Blood Cytokine Profiles Associated with Distinct Patterns of Bronchopulmonary Dysplasia among Extremely Low Birth Weight Infants

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Objective To explore differences in blood cytokine profiles among distinct bronchopulmonary dysplasia (BPD) patterns.

Study design We evaluated blood spots collected from 943 infants born at ≤1000 g and surviving to 28 days on postnatal days 1, 3, 7, 14, and 21 for 25 cytokines. Infants were assigned to the following lung disease patterns: (1) no lung disease (NLD); (2) respiratory distress syndrome without BPD; (3) classic BPD (persistent exposure to supplemental oxygen until 28 days of age); or (4) atypical BPD (period without supplemental oxygen before 28 days). Median cytokine levels for infants with BPD were compared with the IQR of results among infants with NLD.

Results The distribution of enrolled infants by group was as follows: 69 (NLD), 73 (respiratory distress syndrome), 381 (classic BPD), and 160 (atypical BPD). The remaining 260 infants could not be classified because of missing data (104) or not fitting a predefined pattern (156). Median levels of 3 cytokines (elevated interleukin [IL]-8, matrix metalloproteinase-9; decreased granulocyte macrophage colony-stimulating factor) fell outside the IQR for at least 2 time points in both infants with atypical and classic BPD. Profiles of 7 cytokines (IL-6, IL-10, IL-18, macrophage inflammatory protein-1α, C-reactive protein, brain-derived neurotrophic factor, regulated on activation, normal T cell expressed and secreted) differed between infants with classic and atypical BPD.

Conclusions Blood cytokine profiles may differ between infants developing classic and atypical BPD. These dissimilarities suggest the possibility that differing mechanisms could explain the varied patterns of pathophysiology of lung disease in extremely premature infants. *(J Pediatr 2016;174:45-51)*.

Many investigators have described the association of altered lung fluid cytokine levels with the development of bronchopulmonary dysplasia (BPD), but these studies are limited by their small size and their inability to obtain samples from nonintubated infants.[1-8] Fewer studies have explored the potential association between variations in blood cytokine levels and BPD.[9-12]

Some authors have described a "new" BPD associated with improved survival in smaller infants and decreased incidence of the more classic, severe form of BPD because of the availability of newer management approaches.[13,14] We have previously described 4 patterns of respiratory course among infants ≤1250 g birth weight, based on oxygen administration: (1) normal–no clinical or radiologic evidence of lung disease; (2) respiratory distress syndrome (RDS)–typical RDS, with no supplemental oxygen administration at 28 days of

BDNF	Brain-derived neurotrophic factor	MIP	Macrophage inflammatory protein
BPD	Bronchopulmonary dysplasia	MMP	Matrix metalloproteinase
CRP	C-reactive protein	NLD	No lung disease
EPPD	Early and persistent pulmonary disease	PD	Pulmonary deterioration
		PMA	Postmenstrual age
FiO_2	Inspired concentration of oxygen	RANTES	Regulated on activation, normal T cell expressed and secreted
GM-CSF	Granulocyte macrophage colony-stimulating factor		
		RDS	Respiratory distress syndrome
IL	Interleukin	TNF	Tumor necrosis factor

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The Neonatal Research Network's Cytokines Study was supported by the National Institutes of Health (NIH; M01 RR30, M01 RR32, M01 RR39, M01 RR70, M01 RR80, M01 RR633, M01 RR750, M01 RR997, M01 RR6022, M01 RR7122, M01 RR8084, M01 RR16587), the NICHD (U01 HD36790, U10 HD21364, U10 HD21373, U10 HD21385, U10 HD21397, U10 HD21415, U10 HD27851, U10 HD27853, U10 HD27856, U10 HD27871, U10 HD27880, U10 HD27881, U10 HD27904, U10 HD34216, U10 HD40461, U10 HD40492, U10 HD40498, U10 HD40689), and the Centers for Disease Control and Prevention (CDC; Interagency Agreement Y1-HD-5000-01). C.D. is supported by NICHD (1 U10 HD 068263). Participating sites collected data and transmitted it to RTI International, the data coordinating center for the network, which stored, managed, and analyzed the data for this study. Although NICHD and CDC staff did have input into the study design, conduct, analysis, and manuscript drafting, the content is solely the responsibility of the authors and does not necessarily represent the official views of NICHD or CDC. The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2016.03.058

age; (3) classic BPD–acute lung disease that does not resolve, leading to oxygen administration and radiologic evidence of BPD at 28 days; and (4) atypical BPD–initial resolution of RDS, if present, followed by subsequent development of oxygen requirement that persists through 28 days.[13] Although it would not be appropriate to conflate "new" BPD with the atypical group we describe, these infants may represent a unique group in which to study the pathogenesis of the "new" BPD.

The *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development's Neonatal Research Network has performed cytokine assays on blood spots from over 1000 premature infants. We have previously reported that adding cytokine results to clinical predictors improved the ability of a multivariate model to predict the later development of BPD.[11] However, the modeling did not take into account potential subtypes of BPD. We re-analyzed the cytokine data, specifically dividing subjects into groups with classic and atypical BPD. We hypothesized that the patterns of expression of a limited number of blood cytokines would differ between infants with classic BPD, infants with atypical BPD, and infants without BPD. We speculated that these differences in cytokine levels could serve as biomarkers of disease type and form the focus of further, hypothesis-driven exploration of the pathogenesis of BPD.

Methods

This study analyzed data from a prospective cohort study of blood cytokine levels among premature infants that has been reported previously.[11,15] Briefly, whole blood spots were collected on filter paper from infants with birth weights between 401 and 1000 g born at one of 17 Neonatal Research Network centers between 1999 and 2002. Samples were collected within 4 hours after birth (day 1) and then between 2-4 days (day 3), 6-8 days (day 7), 11-17 days (day 14), and 18-24 days (day 21). Samples were dried using a desiccator, frozen immediately, and analyzed later for levels of 25 cytokines using a multiplex, bead-based luminescent immunoassay (Luminex, Austin, Texas), as previously described.[16] The cytokine levels measured included interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8 (CXCL8), IL-10, IL-12, IL-17, IL-18, tumor necrosis factor (TNF)-α, TNF-α, Regulated on activation, normal T cell expressed and secreted (RANTES), brain-derived neurotrophic factor (BDNF), C-reactive protein (CRP), granulocyte macrophage colony-stimulating factor (GM-CSF), interferon-γ, macrophage chemoattractant protein-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, matrix metalloproteinase (MMP)-9, neurotrophin 4, soluble IL-6 receptor, transforming growth factor-β, and triggering receptor expressed on myeloid cells 1. Clinical data, including inspired concentration of oxygen (FiO_2) on days 1, 3, 7, 14, 21, and 28, and at 36 weeks postmenstrual age (PMA), were collected from the medical record. The original study was approved by each center's Institutional Review Board, and parents gave informed permission for the study. This analysis used only deidentified data collected at the time of the original study.

Infants were divided into groups to correspond with our previously reported patterns of lung disease,[13] according to oxygen administration at 1, 4, 7, 14, 21, and 28 days of age. The means of oxygen administration was not included in the definition. A group with no lung disease (NLD) was defined by having FiO_2 <0.25 at days 1, 4, 7, 14, 21, and 28 days. A group was identified as having RDS without BPD (RDS) by having FiO_2 ≥0.25 within the first 21 days, FiO_2 <0.25 at day 3 and/or day 7, and FiO_2 <0.25 at day 28. Infants with FiO_2 ≥0.25 at days 1, 3, 7, 14, 21, and 28 (ie, persistent supplemental oxygen administration) were classified as having "classic BPD." Infants with FiO_2 <0.25 at either day 3 or day 7, but FiO_2 ≥0.25 at day 28 (ie, intervening period of low or absent supplemental oxygen administration) were defined as having "atypical BPD." The BPD groups were further subdivided into "mild BPD" (FiO_2 = 0.21 at 36 weeks' PMA) or "moderate-to-severe BPD/CLD" (FiO_2 >0.21 at 36 weeks' PMA), in a classification roughly approximating consensus guidelines on BPD severity, to allow for evaluation of the common clinical definition that considers only FiO_2 at 36 weeks' PMA.[14] The data predated the use of physiological testing to determine FiO_2 required at 36 weeks' PMA.[17] Subjects receiving nasal cannula flow at FiO_2 0.21 were considered as receiving room air. Results for both any severity of BPD and moderate-to-severe BPD (with mild BPD removed) were analyzed. Subjects who did not fit into one of the classifications or who died before 28 days of age were not included in the analysis.

Baseline and clinical characteristics were analyzed by Kruskal-Wallis test or median test (Apgar scores) for continuous variables, and Fisher exact test for categorical variables. The median values and IQR were determined for each cytokine among the NLD group at each of the sampled times. The median values for each of other groups were plotted against the NLD group. Cytokines for which 2 median values fell outside the IQR for NLD group were defined a priori as different from the pattern for the NLD group. Because the purpose of this study was to generate hypotheses about potential differences in cytokine patterns among various types of lung diseases, no statistical hypothesis testing was performed.

Results

Of the original 1067 subjects in the observational cytokine cohort, 943 survived to 28 days, and 683 of these could be categorized into 1 of the 4 predefined groups (**Figure 1**; available at www.jpeds.com). Of these, 69 (10%) qualified as having NLD, 73 (11%) were categorized as having RDS without BPD, 160 (23%) as having atypical BPD, and 381 (56%) as having classic BPD. When analyzed at 36 weeks' PMA, among infants with atypical BPD, 4 (3%) had died, 39 (24%) had moderate/severe BPD (continued FiO_2