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Blood protein concentrations in the first two postnatal weeks that predict bronchopulmonary dysplasia among infants born before the 28th week of gestation

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Abstract

Lung inflammation contributes to the pathogenesis of bronchopulmonary dysplasia (BPD) and may be accompanied by a systematic inflammatory response. The objective of this study was to investigate the role of systemic inflammation in the development of BPD in a cohort of extremely low gestational age newborns (ELGANs) by examining the relationships between inflammation-associated proteins in neonatal blood samples and pulmonary outcomes. Proteins were measured in blood specimens collected on postnatal days 1–3, 5–8 and 12–15 from 932 ELGANs. Increased risk of BPD was associated with elevated blood concentrations of a variety of pro-inflammatory cytokines, adhesion molecules and proteases. Reduced risk was prominently associated with increased concentrations of one chemokine, RANTES. Elevations of inflammatory proteins associated with BPD risk occurred during the first days following birth, and inflammation intensified thereafter. Therefore, exposures that promote inflammation after the first postnatal days may be more critical in the pathogenesis of BPD. Fetal growth restriction, a known BPD risk factor, was not accompanied by proteins elevations and therefore does not appear to be mediated by systemic inflammation. By contrast, mechanical ventilation altered protein levels and may be associated with systemic inflammation.

Introduction

Support for the claim that early postnatal inflammation contributes to the pathogenesis of bronchopulmonary dysplasia (BPD) comes from several sources. First, the number of inflammatory cells in the airways of preterm infants increases during acute lung disease, and this increase persists in infants who develop BPD (1, 2). Second, preterm infants who develop BPD are more likely than their peers to have elevated concentrations of

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inflammatory mediators in their tracheal fluid (3, 4). Finally, in animal models, exposures associated with BPD (e.g. mechanical ventilation and increased ambient oxygen) increase gene expression of inflammation-associated proteins and increase levels of these proteins in the lung (5, 6).

The finding of altered levels of inflammation-associated proteins in the blood of infants who develop BPD (7, 8) indicates that BPD may be associated with a systemic inflammatory response, but it is not clear if these proteins originate in the lung or elsewhere. In either case, measurement of biomarkers of inflammation in the blood may provide insight about the role of inflammation in the development of BPD.

The objective of this study was to investigate the role of inflammation in the development of BPD in a cohort of extremely low gestational age newborns (ELGANs) by examining the relationships between biomarkers in neonatal blood samples and pulmonary outcomes. We measured biomarker levels in serial blood specimens collected soon after birth and during the subsequent two weeks. Our goal was to investigate the role of inflammation before and following neonatal exposures that might promote inflammation. Of particular interest were changes in levels of biomarkers among infants with factors known to increase the likelihood of BPD, including fetal growth restriction and mechanical ventilation. Identifying relationships between these risk factors and specific biomarkers might suggest mechanisms through which these exposures impart risk. We examined these relationships separately in infants with mild/moderate and severe BPD to evaluate the possibility that the pathophysiology and the role of inflammation might be distinct in these two entities.

Methods

This study was a component of the ELGAN Study, a multi-center study designed to identify characteristics and exposures that increase the risk of neurologic disorders in ELGANs (9). During the years 2002–2004, women delivering before 28 weeks gestation at one of 14 institutions were enrolled in the study. The study was approved by the individual institutional review boards, and informed consent was obtained from participating mothers. The original consent and approval included the provision for secondary analyses of de-identified data.

Our study cohort consisted of all infants in the ELGAN Study who survived to 36 weeks postmenstrual age (PMA) for whom protein measurements were available in one or more blood specimens obtained during the first two postnatal weeks. Because of financial constraints, measurement of proteins was limited to specimens from infants who survived until the 24-months adjusted age and for whom all elements of the follow-up evaluation at 24 months were completed, including developmental testing, neurologic examination and measurement of head circumference. A full description of the methods of the ELGAN Study is provided elsewhere (9). Here we focus on those most relevant to this report.

Maternal, Pregnancy and Newborn Variables

Selected characteristics of the pregnancy, intrapartum and newborn period were recorded. The gestational age (GA) estimates were based on a hierarchy of the quality of available information, that included (in order of priority): 1) dates of embryo retrieval or intrauterine insemination or fetal ultrasound before the 14^{th} week (62%), 2) fetal ultrasound at 14 or more weeks (29%), 3) last menstrual period without fetal ultrasound (7%), and 4) GA recorded in the log of the NICU (1%). The birth weight birth weight Z-score for each infant was calculated using a standard data set (10).

Data describing the highest level of respiratory support, categorized as no support, increased ambient oxygen, nasal continuous positive airway pressure, conventional mechanical ventilation or high frequency ventilation, was collected daily during the first week and on days 7, 14, 21, and 28, and at 36 weeks PMA. We did not record the specific devices used.

BPD was defined as oxygen therapy at 36 weeks PMA. Decisions to administer supplemental oxygen were made by infants' clinical providers and were not based on a uniform threshold of blood oxygenation. We subdivided these infants into those who were not ventilator dependent (mild/moderate BPD) and those who were ventilator dependent (severe BPD).

Placenta Morphology

Samples were collected from placentas for histologic examination, and were processed for morphologic assessment as part of routine clinical care. Details describing the criteria for each histologic lesion are presented elsewhere (11).

Blood Collection and Protein Measurements

Blood was collected on filter paper (Schleicher & Schuell 903). The times of collection are designated as Day 1, 7 and 14, and were defined by the following ranges of postnatal age at collection: Day 1 range = Days 1–3; Day 7 range = Days 5–8; Day 14 range = Days 12–15. Dried blood spots were stored at −70°C in sealed bags with desiccant until processed.

Analyses of proteins were performed in the Laboratory of Genital Tract Biology, Brigham & Women's Hospital. For protein elution, 12mm punched biopsies of the frozen blood spots were submerged in 300 μL PBS containing 0.1% Triton X100 (Sigma-Aldrich, St. Louis, MO) and 0.03% Tween-20 (Fisher, Hampton, NH), vortexed for 30 seconds, and incubated on a shaker for 1 hour at 4°C. The buffer and biopsy were then transferred over the filter of a SpinX tube (Corning Fisher), centrifuged at 2000 × g followed by collection of the filtered eluted blood. An additional wash of the punch was performed in 100 μL for a final elution volume of 400 μL.

Proteins were measured in duplicate using the Meso Scale Discovery multiplex platform and Sector Imager 2400 (MSD, Gaithersburg, MD). This electrochemiluminescence system has been validated by comparisons with traditional ELISA (12). The MSD Discovery Workbench Software was used to convert relative luminescent units into protein concentrations using interpolation from several log calibrator curves. Split quality control blood pools tested on each plate showed inter-assay variation of 10–20% for each protein. The total protein concentration in each eluted sample was determined by BCA assay (Thermo Scientific, Rockford, IL) using a multi-label Victor 2 counter (Perkin Elmer, Boston, MA) and the measurement of each analyte was normalized to mg total protein.

We measured the following 25 proteins: IL-1β, IL-6, IL-6R (R=receptor), TNF-α, TNF-R1, TNF-R2, IL-8 (CXCL8; interleukin-8), MCP-1 (CCL2; monocyte chemotactic protein-1), MCP-4 (CCL13; monocyte chemoattractant protein-4), MIP-1β (CCL4; macrophage inflammatory protein-1β), RANTES (CCL5; regulated upon activation, normal T-cell expressed, and secreted), I-TAC (CXCL11; interferon-inducible T cell alpha-chemoattractant), ICAM-1 (CD54), ICAM-3 (CD50), VCAM-1 (CD106; vascular cell adhesion molecule-1), E-selectin (CD62E; E-SEL), MMP-1 (matrix metalloproteinase-1), MMP-9 (matrix metalloproteinase-9), CRP (C-reactive protein), SAA (serum amyloid A), MPO (myeloperoxidase), VEGF, VEGF-R1, VEGF-R2, and IGFBP-1 (insulin growth factor binding protein-1).

Data Analysis

We evaluated the generalized null hypothesis that the risks of mild/moderate and severe BPD are not associated with blood protein concentrations in the highest quartile for GA and the day the blood was collected. We began our analyses by exploring the frequency of each entity in the four quartiles of the concentration of each protein. Linear trends were rare so our final analyses are limited to comparisons of infants whose protein concentration was in the highest quartile to infants whose protein concentration was in the lower three quartiles. An association of risk of each BPD entity with a protein concentration in the highest quartile was defined by an odds ratio and the 99% confidence interval. We selected this confidence interval rather than the conventional 95% confidence interval because we wanted to modify our analyses for multiple comparisons, while not appreciably increasing the risk of a type 2 error (13).

Because the two BPD entities are mutually exclusive and each is compared to the same referent group (infants who did not develop BPD), we used multinomial logistic regression (13) and controlled for GA (23–24, 25–26, 27 weeks) to evaluate protein-BPD relationships at each time point (Days 1, 7 and 14).

We evaluated the antecedents of lung injury in the order they occur by creating time-oriented logistic regression models that first considered endogenous characteristics of the infant (e.g., GA and birth weight Z-score). Then we sequentially added information conveyed by variables for an elevated concentration of each protein in the temporal order the blood was collected. In one model, mechanical ventilation was added at Day 7 if the infant was being treated with any form of mechanical ventilation on that day. In these time-oriented models, significant variables ($p < 0.01$) are retained for inclusion in the model with information from the next time interval or epoch. The final model included variables from each of the three specimen collection intervals. We used a step down procedure seeking a parsimonious solution without interaction terms.

Results

During the study period, 1249 mothers of 1506 infants consented to participate in the ELGAN Study. Among these infants, 255 died before 36 weeks PMA and 51 died between 36 weeks PMA and two years adjusted age (Figure 1). An additional 258 were excluded from our study because either follow-up evaluation was incomplete or blood specimens were not available. Our cohort included the remaining 932 infants. Frequency of maternal, pregnancy and newborn features within each category are listed in Table 1. Four hundred infants (43%) were oxygen dependent but not ventilator dependent at 36 weeks PMA, and an additional 86 infants (9%) were both oxygen and ventilator dependent.

Among the 258 infants who survived to 24 months adjusted age, but for whom measurements of proteins were not available, the incidence of mild/moderate BPD was 39%, and the incidence of severe BPD was 5%. Among the 51 infants who died between 36 weeks PMA and 24 months adjusted age the incidence of mild/moderate BPD and severe BPD was 39% and 47%, respectively.

Protein measurements were available for 855 infants on Day 1, 860 infants on Day 7 and 781 infants on Day 14. Among all infants, concentrations of most proteins tended to increase with advancing postnatal age (data not shown). In multinomial analysis, infants who developed mild/moderate BPD, compared to infants with no BPD, had elevated Day 1 concentrations of TNF- α, elevated Day 7 concentrations of TNF-R2, MCP-1, and ICAM-1, and elevated Day 14 concentrations of IL-1b, IL-6, TNF- α, TNF-R1, IL-8, MCP-1,

ICAM-1, and MMP-9 (Table 2). In contrast, these infants had concentrations of RANTES that were significantly reduced on Days 7 and 14 and MIP-1β on Day 7.

Compared to infants with no BPD, infants who developed severe BPD did not have an appreciable inflammation signal in their Day 1 blood (Table 2). Rather, the signal was first evident in Day 7 blood with elevated concentrations of TNF-R2, MCP-1, and ICAM-1. The inflammation signal was even more apparent on Day 14 with elevated concentrations of IL-6, TNFα, TNF-R1, MCP-1, ICAM-1, E-selectin1 and MMP-9. Severe BPD was also preceded by reduced concentrations of RANTES (Days 7 and 14) and MMP-1 (Days 1 and 14).

We created three time-oriented risk models for the two BPD severity levels. All included GA in three strata (23–24, 25–26, 27 weeks); the second also included birth weight Z-score < −1 and the third included birth weight Z-score < −1 and mechanical ventilation on Day 7. These models were created to identify the influence of fetal growth restriction and mechanical ventilation on blood protein concentrations. Acquired sepsis and receipt of postnatal steroids did not predict BPD nor did their addition change the proteins selected in the modeling process.

Time-oriented risk model with Gestational Age Only (Table 3)

In this model, increased risk of mild/moderate BPD was associated with an elevated concentration of TNF-α on Day 1. An elevated concentration of TNF-R2 on Day 7 was also associated with increased risk, whereas infants who had elevated concentrations of RANTES and MIP-1β on Day 7 were at reduced risk. The Day 7 RANTES risk information was diminished by the addition of Day 14 RANTES information. IL-1β and ICAM-1 were the two Day 14 proteins whose elevated concentrations provided supplemental information about an increased risk of the mild/moderate BPD.

Elevated concentrations of VCAM-1 on Day 1 were associated with an increased risk of severe BPD, while elevated concentrations of MMP-1 on Day 1 were associated with decreased risk. Both of these proteins continued to convey information about risk even when the information conveyed by Day 7 and Day 14 proteins was added to the model. Elevated concentrations of Day 7 ICAM-1 and MCP-1 were associated with increased risk, whereas increased concentrations of RANTES were associated with reduced risk. The risk information provided by the RANTES Day 7 concentrations was diminished by the addition of Day 14 RANTES concentrations to the model. Similarly, the association with Day 7 ICAM-1 concentrations was attenuated when the Day 14 ICAM-3 concentrations were added to the model.

Time-oriented risk model with Gestational Age and Birth Weight Z-score < −1 (Table 4)

Birth weight Z-score < −1 predicted both mild/moderate and severe BPD in all epochs even after adjustment for GA and protein levels. The inclusion of birth weight Z-score resulted in relatively minor changes in the Day 1 protein levels that had been identified as predictors in the model that excluded birth weight Z-score. TNF-α remained in the model, as did RANTES on Day 14, but RANTES on Day 7 did not. The predictors of severe BPD changed minimally. ICAM-1 was not added on Day 7 as it had been in the previous model, while on Day 14, ICAM-1 replaced ICAM-3.

Time-oriented risk model with Gestational Age, Birth Weight Z-score < −1 and Mechanical Ventilation on Day 7 (Table 5)

Mechanical ventilation on Day 7 was a predictor of both mild/moderate and severe BPD and was a stronger predictor of severe BPD than all other risk factors, including protein levels.

Adjusting for mechanical ventilation on Day 7 altered the model for mild/moderate BPD by dropping ICAM-1 on both Days 7 and 14, and RANTES on Day 14. Adjusting for mechanical ventilation altered the model for severe BPD by eliminating RANTES on both Days 7 and 14, and MCP-2 on Day 7. ICAM-1 on Day 14 was replaced by ICAM-3.

Discussion

Our study demonstrates that the risk of BPD among infants born before the 28th week of gestation is associated with elevated blood concentrations of a variety of proteins integral to inflammation. These include pro-inflammatory cytokines, adhesion molecules and proteases. Reduced risk is prominently associated with increased concentrations of one chemokine, RANTES. These findings suggest that an inflammatory process involving a variety of mediators is critical in the development of BPD. Elevations of inflammatory proteins associated with BPD risk occur during the first days following birth. However, inflammation intensifies thereafter. Therefore, exposures that promote inflammation after the first postnatal days may be more critical in the development of BPD.

Our observations are in general agreement with previous reports. For example, in a cohort of ELGANs, death or BPD at 36 weeks PMA was predicted by elevated blood concentrations of a variety of cytokines (7). The notable similarities to our findings were the increased risk associated with high levels of TNFα and decreased risk with high levels of RANTES. In contrast to that study, we did not find a distinct pattern of decreased concentrations of proteins that the authors associated with adaptive (as opposed to innate) immunity in infants who developed BPD, with the exception of RANTES. Our choice of proteins might have masked these trends. For example, we did not measure IFNγ or TNFβ, cytokines associated with innate immunity, or IL-10, a cytokine associated with adaptive immunity (14). A more important difference between these studies might be our exclusion of infants who died prior to 36 weeks PMA. In some studies of BPD, death prior to 36 weeks PMA is included as an outcome measure because it competes with BPD. However, because a relatively small proportion of infants die from lung disease, and some die of multi-system organ failure or sepsis, processes with a strong systemic inflammatory component, we believe that it is more appropriate to exclude infants who die prior to 36 weeks PMA when investigating the role of inflammation in lung disease.

We observed a doubling of risk of less severe BPD among infants with concentrations of TNFα in the highest quartile on the first postnatal day. A similar association was not seen in another cohort of premature infants (15). Several important differences in study design might explain this lack of agreement. For example, that study included infants born before the 33rd week of gestation, while our study did not include any infant born between weeks 28 to 32. The differences may also have resulted from different methods to account for the potential contribution of chorioamnionitis. We did not adjust for the presence of chorioamnionitis in our regression models because in a previous study of BPD risk in this cohort, we did not identify chorioamnionitis as a risk factor (16). In addition, that study measured concentrations in cord blood only, while the specimens in our study were obtained postnatally. In both humans and animals, pro-inflammatory cytokines appear in the lungs soon after early neonatal exposures (e.g. mechanical ventilation) that promote inflammation (17, 18), raising the possibility that the early elevations in TNFα in our infants are a consequence of postnatal exposures.

The chemokine RANTES was one of the few proteins associated with reduced risk of both mild/moderate and severe BPD. As a chemotactic agent it attracts inflammatory cells to the site of infection or injury (19). Therefore, one might expect elevated levels to intensify the inflammatory process and increase the likelihood of BPD. In fact, elevated levels of other

chemotactic agents, including IL-8 and MIP-1, have been observed in bronchial alveolar lavage fluid in infants who develop BPD (20). However, there is evidence that RANTES actually protects against organ damage in animal models of inflammation-mediated diseases (21, 22). In addition, an *in vitro* study provides support that RANTES reduces inflammation (23). Thus the association of reduced risk with elevated concentrations of RANTES might reflect anti-inflammation and protection.

Other proteins highly associated with both mild/moderate and severe BPD are adhesion molecules, most notably ICAM-1 and ICAM-3. As a group, adhesion molecules promote migration of inflammatory cells from the blood to sites of injury in the lung, and their primary site of action is at the blood-tissue interface (24). Because of this site of action, blood levels of adhesion molecules might be elevated in organ-specific inflammation. Therefore, it is not surprising that increased concentrations in blood are also associated with BPD risk. Other circulating inflammatory proteins that are elevated in BPD may not be of pulmonary origin. Rather, their presence may result from a systemic response to injury or inflammation in the lung (e.g., SAA or CRP). It is also possible that under certain circumstances proteins produced by cells in the lung remain in the lung compartment, while under other circumstances (e.g., intense pulmonary inflammation), they may "leak" into the circulation (25). Because of these uncertainties, we advise caution in drawing inferences about the observed changes in blood proteins and pulmonary pathology.

Some important observations resulted from developing time-oriented risk models for BPD that both included and excluded specific, known clinical risk factors. In one model, we included birth weight Z-score, a marker of fetal growth restriction, because it is both a known BPD risk factor (16, 26) and is associated with decreased placental expression of selected inflammatory cytokines (27). The associations between protein concentrations and BPD risk in this model were similar to those in the model that excluded birth weight Z-score. This finding suggests that the increased BPD risk associated with fetal growth restriction is probably not mediated by circulating inflammation-associated proteins. By contrast, the addition of mechanical ventilation at 7 days changed the apparent influence of ICAM-1 and RANTES, suggesting that mechanical ventilation may influence pathogenesis by altering expression of these proteins.

This study has a number of strengths. We included a large number of infants, making it unlikely that we missed important associations due to lack of statistical power, and we collected all of our data prospectively. We selected infants based on GA, not birth weight, in order to minimize confounding due to factors related to fetal growth restriction, and with regression models we adjusted for fetal growth restriction. We did not include infants who died prior to 36 weeks PMA, thereby restricting this to a study of BPD and not the composite outcome of BPD or death.

This study also has limitations. For practical reasons, our cohort was limited to infants who had complete neurodevelopmental evaluation at 24 months. This resulted in the non-random exclusion of two potentially important cohorts. The incidence of both mild/moderate and severe BPD in the cohort of infants who survived to 24 months but for whom protein measurements were not available was only slightly lower than in our study cohort. Therefore, their exclusion is not likely to have resulted in a significant bias in the results. The incidence of severe BPD among infants who died between 36 weeks PMA and 24 months was significantly higher (47%) than in our study cohort (9%). The effect of exclusion of these infants, however, is likely to be minimal because they represent a small proportion of the ELGAN Study cohort (≈ 4%). The diagnosis of BPD was made on the basis of clinicians' decisions to treat with mechanical ventilation or supplemental oxygen, not on the basis of physiologic disturbances, and clinical practices almost certainly varied

among centers. Finally, because this was an observational study, we cannot know whether the protein elevations were associated with BPD in a cause and effect relationship or whether the elevations are surrogates for other processes that might have influenced BPD risk.

Our study has several implications for researchers interested in preventing BPD. First, the different risk factor profile for mild/moderate and severe BPD suggest that BPD is a heterogenous condition. Consideration of this heterogeneity could lead to more informative epidemiologic studies. Second, our findings suggest the possibility that inflammation-associated proteins in neonatal blood could serve as biomarkers of modifiable biological processes involved in the development of BPD. If so, these biomarkers could be used as response measures in intervention studies. Third, at least some aspect of the fetal and neonatal inflammatory response is associated with a lower risk of BPD. Thus one approach to prevention might be the enhancement of endogenous protectors.

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Abbreviations

BPD	bronchopulmonary dysplasia
ELGAN	extremely low gestational age newborn
PMA	postmenstrual age
R	receptor

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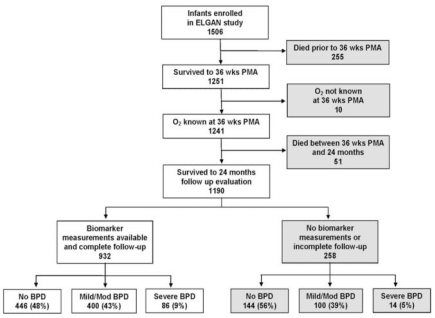

Figure 1.
Derivation of the study population

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Table 1

Antenatal, delivery, and neonatal characteristics of infants classified by their oxygen and ventilator dependency at 36 weeks PMA.

Characteristics		Bronchopulmonary dysplasia*			
		None	Mild/mod **	Severe §	N
Maternal race	White	59	64	62	561
	Black	28	26	32	251
Antenatal corticosteroid course	Complete	67	62	60	599
	Partial	22	28	25	233
Cesarean delivery	Yes	67	66	71	621
Gestational age (wks)	23–24	10	30	31	188
	25–26	44	49	50	433
Birth weight (g)	≤ 750	19	51	61	342
	751–1000	52	38	31	409
Birth weight Z-score	< −2	3	7	13	50
	≥ −2, < −1	7	18	51	121
Sex	Male	51	55	49	490
Fetuses	Multiple	35	32	36	316
Chorioamnionitis	Yes	36	37	29	305
Funisitis	Yes	17	18	11	142
Maximum number		446	400	86	932

* numbers in cells are column percentages

** oxygen dependent but not ventilator dependent at 36 weeks PMA

§ oxygen and ventilator dependent at 36 weeks PMA

Table 2

Odds ratios (99% confidence intervals) from a multinomial analysis for mild/moderate BPD and severe BPD associated with highest quartile of the distribution of inflammation associated proteins collected at specific postnatal ages and adjusted for gestational age (23–24, 25–26, 27 weeks). The referent group consists of infants who had no BPD.

	Mild/moderate BPD			Severe BPD		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
CRP	1.5 (0.96, 2.3)	1.3 (0.9, 2.0)	1.4 (0.9, 2.2)	1.3 (0.6, 2.7)	2.0 (0.99, 3.9)	1.4 (0.6, 3.0)
SAA	1.6 (1.00, 2.4)	0.8 (0.5, 1.2)	1.1 (0.7, 1.8)	0.9 (0.4, 2.1)	0.7 (0.3, 1.5)	0.8 (0.3, 1.8)
MPO	1.0 (0.7, 1.6)	1.1 (0.7, 1.6)	1.5 (0.95, 2.3)	1.4 (0.7, 2.7)	1.0 (0.5, 2.0)	1.3 (0.6, 2.7)
IL-1beta	1.2 (0.8, 1.9)	1.2 (0.8, 1.8)	2.4 (1.5, 3.7)	0.8 (0.4, 1.7)	1.4 (0.7, 2.8)	1.8 (0.9, 3.8)
IL-6	1.0 (0.7, 1.6)	0.9 (0.6, 1.4)	1.8 (1.1, 2.8)	0.7 (0.3, 1.5)	1.3 (0.7, 2.6)	2.0 (0.96, 4.1)
IL-6R	1.0 (0.7, 1.6)	1.4 (0.9, 2.2)	1.3 (0.9, 2.1)	1.2 (0.6, 2.4)	1.6 (0.9, 3.2)	1.0 (0.5, 2.2)
TNF-alpha	1.8 (1.2, 2.7)	1.3 (0.9, 2.0)	1.9 (1.2, 3.0)	1.9 (0.96, 3.9)	1.6 (0.8, 3.2)	2.3 (1.1, 4.8)
TNF-R1	1.1 (0.7, 1.7)	1.4 (0.9, 2.2)	2.1 (1.3, 3.4)	1.3 (0.6, 2.7)	1.9 (0.96,3.8)	3.2 (1.5, 6.5)
TNF-R2	1.3 (0.8, 2.0)	1.6 (1.02, 2.5)	1.4 (0.9, 2.2)	1.7 (0.9, 3.5)	2.1 (1.1, 4.2)	1.9 (0.9, 3.9)
IL-8 (CXCL8)	1.5 (0.97, 2.3)	1.2 (0.8, 1.9)	2.2 (1.4, 3.5)	1.3 (0.6, 2.7)	1.4 (0.7, 2.8)	2.1 (0.98, 4.4)
MCP-1 (CCL2)	1.3 (0.9, 2.0)	1.6 (1.04, 2.5)	1.7 (1.1, 2.7)	1.3 (0.6, 2.6)	3.0 (1.5, 5.9)	2.7 (1.3, 5.6)
MCP-4 (CCL13)	1.2 (0.6, 2.6)	1.4 (0.9, 2.2)	1.1 (0.7, 1.7)	0.9 (0.4, 2.0)	1.7 (0.9, 3.4)	1.0 (0.4, 2.1)
MIP-1B (CCL4)	1.0 (0.7, 1.6)	0.6 (0.4, 0.95)	0.8 (0.5, 1.3)	0.7 (0.3, 1.6)	1.7 (0.3, 1.4)	0.6 (0.3, 1.4)
RANTES (CCL5)	0.8 (0.5, 1.2)	0.6 (0.4, 0.95)	0.5 (0.3, 0.8)	0.5 (0.2, 1.2)	0.3 (0.1, 0.7)	0.3 (0.1, 0.8)
I-TAC (CXCL11)	1.3 (0.8, 2.0)	1.1 (0.7, 1.7)	1.0 (0.6, 1.6)	1.2 (0.6, 2.5)	1.4 (0.7, 2.8)	1.1 (0.5, 2.3)
ICAM-1 (CD54)	1.4 (0.9, 2.2)	1.7 (1.1, 2.7)	3.5 (2.1, 5.8)	1.6 (0.8, 3.4)	2.5 (1.3, 5.0)	9.1 (4.3, 19)
ICAM-3 (CD50)	0.9 (0.6, 1.3)	1.0 (0.6, 1.5)	1.5 (0.9, 2.3)	1.0 (0.5, 2.1)	0.6 (0.3, 1.4)	1.5 (0.7, 3.2)
VCAM-1 (CD106)	1.5 (0.95, 2.3)	1.0 (0.6, 1.5)	1.2 (0.8, 1.9)	1.7 (0.8, 3.4)	1.2 (0.6, 2.4)	1.9 (0.9, 3.9)
E-SEL (CL62E)	1.4 (0.9, 2.1)	1.4 (0.9, 2.1)	1.4 (0.9, 2.3)	0.8 (0.4, 1.8)	1.4 (0.7, 2.8)	2.5 (1.2, 5.1)
MMP-1	0.8 (0.5, 1.3)	0.7 (0.5, 1.1)	0.7 (0.4, 1.1)	0.4 (0.2, 0.9)	0.5 (0.2, 1.2)	0.3 (0.1, 0.8)
MMP-9	0.9 (0.6, 1.5)	1.3 (0.8, 2.0)	2.0 (1.2, 3.2)	1.2 (0.6, 2.5)	0.6 (0.3, 1.5)	2.1 (1.02, 4.5)
VEGF	1.0 (0.6, 1.5)	1.0 (0.7, 1.6)	1.0 (0.6, 1.6)	0.8 (0.4, 1.8)	0.4 (0.2, 1.04)	0.6 (0.3, 1.4)
VEGF-R1	1.4 (0.9, 2.2)	1.3 (0.8, 1.9)	1.3 (0.8, 2.0)	0.9 (0.4, 2.0)	1.1 (0.6, 2.2)	1.3 (0.6, 2.6)
VEGF-R2	1.0 (0.7, 1.5)	1.1 (0.7, 1.7)	1.3 (0.9, 2.0)	1.0 (0.5, 2.0)	0.9 (0.5, 1.9)	1.7 (0.8, 3.5)

	Mild/moderate BPD			Severe BPD		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
IGFBP-1	1.5 (0.95, 2.3)	1.3 (0.8, 2.1)	1.3 (0.9, 2.1)	1.2 (0.6, 2.5)	1.4 (0.7, 2.9)	2.0 (0.95, 4.1)
N BPD	374	363	342	77	80	71
N total	855	860	781	855	860	781

Table 3

Odds ratios (and 99% confidence intervals) of mild/moderate BPD and severe BPD (vs no BPD) and associated with a concentration of the protein on the left in the highest quartile for gestational age and day blood was obtained relative to the risk among infants whose concentration was in the lower 3 quartiles. These are time-oriented logistic risk models that include *gestational age* (23–24, 25–26, 27 weeks).

Model for Mild/Moderate BPD				
Day	**Protein**	**Day 1**	**Days 1 and 7**	**Days 1, 7, 14**
1	GA 23–24	6.4 (3.7, 12)	7.8 (4.1, 15)	9.2 (4.5, 19)
	GA 25–26	2.5 (1.6, 3.9)	2.7 (1.6, 4.3)	2.9 (1.7, 5.0)
	TNF-α	1.8 (1.2, 2.8)	2.1 (1.3, 3.3)	2.0 (1.2, 3.5)
7	RANTES		0.6 (0.4, 0.9)	0.7 (0.4, 1.2)
	TNF-R2		2.1 (1.3, 3.5)	1.8 (1.03, 3.3)
	MIP-1β		0.5 (0.3, 0.8)	0.5 (0.3, 0.9)
14	IL-1β			2.0 (1.2, 3.4)
	RANTES			0.5 (0.3, 0.9)
	ICAM-1			2.8 (1.6, 5.0)

Model for Severe BPD				
Day	**Protein**	**Day 1**	**Days 1 and 7**	**Days 1, 7, 14**
1	GA 23–24	9.0 (3.3, 24)	11 (3.5, 32)	15 (3.8, 60)
	GA 25–26	3.0 (1.3, 7.2)	3.0 (1.2, 7.7)	5.1 (1.6, 16)
	VCAM-1	2.5 (1.1, 5.4)	3.2 (1.3, 7.6)	3.8 (1.3, 11)
	MMP-1	0.3 (0.1, 0.9)	0.4 (0.1, 0.98)	0.2 (0.1, 0.9)
7	ICAM-1		2.4 (1.1, 5.3)	1.6 (0.6, 4.2)
	MCP-1		2.3 (1.1, 5.0)	2.9 (1.1, 7.3)
	RANTES		0.3 (0.1, 0.7)	0.5 (0.1, 1.7)
14	RANTES			0.3 (0.1, 0.9)
	ICAM-3			9.0 (3.3, 25)

Table 4

Odds ratios (and 99% confidence intervals) of mild/moderate BPD and severe BPD (vs no BPD) and associated with a concentration of the protein on the left in the highest quartile for gestational age and day blood was obtained relative to the risk among infants whose concentration was in the lower 3 quartiles. These are time-oriented logistic risk models that include *gestational age* (23–24, 25–26, 27 weeks) and *birth weight Z-score <−1*.

Model for Mild/Moderate BPD				
Day	**Protein**	**Day 1**	**Days 1 and 7**	**Days 1, 7, 14**
1	GA 23–24	7.5 (4.1, 13)	8.3 (4.4, 16)	9.5 (4.7, 19)
	GA 25–26	2.5 (1.6, 4.0)	2.8 (1.6, 4.4)	2.9 (1.7, 5.0)
	BWZ* < −1	3.1 (1.8, 5.4)	2.8 (1.5, 4.9)	2.1 (1.1, 4.0)
	TNF-α	1.8 (1.2, 2.9)	1.9 (1.2, 3.1)	2.0 (1.2, 3.4)
7	ICAM-1		1.9 (1.2, 3.2)	1.5 (0.9, 2.7)
	MIP-1β		0.5 (0.3, 0.8)	0.5 (0.3, 0.9)
14	ICAM-1			2.7 (1.5, 5.0)
	IL-1β			1.9 (1.2, 3.2)
	RANTES			0.5 (0.3, 0.8)

Model for Severe BPD				
Day	**Protein**	**Day 1**	**Days 1 and 7**	**Days 1, 7, 14**
1	GA 23–24	9.9 (3.5, 28)	13 (4.1, 39)	18 (4.3, 78)
	GA 25–26	2.8 (1.1, 6.8)	2.8 (1.1, 7.2)	4.9 (1.5, 16)
	BWZ < −1	4.6 (2.0, 11)	4.3 (1.8, 11)	4.2 (1.4, 12)
	VCAM-1	2.7 (1.2, 6.1)	3.7 (1.5, 9.0)	4.5 (1.5, 13)
	MMP-1	0.3 (0.1, 0.9)	0.4 (0.1, 0.98)	0.2 (0.1, 0.9)
7	RANTES		0.3 (0.1, 0.9)	0.6 (0.2, 2.2)
	MCP-1		2.6 (1.2, 5.7)	3.2 (1.2, 8.3)
14	ICAM-1			10 (3.8, 28)
	RANTES			0.2 (0.1, 0.8)

* BWZ = birth weight Z-score

Table 5

Odds ratios (and 99% confidence intervals) of mild/moderate BPD and severe BPD (vs no BPD) of the protein on the left and associated with a concentration of the protein on the left in the highest quartile for gestational age and day blood was obtained relative to the risk among infants whose concentration was in the lower 3 quartiles. These are time-oriented logistic risk models that include *gestational age* (23–24, 25–26, 27 weeks), *birth weight Z-score <1* and *mechanical ventilation on Day 7*.

Model for Mild/Moderate BPD				
Day	**Protein**	**Day 1**	**Days 1 and 7**	**Days 1, 7, 14**
1	GA 23–24	7.5 (4.1, 13)	4.1 (2.1, 8.3)	5.6 (2.6, 12)
	GA 25–26	2.5 (1.6, 4.0)	1.9 (1.1, 3.2)	2.1 (1.2, 3.8)
	BWZ*< −1	3.1 (1.8, 5.4)	2.4 (1.3, 4.5)	2.0 (1.02, 3.9)
	TNF-α	1.8 (1.2, 2.9)	2.1 (1.3, 3.5)	2.0 (1.2, 3.5)
7	Mech vent**		4.2 (2.6, 6.9)	2.9 (1.7, 5.1)
	TNF-R2		2.2 (1.3, 3.9)	2.1 (1.1, 3.9)
	SAA		0.5 (0.3, 0.9)	0.5 (0.3, 0.9)
	MIP-1β		0.5 (0.3, 0.9)	0.6 (0.3, 1.02)
14	IL-1β			1.9 (1.1, 3.2)
	ICAM-3			2.4 (1.3, 4.4)

Model for Severe BPD				
Day	**Protein**	**Day 1**	**Days 1 and 7**	**Days 1, 7, 14**
1	GA 23–24	9.9 (3.5, 28)	4.3 (1.3, 13)	5.0 (1.3, 19)
	GA 25–26	2.8 (1.1, 6.8)	1.8 (0.7, 4.8)	2.4 (0.9, 7.6)
	BWZ < −1	4.6 (2.0, 11)	3.8 (1.5, 9.4)	3.0 (1.1, 8.1)
	VCAM-1	2.7 (1.2, 6.1)	4.1 (1.6, 11)	4.0 (1.4, 12)
	MMP-1	0.3 (0.1, 0.9)	0.4 (0.1, 0.98)	0.2 (0.1, 0.9)
7	Mech vent		15 (4.2, 56)	11 (2.7, 50)
14	ICAM-3			5.6 (2.2, 14)

*BWZ = birth weight Z-score

**Mech vent = mechanical ventilation