Original Research

Serum cytokine concentrations, chorioamnionitis and the onset of bronchopulmonary dysplasia in premature infants.

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Running Title: Cytokines, CAM, and BPD

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List of abbreviations: BPD, bronchopulmonary dysplasia; CAM, chorioamnionitis; EGF, epidermal growth factor; FGF, fibroblast growth factor; FiO_2, fraction of inspiratory oxygen; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; IP, interferon gamma-induced protein; MCP, monocyte chemotactic protein; MIG, monokine induced by interferon-γ; MIP, macrophage inflammatory protein; NBPD, free from bronchopulmonary dysplasia; NCAM, free from chorioamnionitis; PIH, pregnancy induced hypertension; PIP, peak inspiratory pressure; PROM, premature rupture of membrane; RANTES, regulated on activation normal T-cell expressed and secreted; RDS, respiratory distress syndrome; TNF, tumor necrosis factor; VGEF, vascular endothelial growth factor.

ABSTRACT

Objective: To investigate the relationships between serum cytokine levels and chorioamnionitis (CAM) and CAM-related bronchopulmonary dysplasia (BPD) in premature infants.

Methods: Serum was collected at 0 and 7 days after birth from 36 premature infants born at < 32 weeks of gestation. We examined the relationships between 30 cytokine levels and CAM, BPD, and other perinatal factors.

Results: On day 0, GM-CSF, IL-15, IL-17, IL-2, IL-2R, VEGF, and MIG levels were significantly higher in the CAM group (n = 17) than in the non-CAM group (n = 19). These levels had decreased by day 7 and were similar in both groups. The IL-12p70 level on day 0 was significantly lower in the BPD group (n = 16) than in the non-BPD group (n = 15). BPD incidence was similar between the CAM and non-CAM groups.

Conclusions: These data support the hypothesis that intrauterine inflammation is not a primary risk factor for BPD. The immunological environment at birth or soon after, rather than intrauterine fetal inflammation (e.g., CAM), is a primary risk factor for BPD onset in preterm infants. Decreased inflammatory responses are particularly relevant, as indicated by the relationship between BPD and low serum IL-12p70 levels on day 0.

Introduction

Several studies have investigated the factors underlying the onset of bronchopulmonary dysplasia (BPD) in premature infants, and the suggested pathogenic pathways for BPD include lung immaturity in addition to hypercytokinemia in the lungs. Hypercytokinemia is triggered by intrauterine inflammation and causes arrested development of the alveoli of the fetus [1]. In premature infants born at < 32 gestational weeks, the alveoli formation begins and is incomplete; chorioamnionitis (CAM) [2], tissue apoptosis, and the accumulation and activation of inflammatory cells in the lungs due to various inflammatory cytokines are possible contributors to BPD.

CAM presents with elevated inflammatory cytokine and chemokine levels in umbilical cord blood [3–5]. In contrast, previous studies have shown that prenatal inflammatory processes such as CAM protect the fetus from respiratory distress syndrome (RDS), which is a risk factor of BPD [6–8].

The aim of this study was to determine whether changes in cytokine levels associated with CAM influence the development of BPD and to determine whether cytokine levels influence the development of BPD even in the absence of CAM.

Methods

Subjects: Premature infants who were born at < 32 weeks of gestation between April 2008 and March 2013 at our institution and underwent mechanical ventilation management after birth were included in this study. Infants were excluded if they had chromosomal abnormalities, had congenital major organ malformations, or were transferred to another hospital before they were 7 days old.

Ethical considerations: All protocols in the present study were approved by the ethics committee of Fukushima Medical University. At the time of participation, both parents of the infants received a written explanation in advance, and only infants whose parents provided written consent were included. The personal information of all patients was collected anonymously and separated from any personal identifying information.

Definitions: Placental pathological diagnoses were conducted by an independent pathologist, and the histological criterion for CAM was the presence of accumulated leukocytes extending through the fetal membranes according to the recommendations of Blanc [9]. Using the same criteria, the severity of CAM was determined based on the degree of maternal polymorphonuclear lymphocyte infiltration into the subchorionic space (stage I), intervillous space (stage II), or amniotic cavity (stage III). RDS was comprehensively diagnosed by a clinical attending physician based on symptoms of respiratory distress after hospitalization, chest radiography, and a microbubble test of gastric aspirate. Subjects who received surfactant replacement therapy were also considered to have RDS. BPD was defined according to the National Institute of Child Health and Human Development guidelines [10], i.e., when a respirator or oxygen was required to maintain SpO_2 at 94–96% for at least 36 weeks after birth; we did not perform oxygen reduction tests.

Clinical information: Clinical data were retrieved retrospectively from medical records. Premature rupture of membrane, pregnancy-induced hypertension, caesarian section, prenatal steroid administration, and CAM were included as maternal factors. Perinatal information included sex; gestational age; birth weight; fetal growth restriction; onset of RDS; inhaled steroid therapy at < 7 days of age; maximum fraction of inspiratory oxygen and peak inspiratory pressure during day 0; onset of BPD; and death at < 36 weeks postmenstrual age.

Serum cytokine concentration measurement: Serum was collected from the infant subjects for serum cytokine concentration measurements using 0.3 mL of venous blood that was sampled for medical purposes within 1 hour after birth (0 days), to reflect the intrauterine environment, and 7 days after birth. The collected blood was immediately centrifuged at 4000 rpm for 10 min, and 50 μL of serum was transferred to a serum tube, following which it was frozen and stored at -80°C until cytokine concentrations were measured.

Cytokine concentrations were measured by flow cytometry using the Luminex® 200™ System (Luminex Corporation, Austin, TX, USA) in accordance with a previous report [11]. In brief, antibodies of each of the cytokines were bound to carboxylated beads, washed, and then labeled by binding to biotinylated antibodies using the Human Thirty-Plex Antibody Bead Kit (Invitrogen Corporation, Carlsbad, CA, USA). This kit can detect the following 30 cytokines simultaneously in a single sample: epidermal growth factor (EGF), eotaxin, fibroblast growth factor (FGF)-basic, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), hepatocyte growth factor (HGF), interferon (IFN)-α, IFN-γ, interleukin (IL)-10, IL-12p70, IL-13, IL-15, IL-17, IL-1β, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), monocyte chemoattractant protein (MCP)-1, monokine induced by interferon-γ (MIG), macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation normal T-cell expressed and secreted (RANTES), interferon gamma-induced protein (IP)-10, and IL-1Ra. Cytokine concentrations (pg/mL) were determined from standard curves prepared on each plate.

Statistics: The subjects were initially divided into two groups: the CAM group, including infants from mothers who had CAM, and the NCAM group, including infants from mothers free of CAM. Then, patients who died were excluded from the sample, and to investigate the possibility that different cytokines were involved in BPD onset, the subjects were divided into two groups: the BPD group, including infants with BPD onset, and the NBPD group, including infants without BPD onset. The data were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Categorical or continuous data were compared between the two groups using chi-square tests or Mann-Whitney *U* tests, respectively; the comparisons included perinatal factors as well as serum cytokine concentrations at 0 days and 7 days of age. Because of the number of cytokines investigated, the differences in cytokine concentrations between the groups were adjusted using Bonferroni correction (the corrected *p*-values were 30 fold that of the crude *p*-value). Differences in serum cytokine concentrations were compared between 0 and 7 days of age using paired Student's *t*-tests. Stepwise logistic regression analysis was subsequently used to test the ability of a single cytokine to

predict the risk of BPD. The other risk factors evaluated were gestational age, fetal growth restriction, sex, and severity of respiratory distress (maximum fraction of inspiratory oxygen and maximum peak inspiratory pressure during day 0). The level of significance was set at $p < 0.05$.

Results

Subjects: Of the 48 premature infants born at < 32 weeks of gestation at our institution during the study period, 43 underwent mechanical ventilation management in the neonatal intensive care unit. We excluded one infant with a chromosomal abnormality, two with congenital major organ malformations, and four whose parents chose not to participate in the study, resulting in 36 subjects (18 male infants, 50.0%) included in the analyses.

Of the maternal factors assessed, premature rupture of the membrane occurred in nine (25.0%) mothers, pregnancy-induced hypertension in five (13.9%), and caesarian section in 32 (88.9%). Prenatal steroids were used by 22 (61.1%) mothers.

The median gestational age and birth weight were 25.9 weeks (range, 23.3–31.9 weeks) and 859 g (374–1670 g), respectively. RDS occurred in 34 (94.4%) infants, and 22 (61.1%) infants received inhaled steroid therapy. There were five deaths at < 36 weeks after birth.

Perinatal information: The CAM group included 17 (47.2%) infants, and the NCAM group included 19 (52.8%) infants. After excluding the five infants who died (n = 2, CAM; n = 3, NCAM), BPD occurred in 16 (51.6%) infants; the incidence of BPD was similar between the CAM and NCAM groups. Significant differences were observed between the BPD and NBPD groups with respect to gestational age and birth weight (Table 1).

Serum cytokine concentrations: Compared with the NCAM group, the CAM group had significantly higher values for 22 cytokines at 0 days of age: FGF-basic, G-CSF, GM-CSF, IFN-α, IL-13, IL-15, IL-17, IL-1β, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, VEGF, MCP-1, MIG, MIP-1α, RANTES, IP-10, and IL-1Ra. Moreover, the levels of seven cytokines (GM-CSF, IL-15, IL-17, IL-2, IL-2R, VEGF, and MIG) remained higher in the CAM group after Bonferroni correction. However, by 7 days of age, the levels of all of these cytokines had decreased and were similar in both groups (Table 2). Of the 22 cytokines with higher concentrations in the CAM group at 0 days of age, the concentrations of 12 (G-CSF, IFN-α, IL-15, IL-2R, IL-5, IL-6, IL-7, IL-8, VEGF, MCP-1, IP-10, and IL-1Ra) were higher in stage-III CAM than in the less severe stages (I or II); however, this difference did not reach statistical significance (Table 3). At 0 days of age, the IL-12p70 concentration was significantly lower in the BPD group than in the NBPD group. At 7 days of age, IL-12p70 levels had decreased in both groups (both, $p < 0.001$). Although the IL-12p70 concentration remained lower in the BPD group at 7 days of age, this was not significant after Bonferroni correction. Eotaxin, IL-13, IL-2R, MIG, and IL-1Ra concentrations were also lower in the BPD group at 7 days of age, and these differences were also insignificant after Bonferroni correction. There were no other significant differences between the two groups (Table4). High IL-12p70 concentrations at day 0 decreased the risk of BPD after adjusting for potential confounders (odds ratio, 0.98; 95%

confidence interval, 0.96–0.99; $p = 0.015$) as well as the gestational ages (Table 5). Because the gestational age and birth weight were lower in the BPD group than in the NBPD group, correlations between neonatal serum IL-12p70 concentration and gestational age and birth weight were examined using regression analysis. Statistically significant correlations were not present at 0 days of age (gestational age, $R^2 = 0.071$, $p = 0.146$; birth weight, $R^2 = 0.094$, $p = 0.094$).

Discussion

In previous studies using umbilical cord blood, CAM was associated with significantly higher levels of pro-inflammatory cytokines, such as IL-6 [3, 5], IL-8 [3, 5], TNF-α [4], G-CSF [5], and IL-1β [12]. These pro-inflammatory cytokines, elevated by CAM, are considered to enhance the inflammatory cascade in the alveoli and represent the primary factor for the development of BPD [13, 14]. However, Paananen *et al.* [5] reported that transient inflammation associated with CAM was reversible by the 24th hour after birth. Several studies have reported that CAM decreases the risk of RDS; therefore, because RDS is considered a risk factor for BPD, CAM might be considered protective against BPD [6–8]. Furthermore, Kaukola *et al.* [8] reported that extensive analysis of inflammatory biomarkers in cord blood at birth failed to reveal any relationships between common inflammatory cytokines and BPD. In our study, by investigating the concentrations of 30 cytokines/chemokines in premature infants born at < 32 weeks of gestation, we confirmed that during the first day of life, infants in the CAM group had significantly higher levels of seven of the 30 cytokines. Therefore, that inflammation in the uterus leads to systemic inflammation in preterm infants with CAM. However, this inflammation is transient and no longer present on day 7, as evidenced by decreased concentrations of all seven cytokines. At this point, the levels were similar between the two groups, and the rate of subsequent BPD onset was also similar between the two groups.

The BPD group had lower serum IL-12p70 concentrations at 0 days compared with those without BPD, and these results were the same in both the CAM group and the non-CAM group. Moreover, this tendency was also observed on day 7, an observation that has not been reported previously. In a previous report, Kaukola *et al.* [8] failed to demonstrate an association between BPD and IL-12p70 levels. In the present study, all of the infants underwent mechanical ventilation management, and the majority of the infants showed signs of RDS (34 of 36 subjects, 94.4%), in contrast to 64.4% incidence of RDS in the participants in the study by Kaukola *et al*. We therefore speculated that the severity of CAM or BPD in the present study was different, despite similar definitions of BPD.

IL-12p70 is produced by macrophages [15], activates natural killer cells to induce IFN-γ production [16], and is believed to have an important role in protective immunity against intracellular microbes, defending the body from predators [17]. In neonates, the immune response is dominated by Th2 cell differentiation, and appropriate stimuli and bacterial exposure from the environment, in association with growth, is thought to stimulate macrophages to secrete IL-12p70. IL-12 then inhibits the Th2 immune response, induces Th1 cell differentiation, and establishes the Th1/Th2 cell balance [18]. We speculated that the lower serum IL-12p70 levels observed in the BPD group resulted in inadequate inhibition of Th2

cytokines, producing an abnormal cytokine balance and leading to the onset of BPD. Similarly, Thompson *et al.* [19] reported that an abnormal Th1/Th2 cytokine balance in lung tissue contributes to the onset of BPD. In the present study, there were no correlations between serum IL-12p70 and gestational age and birth weight on the day of birth. In a study of 927 premature infants, gestational age was not related with umbilical cord blood IL-12 levels [20], and there was no difference in IL-12 levels in umbilical cord blood between premature and full-term infants [21]. Therefore, the lower IL-12p70 levels associated with BPD in the present study may not have been caused by younger gestational age.

The present study has several limitations. First, the sample size was small, limiting the statistical power of the findings. In the present study, of the 22 cytokines with higher concentrations in patients with CAM, 12 cytokines were higher in those with stage-III CAM than in those with less severe CAM. However, we were unable to perform statistical analyses to determine the significance of these differences. Future studies should investigate the relationship between the severity of CAM and cytokines, because the severity of CAM is associated with the severity of BPD [22]. Second, because the serum samples were from premature infants, they were reflective of systemic inflammation in neonates, but not necessarily of pulmonary inflammation. None of the serum concentrations of the seven cytokines that were higher in CAM were associated with the onset of BPD, and we were unable to demonstrate a link between systemic/pulmonary inflammation and BPD onset in this study.

Conclusion

In this study with premature infants, the concentrations of several inflammatory cytokines were higher in the patients with CAM. However, these concentrations were elevated only transiently (for a few days), and there were no differences in the cytokine concentrations according to BPD onset. However, serum IL-12p70 concentrations at birth were significantly lower in infants with BPD, suggesting that a decrease in the fetal or neonatal immune response might be associated with the onset of BPD in these infants, but that intrauterine inflammation is not a key factor in the development of BPD. Further studies should be conducted with larger samples to determine if a persistent decrease in IL-12p70 contributes to the development of BPD.

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Conflict of Interest

The authors report no potential conflicts of interest.

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Table 1. Comparison of clinical backgrounds between premature infants with CAM and without CAM (NCAM), and with BPD and without BPD (NBPD).

	CAM (n = 17)	NCAM (n = 19)	*p-value*	BPD (n = 16)	NBPD (n = 15)	*p-value*
Maternal Factors						
PROM	5 (29.4)	4 (21.1)	0.563	4 (25.0)	3 (20.0)	0.739
PIH	2 (11.8)	3 (15.8)	0.727	3 (18.8)	2 (13.3)	0.682
Caesarian section	16 (94.1)	16 (84.2)	0.345	15 (93.8)	13 (86.7)	0.505
Prenatal steroids	10 (58.8)	12 (63.2)	0.790	10 (62.5)	9 (60.0)	0.886
Histological CAM				6 (37.5)	9 (60.0)	0.210
Perinatal Factors						
Male sex	8 (47.1)	10 (52.6)	0.738	9 (56.3)	5 (33.3)	0.200
Gestational age (weeks)	25.1 (23.3–31.6)	27.0 (23.4–31.9)	0.074	24.8 (23.4–31.1)	28.1 (23.3–31.9)	0.006
Birth weight (g)	830 (526–1670)	1045 (374–1656)	0.196	737 (374–1440)	1184 (594–1670)	0.002
Fetal growth restriction	2 (11.8)	4 (21.1)	0.455	4 (25.0)	2 (13.3)	0.411
RDS	16 (94.1)	18 (94.7)	0.935	16 (100.0)	13 (86.7)	0.131
Steroid inhalation	9 (47.4)	13 (76.5)	0.074	11 (68.8)	7 (46.7)	0.213
Maximum FiO_2 during day 0 (%)	43 (25–100)	40 (21–100)	0.731	45 (25–100)	35 (21–100)	0.313
Maximum PIP during day 0 (cmH_2O)	22 (18–25)	20 (18–30)	0.909	22 (18–26)	20 (18–25)	0.131
BPD	6 (35.3)	10 (52.6)	0.292			
Death at PMA < 36 weeks	2 (11.8)	3 (15.8)	0.727			

Values are reported as n (%) or median (range). The data were compared between groups using χ-square tests or Mann-Whitney *U* tests. Deaths at < 36 weeks PMA were excluded in the comparisons between BPD and NBPD.

PROM, premature rupture of membrane; PIH, pregnancy induced hypertension; CAM, chorioamnionitis; RDS, respiratory distress syndrome; FiO_2, fraction of inspiratory oxygen; PIP, peak inspiratory pressure; BPD, bronchopulmonary dysplasia; PMA, postmenstrual age.

Table 2. Comparison of neonatal serum cytokine concentrations (pg/mL) between premature infants with CAM and without CAM (NCAM).

	Age (days)	CAM (n = 17)	NCAM (n = 19)	crude *p*	corrected *p*
EGF	0	45.8 (15.5–327.2)	38.0 (13.9–188.1)	0.501	
	7	45.5 (13.9–170.7)	44.5 (2.3–202.6)	0.619	
Eotaxin	0	30.0 (7.9–109.3)	28.8 (5.9–131.2)	0.447	
	7	32.6 (17.3–85.1)	40.7 (14.2–208.0)	0.430	
FGF-basic	0	28.9 (18.7–59.1)	22.3 (13.7–28.4)	0.024	0.708
	7	25.3 (13.7–39.1)	26.8 (15.2–45.4)	0.895	
G-CSF	0	615.1 (12.2–13774.5)	32.7 (12.2–413.0)	0.003	0.101
	7	49.9 (8.6–214.9)	51.1 (45.2–145.4)	0.142	
GM-CSF	0	21.4 (21.2–22.7)	21.3 (21.0–21.4)	0.001	0.035
	7	18.2 (18.2–21.3)	18.2 (17.2–18.5)	0.135	
HGF	0	533.1 (95.2–3400.0)	503.7 (85.1–3400.0)	0.715	
	7	614.0 (39.6–2557.5)	420.8 (112.6–1318.7)	0.099	
IFN-α	0	72.4 (0.0–162.2)	13.7 (0.0–99.0)	0.012	0.366
	7	0.0 (0.0–204.9)	0.0 (0.0–175.2)	0.492	
IFN-γ	0	0.0 (0.0–26.2)	0.0 (0.0–1.7)	0.056	
	7	2.7 (0.0–9.5)	3.1 (0.6–9.0)	0.872	
IL-10	0	34.0 (27.7–496.2)	31.8 (27.2–636.9)	0.313	
	7	12.4 (12.4–29.0)	12.4 (12.4–12.5)	0.061	
IL-12p70	0	570.4 (235.9–993.6)	464.8 (163.0–983.7)	0.198	
	7	197.0 (113.2–539.4)	335.4 (90.6–512.7)	0.102	
IL-13	0	34.2 (31.8–36.5)	32.4 (31.8–37.4)	0.048	1.430
	7	37.3 (31.8–39.9)	37.7 (35.9–47.2)	0.380	
IL-15	0	31.2 (0.0–221.1)	0.0 (0.0–38.5)	<0.001	0.014
	7	30.6 (0.0–99.5)	27.0 (0.0–61.2)	0.229	
IL-17	0	72.2 (71.8–73.3)	71.8 (71.5–72.2)	<0.001	0.013
	7	4.9 (44.5–71.7)	4.8 (44.1–50.9)	0.201	
IL-1β	0	22.1 (17.3–229.1)	19.7 (16.1–33.0)	0.012	0.355
	7	0.0 (0.0–91.4)	0.0 (0.0–83.6)	0.261	
IL-2	0	27.5 (27.1–31.3)	27.1 (26.8–27.9)	0.001	0.032
	7	0.0 (0.0–27.1)	0.0 (0.0–0.9)	0.238	
IL-2R	0	519.0 (277.8–859.2)	344.3 (28.3–572.4)	<0.001	0.019

	7	525.1 (198.87–1086.0)	590.6 (84.9–3457.3)	0.792	
IL-4	0	73.9 (68.5–83.6)	71.9 (66.1–73.5)	0.039	1.168
	7	72.4 (66.9–78.1)	71.7 (65.5–75.5)	0.402	
IL-5	0	12.2 (10.6–37.0)	10.6 (10.2–11.6)	0.002	0.066
	7	0.5 (0.0–10.6)	0.0 (0.0–5.8)	0.307	
IL-6	0	324.4 (12.6–5150.0)	47.1 (10.3–315.5)	0.010	0.298
	7	9.0 (0.0–400.6)	0.6 (0.0–162.1)	0.485	
IL-7	0	50.8 (0.0–121.1)	16.0 (0.0–63.4)	0.007	0.209
	7	4.5 (0.0–87.4)	0.0 (0.0–75.0)	0.124	
IL-8	0	641.2 (61.9–24756.6)	217.0 (50.6–9234.5)	0.044	1.310
	7	151.8 (43.3–27296.3)	196.1 (57.4–2432.7)	0.413	
TNF-α	0	7.3 (5.8–35.8)	6.8 (5.8–17.4)	0.180	
	7	0.0 (0.0–48.3)	0.0 (0.0–124.2)	0.285	
VEGF	0	11.8 (1.7–67.1)	3.4 (1.5–11.5)	0.001	0.032
	7	4.9 (1.5–20.3)	4.6 (1.4–16.7)	0.559	
MCP-1	0	1195.1 (559.0–58776.9)	814.0 (189.1–10820.9)	0.026	0.789
	7	720.3 (286.5–3521.6)	792.0 (360.1–1753.3)	0.128	
MIG	0	20.3 (9.3–61.9)	14.9 (0.0–29.6)	0.001	0.034
	7	19.3 (9.3–29.2)	21.8 (15.7–37.7)	0.207	
MIP-1α	0	67.6 (44.0–1729.5)	47.7 (42.5–739.1)	0.007	0.223
	7	65.6 (41.7–8240.8)	63.9 (61.1–1510.1)	0.520	
MIP-1β	0	187.1 (61.5–2606.6)	166.3 (33.2–1475.8)	0.413	
	7	173.3 (47.5–12368.7)	141.7 (45.6–1955.4)	0.501	
RANTES	0	10792.4 (1584.3–32623.3)	7515.5 (1317.8–32623.3)	0.038	1.127
	7	7368.2 (2096.2–43255.3)	6907.1 (559.3–69373.2)	0.792	
IP-10	0	40.8 (12.5–277.9)	17.2 (9.7–88.9)	0.002	0.064
	7	16.1 (8.8–91.0)	24.4 (10.0–50.7)	0.599	
IL-1Ra	0	1084.6 (289.6–6650.0)	448.8 (179.2–2313.5)	0.012	0.373
	7	291.8 (119.1–1794.9)	293.7 (101.3–671.5)	0.661	

Values are reported as median (range). The data were compared between groups using Mann-Whitney U tests.

CAM, chorioamnionitis; EGF, epidermal growth factor; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; MCP, monocyte chemoattractant protein; MIG, monokine induced by interferon-γ; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T-cell expressed and secreted; IP, interferon gamma-induced protein.

Table 3. Comparison of neonatal serum cytokine concentrations (pg/mL) between mild and severe CAM at day 0.

	CAM severity		
	not severe (stage I or II) (n = 6)	**severe (stage III) (n = 11)**	***p*-value**
G-CSF	15.4 (12.2–4204.3)	927.3 (25.8–13774.5)	0.104
IFN-α	0.0 (0.0–155.2)	72.4 (0.0–162.2)	0.574
IL-15	0.0 (0.0–202.3)	43.6 (0.0–221.1)	0.383
IL-2R	399.7 (277.8–639.3)	605.8 (289.4–859.2)	0.052
IL-5	10.6 (10.6–14.9)	14.0 (10.6–37.0)	0.082
IL-6	13.7 (12.6–5150.0)	1462.0 (13.6–5150.0)	0.130
IL-7	13.0 (0.0–77.2)	58.4 (0.0–121.1)	0.082
IL-8	166.2 (61.9–9630.9)	747.9 (150.7–24756.6)	0.234
VEGF	3.9 (1.7–39.2)	22.8 (2.7–67.1)	0.064
MCP-1	816.6 (559.0–37020.2)	2682.4 (772.4–58776.9)	0.234
IP-10	30.3 (17.3–220.7)	52.3 (12.5–277.9)	0.879
IL-1Ra	453.8 (423.8–2428.0)	1663.9 (289.6–6650.0)	0.279

Values are reported as medians (ranges). The data were compared between groups using the Mann-Whitney *U* tests.

CAM, chorioamnionitis; EGF, epidermal growth factor; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; MCP, monocyte chemoattractant protein; MIG, monokine induced by interferon-γ; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T-cell expressed and secreted; IP, interferon gamma-induced protein.

Table 4. Comparison of neonatal serum cytokine concentrations (pg/mL) premature infants with BPD and without BPD (NBPD).

	Age (days)	BPD (n = 16)	NBPD (n = 15)	crude *p*	corrected *p*
EGF	0	52.2 (13.9–327.2)	45.4 (21.4–188.1)	0.306	
	7	45.3 (11.4–202.6)	45.9 (8.5–170.7)	0.826	
Eotaxin	0	27.2 (6.4–96.3)	30.7 (7.9–131.2)	0.483	
	7	28.5 (14.2–208.0)	51.0 (28.4–138.9)	0.007	0.197
FGF-basic	0	22.3 (13.7–59.1)	27.8 (18.7–45.9)	0.264	
	7	24.4 (13.7–33.1)	27.7 (21.2–45.4)	0.063	
G-CSF	0	44.3 (12.2–13774.5)	35.4 (12.2–13774.5)	0.437	
	7	50.5 (8.6–137.1)	51.1 (45.2–214.9)	0.490	
GM-CSF	0	21.3 (21.2–22.7)	21.4 (21.0–22.2)	0.639	
	7	18.2 (17.2–21.3)	18.2 (18.2–18.6)	0.238	
HGF	0	548.7 (187.2–3400.0)	484.8 (85.1–969.3)	0.559	
	7	387.4 (39.6–1139.4)	557.4 (112.6–2557.5)	0.128	
IFN-α	0	52.9 (0.0–162.2)	47.2 (0.0–155.2)	0.706	
	7	0.0 (0.0–175.2)	0.0 (0.0–204.9)	0.662	
IFN-γ	0	0.0 (0.0–25.8)	0.0 (0.0–26.2)	0.357	
	7	2.6 (0.0–9.5)	4.9 (1.3–9.3)	0.165	
IL-10	0	33.7 (27.5–496.2)	30.4 (27.2–262.3)	0.619	
	7	12.4 (12.3–29.0)	12.4 (12.3–15.4)	0.574	
IL-12p70	0	379.8 (188.7–702.9)	641.9 (235.9–993.6)	<0.001	0.009
	7	185.4 (113.2–512.7)	348.9 (140.7–539.4)	0.013	0.389
IL-13	0	32.9 (31.8–37.4)	32.9 (32.0–36.5)	0.446	
	7	37.0 (31.8–39.4)	37.8 (36.3–47.2)	0.018	0.534
IL-15	0	6.1 (0.0–221.1)	0.0 (0.0–100.3)	0.338	
	7	25.5 (0.0–82.8)	34.7 (0.0–99.5)	0.065	
IL-17	0	71.9 (71.5–72.5)	72.0 (71.6–73.3)	0.688	
	7	44.8 (44.2–71.7)	45.0 (44.5–50.9)	0.088	
IL-1β	0	20.3 (16.1–72.8)	20.9 (18.5–229.1)	0.171	
	7	0.0 (0.0–83.6)	0.0 (0.0–91.4)	0.395	
IL-2	0	27.3 (26.8–31.3)	27.3 (27.1–29.0)	0.608	
	7	0.0 (0.0–27.1)	0.0 (0.0–0.9)	1.000	
IL-2R	0	394.5 (28.3–830.3)	436.8 (248.2–859.2)	0.059	
	7	430.8 (210.1–1239.6)	673.9 (198.9–3457.3)	0.019	0.558

IL-4	0	72.3 (66.1–80.3)	73.5 (71.0–83.6)	0.129	
	7	71.7 (66.7–74.3)	73.0 (69.2–78.1)	0.130	
IL-5	0	11.1 (10.2–37.0)	11.1 (10.2–14.9)	0.891	
	7	0.0 (0.0–10.6)	0.5 (0.0–2.3)	0.379	
IL-6	0	82.0 (11.1–5150.0)	128.9 (12.5–5150.0)	0.629	
	7	10.6 (0.0–58.4)	3.2 (0.0–400.6)	0.796	
IL-7	0	27.8 (0.0-121.1)	21.7 (0.0–81.0)	0.548	
	7	0.0 (0.0–82.0)	0.0 (0.0–87.4)	0.265	
IL-8	0	355.1 (120.6–24756.6)	427.9 (50.6–17052.6)	0.579	
	7	182.0 (44.8–2432.7)	165.4 (43.3–27296.3)	0.704	
TNF-α	0	6.8 (5.8–23.7)	7.5 (6.3–35.8)	0.077	
	7	0.0 (0.0–124.2)	0.0 (0.0–48.3)	0.365	
VEGF	0	5.8 (1.5–67.1)	5.1 (1.7–45.3)	0.474	
	7	4.6 (1.5–18.2)	5.9 (1.6–20.3)	0.242	
MCP-1	0	1081.0 (189.1–58776.9)	1030.3 (550.7–37020.2)	0.815	
	7	749.1 (311.0–1666.3)	726.2 (286.5–3521.6)	0.539	
MIG	0	17.3 (0.0–61.9)	16.8 (9.3–43.3)	0.871	
	7	18.8 (9.3–28.8)	22.8 (16.8–37.7)	0.003	0.075
MIP-1α	0	50.9 (42.9–739.1)	57.8 (43.2–1729.5)	0.559	
	7	63.8 (41.7–1510.1)	66.2 (61.1–8240.8)	0.188	
MIP-1β	0	170.5 (61.5–1771.8)	184.5 (33.2–2606.6)	0.682	
	7	108.3 (47.5–1955.4)	202.5 (45.6–12368.7)	0.179	
RANTES	0	8189.8 (1317.8–32623.3)	9091.3 (4370.0–32623.3)	0.192	
	7	7031.3 (1463.6–20970.6)	6985.2 (2096.2–43255.3)	0.770	
IP-10	0	23.8 (9.9–277.9)	24.5 (9.7–133.9)	0.579	
	7	17.6 (10.0–54.1)	27.4 (8.8–91.0)	0.061	
IL-1Ra	0	473.8 (230.7–6650.0)	550.2 (374.4–6041.5)	0.099	
	7	242.0 (119.1–671.5)	338.9 (127.6–1794.9)	0.010	0.290

Values are reported as medians (ranges). The data were compared between groups using the Mann-Whitney *U* tests. Deaths at < 36 weeks post-menstrual age were excluded.

BPD, bronchopulmonary dysplasia; EGF, epidermal growth factor; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; MCP, monocyte chemoattractant protein; MIG, monokine induced by interferon-γ; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T-cell expressed and secreted; IP, interferon gamma-induced protein.

Table 5. Multiple logistic regression model showing independent predictors of BPD (n = 31).

Predictor of BPD	Odds Ratio (95% CI)	*p*-value
Gestational age (weeks)	0.66 (0.44-0.96)	0.048
IL-12p70 on day 0 of life	0.98 (0.96-0.99)	0.015

BPD, bronchopulmonary dysplasia; CI, confidence interval; IL-12p70, interleukin-12p70.