due to the imprecise nature of the diagnoses of RDS and BPD.[45]

Effect of treatment allocation is an unlikely explanation of the results since umbilical cord serum concentrations of the analytes were not significantly different between repeat AC (treatment group) and placebo group, and in our analysis we adjusted for treatment allocation, and effect of treatment allocation was excluded.

Myeloperoxidase

MPO, a leukocyte activation mediator,[56] is a constituent of the neurophil phagosome and contributes to bacterial killing. MPO has been correlated to phagocyte degranulation,[57] and implicated in vascular regulation[56] and vascular disease.[58] MPO is a potential biomarker of PTB and subsequent inflammatory insults, and umbilical cord blood is a suitable source of leukocytes for examination of their adherence in the setting of inflammation. [59] Although in pregnancies delivered preterm elevated concentrations of MPO in umbilical cord serum were associated with PTB,[60–61] they were not associated with CP.[61] In our study population umbilical cord serum concentration of MPO was a marker for PTB < 32 weeks GA, but not a marker for neonatal and infant morbidities. The factors reviewed above for IL-6 and CRP apply to MPO and the results observed in the study.

We can speculate on potential clinical implications of our findings. Pregnancies at risk for PTB are at increased risk for intraamniotic infection, fetal inflammatory response syndrome (FIRS), neonatal sepsis, and their long term consequences. The vast majority of pregnancies that have intraamniotic infection are asymptomatic and lack signs of clinical chorioamnionitis. When tocolysis is administered to patients with preterm labor concerns for possible fetal/neonatal harm due to prolongation of pregnancies with intraamniotic infection, are raised. Our findings, of lack of association between elevated umbilical cord serum concentration of IL-6, CRP, and MPO with poor neurodevelopmental outcomes in patients identified to be at risk for PTB, should be reassuring to clinicians that utilize tocolysis in patients with preterm labor.

Studies to date of the associations between umbilical cord concentrations of IL-6, CP and MPO and adverse neonatal outcome, were in pregnancies delivered preterm, and most were limited to short term neonatal outcomes. The few studies reporting long term outcomes have primarily focused on the risk of CP. In this study we report on the association with both short term morbidities and long term neurodevelopmental outcomes. In pregnancies identified to be at risk for PTB elevated umbilical cord serum concentrations of IL-6, CRP, and MPO were associated with PTB < 32 weeks GA, but not with poor neurodevelopmental outcomes.

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Appendix

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Table I

Umbilical Cord Serum Concentrations at Birth of Analytes in Repeat Steroid (Treatment) and Placebo Group

	All		Placebo		Treatment		
	N		N		N		p-value
CRP	400	56.0 (11.4–4639.2)	200	51.2 (12.2–198.9)	200	65.5 (11.2–5172.4)	0.08
IL6	400	5.6 (0.6–2065.1)	200	5.42 (0.7. 1693,6)	200	5.9 (0.6–2258.4)	0.80
MPO	399	75.9 (9.1–749.4)	200	66.1 (6.9–835.6)	199	87.3 (11.1–650.3)	0.11

Data expressed as median with 3rd and 97th percentiles.

Interleukin-6 (IL-6) concentrations expressed in pg/mL; C-reactive protein (CRP) and myeloperoxidase (MPO) concentrations expressed in ng/mL.

Table II

Logistic Regression Analysis for Elevated Umbilical Cord Analytes (IL-6, CRP and MPO) at Birth and Risk of Neonatal Morbidities

Analyte	N	Odds Ratio	95% CI	P-value
RDS				
IL-6 >75th percentile	400	0.88	0.36–2.14	0.79
CRP >75th percentile	400	0.64	0.26–1.58	0.34
MPO >75th percentile	399	0.50	0.20–1.21	0.12
CLD				
IL-6 >75th percentile	400	0.33	0.09–1.23	0.10
CRP >75th percentile	400	0.10	0.02–0.41	0.002
MPO >75th percentile	399	0.55	0.15–2.07	0.38
Sepsis				
IL-6 >75th percentile	400	0.52	0.14–1.99	0.34
CRP >75th percentile	400	1.06	0.40–2.84	0.91
MPO >75th percentile	399	0.77	0.20–2.93	0.70
NEC				
IL-6 >75th percentile	400	1.85	0.50–6.78	0.35
CRP >75th percentile	400	1.60	0.47–5.41	0.45
MPO >75th percentile	399	1.25	0.38–4.14	0.72
IVH				
IL-6 >75th percentile	387	1.00	0.31–3.19	1.00
CRP >75th percentile	387	1.22	0.45–3.33	0.69
MPO >75th percentile	386	1.29	0.44–3.73	0.64

The logistic regression analysis model included gestational age at delivery, treatment group and analytes. CI, confidence interval; CLD, chronic lung disease; CRP, C-reactive protein; IL-6,

Table III

Logistic Regression Analysis for Elevated Umbilical Cord Serum Analytes (IL-6, CRP and MPO) at Birth and Association with Neurodevelopmental Outcomes (MDI < 70 and PDI < 70)

Analyte	N	Odds Ratio	95% CI	P-value
MDI < 70				
IL-6 >75th percentile	318	1.28	0.62–2.63	0.51
CRP >75th percentile	318	0.78	0.46–1.31	0.35
MPO >75th percentile	317	0.84	0.47–1.51	0.56
PDI < 70				
IL-6 >75th percentile	318	1.15	0.50–2.63	0.75
CRP >75th percentile	318	0.72	0.27–1.87	0.50
MPO >75th percentile	317	0.87	0.36–2.10	0.75

IL-6, interleukin-6; CRP, C-reactive protein; MPO, myeloperoxidase

MDI denotes Bayley II Mental Developmental Index; PDI is Bayley II Psychomotor Developmental Index.

Regression models included gestational age at delivery, treatment group, and analyte.

Table IV

Elevated Umbilical Cord Serum Concentrations at Birth of Analytes (IL-6, CRP and MPO) and Risk of Preterm Birth <32 Weeks of Gestation.

Analyte	>75th Percentile (concentration)	Percent Delivery <32 Weeks (cases/total)	P-value
IL-6	No	19.2% (51/266)	0.002
	Yes (>33.6 pg/ml)	34.1% (31/89)	
CRP N=355	No	19.6% (52/265)	0.008
	Yes (>221.2 ng/ml)	33.3% (30/90)	
MPO N=355	No	19.7% (52/264)	0.01
	Yes (> 181.5) ng/ml	32.2% (29/90)	

IL-6, interleukin-6; CRP, C-reactive protein; MPO, myeloperoxidase

Table V

Logistic Regression Analysis for Elevated Umbilical Cord Serum Analytes (IL-6, CRP and MPO) at Birth and Association with Neonatal Morbidities For Infants born at < 32 weeks' GA

Analyte	N	Odds Ratio	95% CI	P-value
RDS				
IL-6 >75th percentile	93	1.81	0.61–5.33	0.28
CRP >75th percentile	93	0.27	0.08–0.95	0.04
MPO >75th percentile	92	0.63	0.21–1.87	0.40
CLD				
IL-6 >75th percentile	93	0.14	0.01–1.61	0.12
CRP >75th percentile	93	0.06	0.01–0.80	0.03
MPO >75th percentile	92	1.04	0.22–5.06	0.96
Sepsis				
IL-6 >75th percentile	93	0.61	0.15–2.54	0.50
CRP >75th percentile	93	1.53	0.44–5.33	0.51
MPO >75th percentile	93	1.29	0.33–5.05	0.72
NEC				
IL-6 >75th percentile	93	0.88	0.12–6.39	0.90
CRP >75th percentile	93	5.93	1.00–35	0.05
MPO >75th percentile	92	2.40	0.45–12.89	0.31
IVH				
IL-6 >75th percentile	93	1.95	0.54–7.09	0.31
CRP >75th percentile	93	0.51	0.10–2.46	0.40
MPO >75th percentile	92	1.11	0.35–3.51	0.86

The logistic regression analysis model included gestational age at delivery, treatment group and analytes. CI, confidence interval; CLD, chronic lung disease; CRP, C-reactive protein; IL-6, interleukin-6; MPO, myeloperoxidase; NEC, necrotizing enterocoloitis; RDS, respiratory distress syndrome

Table VI

Logistic Regression Analysis for Elevated Umbilical Cord Serum Analytes (IL-6, CRP and MPO) at Birth and Association with Neurodevelopmental Outcomes (MDI < 70 and PDI < 70) For Infants born at < 32 weeks' GA

Analyte	N	Odds Ratio	95% CI	P-value
MDI < 70				
IL-6 >75th percentile	71	0.91	0.27–2.99	0.87
CRP >75th percentile	71	1.05	0.30–3.75	0.94
MPO >75th percentile	70	1.00	0.99–1.01	0.67
PDI < 70				
IL-6 >75th percentile	71	1.76	0.52–5.96	0.37
CRP >75th percentile	71	0.25	0.03–2.10	0.20
MPO >75th percentile	70	1.43	0.33–6.20	0.64

-IL-6, interleukin-6; CRP, C-reactive protein; MPO, myeloperoxidase

-MDI denotes Bayley II Mental Developmental Index; PDI is Bayley II Psychomotor Developmental Index.

-Regression models included gestational age at delivery, treatment group, and analyte.