Blood Cytokines during the Perinatal Period in Very Preterm Infants: Relationship of Inflammatory Response and Bronchopulmonary Dysplasia

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Objective To evaluate the influence of chorioamnionitis (CA) on plasma cytokines and the cytokine-associated risk of bronchopulmonary dysplasia (BPD) during the perinatal period.

Study design Eleven cytokines from 128 very low gestational age infants were analyzed from cord blood and from plasma at ages 1 day and 7 days after birth. The diagnosis of CA was based on histology of the placenta, fetal membranes, and umbilical cord. Neonatal risk factors were recorded.

Results In the 48 infants born with CA, high concentrations of inflammatory cytokines in cord blood decreased during the first postnatal day. Inflammatory cytokines in cord blood was associated with the severity of CA. At 1 day after birth, the concentration of interleukin (IL)-8 predicted the risk of BPD. For the 75 infants born without CA, cytokine concentrations increased after birth. For the 128 infants born with or without CA, at 1 day after birth, the concentrations of IL-8, granulocyte colony-stimulating factor, and anti-inflammatory IL-10 were associated with the risk of BPD, after adjustment for the duration of gestation and severity of respiratory distress during the first day.

Conclusions In infants exposed to CA, insufficient inhibition of high fetal inflammatory cytokine response shortly after birth may increase the risk of BPD. *(J Pediatr 2009;154:39-43)*

Chorioamnionitis (CA) and a fetal inflammatory response are associated with bronchopulmonary dysplasia (BPD) and respiratory distress syndrome (RDS).[1-3] Very low gestational age (VLGA) infants exposed to CA have a decreased risk for RDS and a predisposition to BPD.[4-6] Within 1 to 4 days after birth, concentrations of inflammatory mediators (eg, cytokines, free radicals, proteases) increase in the lungs and air spaces of infants who develop BPD.[7-10] High blood cytokine levels after birth were detected in infants at risk for BPD.[5,11]

In intrauterine inflammation, the fetus is exposed to cytokines, endotoxins, and/or microbes commonly present in amniotic fluid, fetal membranes, and vessels of the chorionic plate.[12] In animal models, administration of cytokines or endotoxin into amniotic fluid has been shown to protect the fetus from respiratory failure at birth.[13-15] Prolonged intrauterine inflammation causes remodeling of small pulmonary arterial vessels.[16] Transgenic animals that overexpress specific proinflammatory cytokines develop simplified, large alveolar structures characteristic of BPD.[17,18] Retardation of vascular growth and inhibition of the formation of alveoli are major pathological features of the new BPD.[19]

We prospectively studied the relationship between blood cytokine concentrations and the risk of BPD in a population of VLGA infants. Placentas were prospectively evaluated, and concentrations of 11 cytokines recovered at birth, on day 1 of life, and on day 7 of life were measured.

METHODS

Study Population

The VLGA infants were born alive at Oulu University Central Hospital between November 1998 and November 2002. The parents signed written informed consent, and

BPD	Bronchopulmonary dysplasia	OI	Oxygenation index
CA	Chorioamnionitis	RDS	Respiratory distress syndrome
CI	Confidence interval	ROC	Receiver operating characteristic
G-CSF	Granulocyte colony-stimulating factor	VLGA	Very low gestational age
IL	Interleukin		

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Supported by the Sigrid Jusélius Foundation, Foundation for Pediatric Research Finland, and Biocenter Oulu. The authors declare no potential conflicts of interest, real or perceived.

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Submitted for publication Dec 21, 2007; last revision received Jun 5, 2008; accepted Jul 8, 2008.

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0022-3476/$ - see front matter

10.1016/j.jpeds.2008.07.012

the study design was approved by the hospital's Ethics Committee. Antenatal and neonatal risk factors were prospectively recorded. The diagnosis of BPD was made at 36 weeks' postmenstrual age. The infants with moderate or severe BPD received either supplemental O_2 to maintain oxygen saturation at 88% to 93% or continuous distending pressure to the airways. Severe BPD was defined as the need for $> 30\%$ of O_2 or mechanical ventilation. RDS was defined as typical chest x-ray findings or the need for either ventilation and supplemental oxygen for at least 48 hours or surfactant therapy for respiratory distress after the chest x-ray. The severity of respiratory distress was expressed as oxygenation index (OI): fraction of inspired $O_2 \times 100 \times$ mean airway pressure/arterial O_2 tension. OI during the first day of life was defined as the mean of the prospectively collected recordings.

Diagnosis of Intrauterine Inflammation

All placentas were fixed in neutral buffered formalin. The rim of membrane was taken from the site of membrane rupture. The umbilical cord specimens were taken from the fetal and placental sides of the umbilical cord and from midway between the sides of insertion. A full-thickness placental specimen was taken from midway between the umbilical cord insertion and the placental margin. Sections were stained with hematoxylin and eosin, and a single investigator (R.H.) blindly assessed the histology. CA was defined as the presence of polymorphonuclear leukocytes in the amnion and chorion decidua. The umbilical cord and the chorionic plate of the placenta also were studied. Each of the 3 tissues was graded for the degree of severity of inflammation based of the density of leukocytes.[20] Salafia grade 1 to 2 was defined as "mild" inflammation; grade 3, as "moderate" inflammation; and grade 4, as "severe" inflammation. The severity of CA was classified based the the most severe histological grade found in the umbilical cord, fetal membranes, or chorionic plate of the placenta.

Analysis of Cytokines

Cord blood was collected from a clamped umbilical cord artery, and arterial blood was collected at 24 hours and at 168 hours after birth. EDTA-plasma samples were separated by centrifugation and stored at $-70°C$ until analysis. Concentrations of the following cytokines were analyzed using the Cytometric Bead Array Kit (BD Biosciences, San Diego, California): interleukin (IL)-12p70, tumor necrosis factor (TNF)-α, IL-6, IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor, eotaxin, IL-4, IL-3, and IL-1β. Bead populations with distinct fluorescence intensities for specific soluble proteins were measured with flow cytometry. The interassay variation coefficients ranged from 8% to 20% (1 to 10 pg/mL), 3% to 10% (70 to 80 pg/mL), and 2% to 6% (527 to 630 pg/mL).

Statistical Analysis

Statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc, Chicago, Illinois). The infants with and without exposure to CA were analyzed together and also as 2 separate populations. In the initial analyses, the correlations among cytokines, risk factors (Table I), and outcome variables were assessed using Spearman's rank correlation. The trends for cytokine concentrations during the perinatal transition in infants with and without BPD were evaluated using gestational age at birth as the continuous independent variable in the covariance analysis. Cytokines and early neonatal risk factors were evaluated for predicting the risk of BPD by defining the receiver operating characteristic (ROC). Stepwise logistic regression analysis was subsequently used to test a single cytokine for predicting the risk of BPD. The other risk factors evaluated were gestational age at birth, intrauterine growth retardation, CA, and severity of respiratory distress during the first day of life. All tests were 2-tailed.

Table I. Characteristics of the 128 VLGA infants included in the study

Variable	Median (min, max) or number of infants (%)
Gestational age at birth, weeks	29.4 (24.1, 31.9)
Birth weight, g	1170 (370, 2210)
Male sex	68/128 (53%)
Small for gestation	29/128 (22.6%)
Antenatal steroid use	108/124 (87.1%)
Histological CA*	48/123 (39.0%)
Rupture of fetal membranes	32/128 (25%)
Preeclampsia	36/128 (28.1%)
Surfactant therapy	77/128 (60.1%)
Mechanical ventilation, days	3.2 (0.0, 95.5)
RDS	84/128 (65.6%)
BPD†	32/128 (25.0%)
Moderate at 36 postmenstrual weeks	27/128 (21.1%)
Severe at 36 postmenstrual weeks	5/128 (3.9%)

*One case revealed mild inflammation in the chorionic plate of the placenta without detectable CA in reflecting membranes or funisitis.
†Eight infants did not have RDS.

RESULTS

Altogether, 232 VLGA infants were born in the in the regional hospital during the study period. A total of 104 infants did not participate (death in the delivery room, n = 3; death during initial hospitalization, n = 15; refusal of consent, n = 6; at least 1 of the 3 blood specimens not available for this analysis, n = 80). The final study group comprised 128 infants. Mean birth weight, mean duration of gestation, outcomes, and major risk factors did not differ significantly between in the final study group and the whole cohort of 214 surviving VLGA infants. Most of the fetuses were exposed to glucocorticoids, and most of the infants with RDS received surfactants (Table I).

IL-6, IL-8, IL-10, and G-CSF were chosen for analysis. The other cytokines were either not detectable or were not associated with BPD or RDS. High IL-6 concentration