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Cytokines Associated with Bronchopulmonary Dysplasia or Death in Extremely Low Birth Weight Infants

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Abstract

Background—Inflammation mediated by cytokines may be important in the pathogenesis of bronchopulmonary dysplasia and the competing outcome of death in extremely low birth weight infants.

Objective—To develop multi-variable logistic regression models for the outcome of bronchopulmonary dysplasia and/or death at 36w post-menstrual age using clinical and cytokine data from the first 28 days.

Methods—1067 extremely low birth weight infants in the Neonatal Research Network of the National Institute of Child Health and Human Development had 25 cytokines measured from blood collected within 4 h of birth and on days 3, 7, 14, and 21. Stepwise regression using peak values of the 25 cytokines and 15 clinical variables identified variables associated with BPD/death. Multi-variable logistic regression was done for bronchopulmonary dysplasia/death using variables selected by stepwise regression. Similar analyses were also done using average cytokine values from days 0–21, days 0–3, and from days 14–21.

Results—Of 1062 infants with available data, 606 infants developed bronchopulmonary dysplasia or died. Combining results from all models, bronchopulmonary dysplasia/death was associated with higher concentrations of interleukins-1 β , -6, -8, -10, and interferon- γ and lower concentrations of interleukin-17, RANTES, and tumor necrosis factor- β . Compared to models with only clinical variables, addition of cytokine data improved predictive ability by a statistically significant but clinically modest magnitude.

Conclusions—The overall pattern of cytokines suggests bronchopulmonary dysplasia/death may be associated with impairment in the transition from the innate immune response mediated by neutrophils to the adaptive immune response mediated by T-lymphocytes.

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Keywords

Logistic models; Infant; premature; Predictive value of tests

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a major cause of morbidity in premature infants.¹ The pathogenesis of BPD is multifactorial, involving factors affecting the severity and management of respiratory distress syndrome, alterations in lung development and maturation, and genetic predisposition.² Cytokines mediate acute lung injury,³ exacerbate ventilator-associated lung injury,⁴ and modulate host defense⁵ while also participating in normal lung development. Elevated cytokine concentrations are observed in the tracheal aspirate^{6,7} and serum^{8,9} of infants with respiratory distress syndrome who subsequently develop BPD. These cytokines may initiate or augment the inflammatory cascade, and thereby predispose to the development of BPD.

Most studies of cytokines in premature infants have focused on a few cytokines from limited numbers of infants. There is currently limited knowledge on which of the many known cytokines mediate normal lung development. Infants who die or develop BPD may demonstrate alterations in the postnatal temporal profile of these critical cytokines, either due to causal involvement of the cytokine in lung injury and subsequent development of BPD, or due to the cytokine serving as a marker of lung injury and inhibited lung development.

Our objective in this study was to develop multi-variable logistic regression models for the outcome of BPD and/or the competing outcome of death at 36w post-menstrual age using clinical and cytokine data from the first 28 days. It was hypothesized that pro-inflammatory cytokines (e.g. IL-1 β , -6, and -8) would be elevated in premature infants who died or developed BPD, while infants who survive without BPD would demonstrate a decline over time of these cytokines.

PATIENTS AND METHODS

This study was a secondary analysis of data from a prospective cohort study (the “Cytokine study”) performed in the 17 centers of the NICHD Neonatal Research Network. Infants 401–1000 g at birth of both genders and all racial/ethnic groups were screened for eligibility. Infants were excluded if they were >72 hours of age. This study was approved by the Institutional Review Boards at participating centers, and written informed consent was obtained from the parent(s). Whole blood spots on filter paper were collected within four hours of birth (day 0), and on days 3 $\pm$ 1, 7 $\pm$ 1, 14 $\pm$ 3, and 21 $\pm$ 3 (date of sampling $\pm$ range of days within which sample could be obtained), and immediately frozen. Clinical data were collected by trained research coordinators, and all analyses were performed at a central data coordinating center (RTI International). The stored blood spots were analyzed in a batch for 25 cytokines (Table 3) using a multiplex Luminex assay (Luminex Corp., Austin, TX) as described previously.^{10, 11} This multiplex assay using approximately 3 microliters of whole blood from stored blood spots has been validated, has low intra-assay (<10%) and inter-assay (7–23%) variation, and the lower limits of detection for this assay are lower than the median concentrations found in normal newborns.¹⁰ Storage of the blood spots appropriately does not change cytokine concentrations.¹⁰

The primary objective of the Cytokine study was to evaluate if early serum cytokine patterns would be useful in predicting cerebral palsy and neurodevelopmental outcome in ELBW infants. The emphasis was therefore on measurement of serum cytokines rather than on

profibrotic/angiogenic mediators or peptide growth factors important in lung development such as vascular endothelial growth factor, platelet derived growth factor, fibroblast growth factors (–2, –10), and epidermal growth factor.

BPD was defined as the use of supplemental oxygen at 36 weeks' post-menstrual age or discharge/transfer on oxygen prior to 36 weeks. As death is a competing outcome for BPD, the primary outcome of BPD or death by 36 weeks' post-menstrual age was used for this secondary study. Stepwise regression using backward elimination ($p > 0.2$ for exit) identified variables associated with BPD/Death, using 15 clinical variables noted by day 28 (Table 1) and peak values of the 25 cytokines (Table 3) as independent variables. Cytokine values were evaluated in all analyses as continuous variables. Multiple logistic regression analysis was done for BPD/Death using the variables selected by stepwise regression. Similar analyses were also done for Death alone and for BPD in survivors. Analyses for BPD/death were also performed using average cytokine values from 0–21 days.

Additional analyses were performed for BPD/Death, Death alone, and BPD in survivors using clinical and average cytokine data from 0–3 days (Early Cytokines Averages model) and data from 14–21 days (Late Cytokines Averages model). For the main analyses (BPD/death in relation to peak, early, or late cytokine models), gestational age, race, gender and center were forced into the model. The predictive abilities (r -squared [max rescaled] and c -statistic) of the models including cytokine data were compared to models including only clinical data (excluding cytokines).

Changes in cytokine concentrations over time in survivors without BPD and in infants who died or developed BPD were plotted using population median values in order to evaluate temporal profiles of the cytokines over the first three postnatal weeks.

RESULTS

Patient characteristics

1067 ELBW infants were enrolled in the study. The mean birth weight (SD) was 761 g (141g), gestational age was 25.8 weeks (2 weeks), 49% were male, and 48% were black. The majority had received antenatal steroids (76%) and were inborn (93%) (Table 2). Of 1062 infants with available data, 606 infants (57%) developed BPD or died. These 606 infants were comprised of 202 [19%] infants who died, and 404 [47%] survivors who developed BPD. The median age at death of infants who died was 19 days [mean 42 days; 9–52 days for 25th–75th centile; range 1–258 days]. Postnatal steroids were used in 25% of enrolled infants. The median age at which steroids were used was 24 days [mean 31 days, 16–40 days for 25th – 75th centile].

Unadjusted comparison of cytokines in infants

An unadjusted comparison of peak cytokine concentrations in infants who developed BPD or died to those who survived without BPD showed that many cytokines were significantly different between the two groups (Table 3). Some of these cytokines, including interleukin-5 (IL-5), c-reactive protein (CRP), monocyte chemoattractant protein (MCP)-1, transforming growth factor –beta (TGF- β), and others were no longer significant after adjustment for other cytokines and clinical variables (Table 4).

Peak cytokine values over days 0–21 in association with BPD/Death (Table 4)

BPD and/or Death were associated with higher peak concentrations of interleukin-1 β (IL-1 β) and interferon- γ (IFN- γ) and with lower interleukin-17 (IL-17) and tumor necrosis factor- β (TNF- β). Clinical variables associated with lower BPD/Death were female gender, days alive and ventilator-free by 28 days, not requiring IMV, and center. When only BPD in survivors

was evaluated, higher IL-1 β and lower IL-17 continued to be significant, as was lower BDNF (Online supplement, Table 1). Models including cytokines were more predictive than those with only clinical variables, but the magnitude of the improvement can be considered clinically modest (r-square 0.56 with cytokines vs. 0.53 without cytokines, c-statistic 0.89 vs. 0.88, $p < 0.001$).

Average cytokine values in association with BPD/Death (Online supplement, Table 2)

BPD and/or Death were associated with higher IL-1 β and lower IL-17 concentrations. Clinical variables associated with lower BPD/Death were female gender, non-black race, surfactant use, days alive and ventilator-free by 28 days, not requiring IMV, and center. The predictive ability of these models were the same as those with the peak cytokine values (r-square 0.56 vs. 0.53, c-statistic 0.89 vs. 0.88, $p < 0.002$).

Early cytokines (0–3 days) averages model (Table 5)

BPD and/or Death were associated with higher concentrations of IL-8 and IL-10 and lower Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES). Protective clinical variables (associated with lower BPD/Death) were female gender, greater gestational age, not being SGA, not requiring IMV, and center. When only BPD in survivors was evaluated, IL-8 continued to be significant (Online supplement, Table 3). Models including cytokines were more predictive than those with only clinical variables (r-square 0.43 vs. 0.38, c-statistic 0.83 vs. 0.82, $p < 0.01$).

Late cytokines averages model (Table 6)

BPD and/or Death were associated with higher IL-6 concentrations. Protective clinical variables were female gender, non-black race, absence of PIH, days alive and ventilator-free by 28 days, not requiring IMV, and center. When only BPD in survivors was evaluated, none of the cytokines were significant (Online supplement, Table 4). Models including cytokines were less predictive than those with only clinical variables (r-square 0.52 vs. 0.53, c-statistic 0.87 vs. 0.88, $p < 0.01$).

Temporal profiles (Figures 1–3)

When all the models were evaluated, the cytokines associated with BPD/Death when present at a higher concentration in any model were IL-1 β , IL-6, IL-8, IL-10, and IFN- γ . The cytokines associated with survival without BPD when present at a higher concentration were IL-17, RANTES, and TNF- β . The median values of these cytokines (un-adjusted for other variables) on day 0, 3, 7, 14, and 21 in survivors free of BPD and in those with BPD/Death were graphed in order to develop temporal profiles of these cytokines (Figure 1). The temporal profile of cytokines not associated with BPD or Death is shown in Figure 2. Marked overlap of cytokine concentrations was noted in infants with BPD/Death and in those surviving without BPD (This overlap is shown for IL-8 in Figure 3, and this pattern of overlap is representative of the overlap noted for other cytokines).

DISCUSSION

Higher serum concentrations of certain cytokines (IL-1 β , IL-6, IL-8, IL-10, IFN- γ) and lower concentrations of other cytokines (IL-17, RANTES, and TNF- β) were associated with the development of BPD/Death in ELBW infants, after adjustment for other clinical variables and concentration of other cytokines. Evaluation of the postnatal course of these and the other cytokines measured reveals complex temporal profiles that may be of physiological and pathophysiological significance. It is possible that these cytokines either serve as a marker for more severe lung injury or are involved in the pathogenesis of BPD. However, addition of

cytokine data did not add much predictive ability to models using only clinical data, suggesting that clinical variables (e.g. mechanical ventilation or its duration) may drive changes in cytokine concentrations rather than *vice versa*. The overall pattern of cytokines associated with BPD/death suggests that this outcome maybe associated with impairment in the transition from the innate immune response mediated by neutrophils to the adaptive immune response mediated by T-lymphocytes.

The strengths of this study include the relatively large sample of ELBW infants, recruited from multiple sites and prospective data collection by trained observers. In addition, rather than evaluation of a few empirically chosen cytokines at a single time-point, we measured multiple cytokines at different time-points and determined which cytokines were statistically associated with BPD/Death. The evaluation of approximately 1000 ELBW infants for 40 variables (15 clinical and 25 cytokine) at five time points (0, 3, 7, 14, and 21 days) yields about 200,000 data points. Due to the tremendous amount of data, these analyses have to be considered primarily exploratory and hypothesis-generating, similar to studies performed using microarrays. The determination of the postnatal temporal profile of these cytokines and the associations of some of these cytokines with BPD/death provides important data for hypothesis-generation and future studies. However, the degree of association of cytokines with outcome, after taking clinical variables into account, is modest and hence cytokine concentrations alone are unlikely to be useful in clinical prediction models.

A limitation is that serum concentrations may not accurately reflect concentrations of the cytokines in the lung, and it is not possible to identify which cells (if any) in the lung are producing or responding to the cytokines, or how these cytokine changes interact with innate immunity which is also developmentally regulated. However, such limitations also exist with tracheal aspirates or bronchoalveolar lavage, which reflect the epithelial lining fluid rather than the alveolar or interstitial milieu. Additional studies are required to determine the relationship between changes in serum cytokines and concomitant lung pathology. It is also possible that changes in some of the serum cytokines reflect the magnitude of lung injury, with more severe lung injury resulting in greater cytokine concentrations. Some cytokine responses may be attempts to compensate or attenuate the lung injury. Early cytokine changes may also reflect the effects of chorioamnionitis rather than lung injury. However, chorioamnionitis is a variable that is difficult to analyze as there is marked variability in the diagnosis of chorioamnionitis, with clinical chorioamnionitis demonstrating poor correlation to histologic chorioamnionitis which varies markedly in its magnitude and extent (deciduitis, amnionitis, funisitis etc). We were unable to evaluate the relationship of clinical and histological chorioamnionitis in relation to cytokines and BPD/death as data were not available for this variable during the years of the study. Postnatal infections may also result in differences in cytokines, but sepsis, multi-organ failure, and subsequent need for mechanical ventilation are confounders. It is known that there is a substantial genetic component to BPD,¹² and it is possible that polymorphisms in cytokine genes may lead to higher cytokine concentrations for the same degree of lung injury. Future studies evaluating the polymorphisms of the important cytokines identified in this study may indicate the contributory role of such genetic variation.

In general, during disease pathogenesis there is usually a relative predominance of either Type 1 (cell mediated immunity; Th1) or Type 2 (humoral immunity; Th2) cytokines.¹³ Immunity develops gradually during gestation, and there is a significant immaturity of the cellular immune response at birth.¹⁴ Our exploratory analyses indicate that infants who develop BPD or die have elevated IL-8 accompanied by a relative predominance of Th2 cytokines (IL-10, IL-6) in comparison to Th1 cytokines (TNF- β) or T-cell products (RANTES), although some Th1 cytokines (IL-1 β , IFN- γ) are also associated with BPD/death. Many of these cytokines have been evaluated in various experimental models, and we can develop hypotheses for their

roles in the development of BPD/death based on the existing literature of their functions in normal development or inflammation.

We analyzed both peak and average cytokine concentrations over the first three weeks as there was insufficient evidence to indicate whether peak or average concentrations would be more closely associated with outcome. Higher IL-1 β and lower IL-17 concentrations were significant in both sets of analyses. IL-1 β is a pro-inflammatory cytokine produced by activated macrophages and many other cell types.¹⁵ IL-17 is produced by a subset of effector CD4⁺ T cells (Th17), and regulates neutrophil production¹⁶ and tissue inflammatory reactions.¹⁷ IL-17 is also pro-angiogenic,¹⁸ and we speculate that a reduction in IL-17 may possibly inhibit angiogenesis and thereby attenuate alveolar development.¹⁹

BPD/Death was also associated with higher peak concentrations of IFN- γ and lower TNF- β . IFN- γ , a Th1 cytokine, contributes to hyperoxia-induced neutrophil recruitment and increased alveolar permeability.²⁰ IFN- γ over-expression leads to emphysema and a macrophage- and neutrophil-rich inflammation.²¹ However, increased IFN- γ reduces fibrosis,²² suggesting that some effects of IFN- γ may be compensatory and others contributory to fibrosis in the setting of lung injury. TNF- β , another Th1 cytokine, is produced mainly by T-lymphocytes²³ and mediates multiple effects²⁴ although its role in lung development or inflammation has not been determined. Higher IL-8 and IL-10, and lower RANTES concentrations in the first four days were the early cytokine associations with BPD/Death. IL-8 (CXCL-8), is produced by many leukocyte types as well as fibroblasts, endothelial cells, and epithelial cells in response to other cytokines, bacterial or viral products, and environmental stressors (e.g. reactive oxygen intermediates).²⁵ In adults with acute lung injury, IL-8 levels correlate with mortality and morbidity, and a greater reduction in plasma IL-8 is noted with smaller tidal volumes, indicating that volutrauma may be involved in IL-8 release.²⁶ IL-8 strongly attracts neutrophils, and antibodies to IL-8 or its receptor reduce neutrophil influx and lung injury in various experimental models.²⁵ RANTES (CCL5) is produced by CD8⁺ T-lymphocytes, epithelial cells, fibroblasts, and platelets.²⁷ RANTES is a late expressed gene in T-lymphocytes and leads to T-lymphocyte activation²⁸ and augmented cytolytic activity of Natural Killer cells.²⁹ The egress of effector CD8⁺ T cells from the lung vascular compartment to the interstitium is driven in part by RANTES.³⁰ We hypothesize that higher IFN- γ and IL-8 may contribute to neutrophil influx and pulmonary edema, and lower TNF- β and RANTES may be the result of a reduction in T-lymphocytes in infants who subsequently develop BPD or die. Genetic polymorphisms are known to increase RANTES production^{31,32} and we speculate that such polymorphisms may reduce the predisposition to BPD.

In the early cytokine model, higher IL-10 was associated with BPD/Death. IL-10, a Th2 cytokine, is generally considered an anti-inflammatory cytokine.³³ However, our data, as well as studies in adults³⁴ indicate that higher plasma IL-10 is associated with worse outcome. Our data support recent observations that IL-10 production within the first 3 days of life by lung inflammatory cells correlates with BPD.³⁵

In the late cytokine model, IL-6 was the only cytokine to be associated with BPD/Death. IL-6 is produced by stimulated monocytes, fibroblasts, and endothelial cells and has multiple biological activities.³⁶ IL-6 is produced at sites of inflammation and plays a key role in the transition from acute to chronic inflammation.³⁷ However, our analysis did not indicate an improvement in predictive ability when cytokine data was combined with clinical variables in the late cytokine model, suggesting that late changes in cytokines may be secondary to changes in clinical variables, such as the duration of mechanical ventilation.

The evaluation of the temporal profiles of cytokine concentrations indicates that certain cytokines that are statistically different by unadjusted analyses in relation to outcome (e.g.

CRP) may no longer be different when analyses are adjusted for clinical variables and other cytokines, while others (e.g. RANTES at 0–3 days) are associated with outcome although the serum concentration at those time points does not differ much between infants with bad and normal outcome. The marked overlap of serum cytokine concentrations between infants with good outcomes and those with bad outcomes indicates that prognosis cannot be based on single cytokine estimations at one time point. The early pattern of serum cytokines and clinical variables may help determine the magnitude of risk for BPD and be useful for recruitment of higher risk infants into future clinical studies. We are currently in the process of evaluating cytokine profiles in distinct patterns of BPD, primarily comparing infants without significant lung disease, with RDS alone, with atypical BPD (late oxygen requirement), and with classic BPD (higher oxygen requirement from soon after birth), in order to determine if these cytokine profiles provide additional information that may yield insight into BPD pathogenesis.

CONCLUSION

The pattern of cytokines suggests an increased early neutrophil influx, a relative decrease in effector T-cells, and impaired angiogenesis maybe associated with BPD/Death. At present it is not known which cytokine responses are contributory and which are compensatory in the setting of BPD, and subsequent basic research using specific inhibitors or agonists or transgenic animal models are required to determine the roles of these cytokines in lung development and injury. Identification of cytokines that contribute to BPD or death in ELBW infants may lead to therapeutic strategies directed against cytokines or their receptors.^{38,39}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BPD	Bronchopulmonary dysplasia
ELBW	extremely low birth weight
IL	interleukin
IFN	interferon

RANTES	Regulated upon Activation, Normal T-cell Expressed, and Secreted
TNF	Tumor necrosis factor

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Figure 1.

Temporal profile of cytokines associated with BPD and/or Death by multi-variable logistic regression analysis in ELBW infants. Values shown are median values, unadjusted for other cytokine or clinical variables.

Figure 2.

Temporal profile of cytokines not associated with the combined outcome of Death or BPD by multi-variable logistic regression analysis in ELBW infants. Values shown are median values, unadjusted for other cytokine or clinical variables.

Figure 3.

Figure showing temporal profile of IL-8 serum concentrations in infants who died or developed BPD (25th to 75th centiles enclosed by pink shading) and in infants who survived without BPD (25th to 75th centiles enclosed by green shading). The IL-8 expression profile as chosen as a representative example to demonstrate the marked overlap of cytokine concentrations in infants with BPD/Death and in those surviving without BPD.

Table 1

Clinical variables used and their definitions.

Variable	Definitions of clinical variables
Birth weight	Birth weight in grams
Gestational Age	Gestational age in completed weeks
SGA	Small for gestational age (below the 10 th percentile). This is determined by the combination of gender, gestational age, and birthweight.
Male gender	Male gender. No infants with ambiguous genitalia were noted.
Black race	Black, not Hispanic
Antenatal steroids	Administration of antenatal steroids to accelerate pulmonary maturity (≥ 1 dose)
PIH	Hypertension/pre-eclampsia/eclampsia
Outborn	Outborn status
C-section	Cesarean section (compared to those with vaginal vertex or breech)
Surfactants	Administration of surfactant to infant (≥ 1 dose)
1 min Apgar (median; 25–75 th)	A three-level variable was created, grouping together scores 0–2, 3–6, and 7–10.
5 min Apgar (median; 25–75 th)	A three-level variable was created, grouping together scores 0–2, 3–6, and 7–10.
Severe IVH	IVH Grades III (Blood/echodensity in ventricle AND ventricular size enlarged) and Grade IV (Blood/echodensity in parenchyma)
IMV	Use of either conventional or high frequency ventilator
Days alive and ventilator-free by day 28	Number of days alive and not on a ventilator, as estimated on day 28

Table 2

Table of patient characteristics (all enrolled infants who were evaluated for BPD/death).

Variable	Overall	Survivors without BPD	BPD and/or Death	p (for survivors without BPD vs. BPD/Death)
Birth weight (Mean±SD)	761 ± 141	804 ± 131	728 ± 138	<0.0001
Gestational Age (Mean±SD)	25.8 ± 2.0	26.6 ± 1.9	25.3 ± 1.8	<0.0001
SGA	14.2 %	19.6 %	10.1 %	<0.0001
Male gender	48.7 %	39.4 %	55.8 %	<0.0001
Black race	47.7 %	47.4 %	47.9 %	0.90
Antenatal steroids	75.9 %	78.4 %	73.9 %	0.10
PIH	24.5 %	31.3 %	19.3 %	<0.0001
Outborn	6.8 %	4.6 %	8.4 %	0.01
C-section	58.8 %	64.6 %	54.5 %	0.001
Surfactants	81.5 %	70.0 %	90.3 %	<0.0001
1 min Apgar (median; 25–75 th)	4 (2 – 6)	5 (3 – 7)	4 (2 – 6)	<0.0001
5 min Apgar (median; 25–75 th)	7 (6 – 8)	7 (6 – 8)	7 (5 – 8)	<0.001
Severe IVH	16.7 %	9.0 %	22.6 %	<0.0001
IMV	92.8 %	83.9 %	99.5 %	<0.0001
Days alive and ventilator-free by day 28	11.1 ± 11.0	18.4 ± 9.7	5.6 ± 8.5	<0.0001

P-values are from Fisher's exact test for categorical variables, and from unadjusted logistic regression for BPD or Death for continuous variables.

Table 3

Unadjusted Comparison of Survivors without BPD to Infants who died or developed BPD (variables significantly different shown in bold font).

Cytokines – peak values (pg/ml)	Survivors without BPD			Death or BPD			p-value*
	Median	Q1	Q3	Median	Q1	Q3	
Interleukin (IL) - 1β	211	92	566	191	92	502	0.23
IL-2	70	42	123	65	39	108	0.06
IL-8	3263	1727	6184	5456	2803	14428	<0.0001
Tumor Necrosis Factor (TNF)-α	89	59	155	86	56	157	0.59
Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES)	163078	110326	243153	131498	85615	209905	<0.0001
Brain-derived neurotrophic factor (BDNF)	10108	5929	16519	6631	3996	10904	<0.0001
C-reactive protein (CRP)	1201910	624065	2362919	1533115	814029	3252155	0.0002
Granulocyte macrophage colony stimulating factor (GM-CSF)	161	98	243	143	78	220	0.0009
Interferon (IFN)-γ	229	132	396	241	132	428	0.45
IL-4	167	112	252	154	94	239	0.03
IL-5	155	94	250	131	79	223	0.001
IL-6	169	94	312	211	113	499	<0.0001
IL-10	529	358	712	613	409	829	<0.0001
IL-12	161	108	237	149	102	235	0.15
IL-17	228	142	477	208	122	345	0.004
IL-18	2261	1576	3171	2313	1549	3313	0.49
Monocyte chemoattractant protein (MCP)	3419	2029	6400	4525	2653	6400	<0.0001
Macrophage Inflammatory Protein (MIP) 1α	257	161	409	280	167	461	0.20
MIP1β	1457	1099	2311	1429	979	2184	0.07
Matrix metalloproteinase (MMP)-9	2112184	1346118	3417132	2646961	1474197	4592850	0.002
Neutrophin (NT) 4	124	73	176	101	61	161	0.002
Soluble IL-6 receptor (sIL-6r)	2319414	1402635	4602881	2669324	1562480	5205577	0.03
Transforming growth factor (TGF)-β	2656	1867	3843	2247	1523	3114	<0.0001
TNF-β	392	211	767	374	203	711	0.30
TREMI	3620	2627	5132	3259	2306	4507	0.0002
Clinical variables	Median	Q1	Q3	Median	Q1	Q3	p-value*

	Survivors without BPD			Death or BPD			p-value*
	Median	Q1	Q3	Median	Q1	Q3	
Cytokines – peak values (pg/ml)							
Gestational Age	26	25	28	25	24	26	<0.0001
Birthweight	818	707	920	713	620	832	<0.0001
Days Alive and Vent-free through 28 d	22	10	26	0	0	9	<0.0001

* p-values are from Wilcoxon Two-Sample Test (t approximation).

Table 4
Peak cytokine values in relation to BPD or Death

All variables selected by stepwise regression at $p < 0.2$ are shown, and the variables significant at $p < 0.05$ by subsequent multivariable logistic regression are shown in bold.

BPD or Death – Peak Cytokine Values: Days 0–21			
N = 1056 (602 with outcome) R-square = 0.42 (0.56 max-rescaled)			
Variable	OR	95% CI	p-value
IL-1β – 1,000 pg/ml	2.42	1.32 – 4.44	<0.01
IL-2 – 100 pg/ml	0.86	0.72 – 1.03	0.10
RANTES – 100,000 pg/ml	0.92	0.82 – 1.04	0.19
BDNF – 10,000 pg/ml	0.90	0.81 – 1.01	0.07
GMCSF – 1,000 pg/ml	3.11	0.83 – 11.66	0.09
IFN-γ – 1,000 pg/ml	2.82	1.27 – 6.27	0.01
IL-10 – 1,000 pg/ml	1.22	0.92 – 1.60	0.16
IL-17 – 1,000 pg/ml	0.42	0.22 – 0.79	<0.01
TNF-β – 1,000 pg/ml	0.60	0.39 – 0.92	0.02
TREM1 – 10,000 pg/ml	0.64	0.35 – 1.17	0.15
Gender – Female vs Male *	0.57	0.41 – 0.81	0.001
Surfactant	0.64	0.38 – 1.10	0.11
Antenatal steroids	1.32	0.86 – 2.01	0.20
Race – Nonblack vs Black *	0.73	0.50 – 1.06	0.10
Days Alive and Vent-free through 28 d	0.87	0.85 – 0.89	<0.0001
IMV	7.02	1.88 – 26.19	<0.01
Center *	varies		<0.0001
GA *	1.00	0.89 – 1.12	0.98

Odds Ratios (OR) are expressed per magnitude of change of the variable (e.g. IL-1 β -1,000 pg/ml indicates that OR are per 1,000 pg/ml change in IL-1 β concentration;

* : variables forced into model.

Table 5
Early Cytokines in relation to BPD or Death: Cytokines in model are averages among all samples within 0–3 days

All variables selected by stepwise regression at $p < 0.2$ are shown, and the variables significant at $p < 0.05$ by subsequent multivariable logistic regression are shown in bold.

BPD or Death – Early Cytokines Averages (0–3 Days)			
N = 1003 (577 with outcome) R-square = 0.32 (0.43 max-rescaled)			
Variable	OR	95% CI	p-value
IL-8 – 100,000 pg/ml	14.98	1.29 – 173.9	0.03
RANTES – 100,000 pg/ml	0.72	0.56 – 0.91	<0.01
BDNF – 10,000 pg/ml	0.87	0.73 – 1.02	0.09
IL-10 – 1,000 pg/ml	2.03	1.11 – 3.72	0.02
MMP-9 – 1,000,000 pg/ml	1.06	0.99 – 1.13	0.12
TREM1 – 10,000 pg/ml	0.53	0.27 – 1.05	0.07
Gestational Age *	0.62	0.55 – 0.69	<0.0001
SGA	1.87	1.09 – 3.19	0.02
Gender – Female vs Male *	0.54	0.40 – 0.74	0.0001
IMV	13.61	3.97 – 46.59	<0.0001
Race – Nonblack vs Black *	0.82	0.58 – 1.15	0.24
Center *	varies		<0.0001

Odds Ratios (OR) are expressed per magnitude of change of the variable (e.g. IL-1 β -1,000 pg/ml indicates that OR are per 1,000 pg/ml change in IL-1 β concentration;

* : variables forced into model.

Table 6
Late Cytokines in relation to BPD or Death: Cytokines in model are averages among all samples within 14–21 days

All variables selected by stepwise regression at $p < 0.2$ are shown, and the variables significant by subsequent multivariable logistic regression at $p < 0.05$ are shown in bold.

BPD or Death – Late Cytokines Averages (14–21 Days)			
N = 830 (453 with outcome) R-square = 0.39 (0.52 max-rescaled)			
Variable	OR	95% CI	p-value
BDNF – 10,000 pg/ml	0.89	0.77 – 1.02	0.10
IFN- γ – 1,000 pg/ml	1.64	0.70 – 3.83	0.25
IL-6 – 1,000 pg/ml	2.42	1.11 – 5.29	0.03
IL-17 – 1,000 pg/ml	0.58	0.22 – 1.55	0.28
TREM1 – 10,000 pg/ml	0.46	0.18 – 1.16	0.10
Gender – Female vs Male *	0.55	0.38 – 0.80	0.002
Surfactant	0.66	0.38 – 1.15	0.14
Race – Nonblack vs Black *	0.66	0.44 – 0.98	0.04
Days Alive and Vent-free through 28 d	0.88	0.86 – 0.90	<0.0001
PIH	1.63	1.02 – 2.61	0.04
IMV	8.59	1.83 – 40.28	0.006
GA *	0.99	0.88 – 1.13	0.93
Center *	Varies		<0.0001

Odds Ratios (OR) are expressed per magnitude of change of the variable (e.g. IL-1 β -1,000 pg/ml indicates that OR are per 1,000 pg/ml change in IL-1 β concentration;

* : variables forced into model.