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T cell cytokines and the risk of blood stream infection in extremely low birth weight infants

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Abstract

Cytokines mediate the host immune response to infectious microorganisms. The objective of this study was to determine whether immune regulatory interleukins (IL-4, IL-5, IL-6 and IL-10) and inflammatory cytokines (Interferon- [INF-], tumor necrosis factor- [TNF-], IL-2, and IL-17) are associated with an increased risk of developing blood stream bacterial/fungal infection (BSI) in extremely low birth weight (ELBW) infants. ELBW infants from 17 NICHD Neonatal Research Network centers without early onset sepsis were studied. Cytokines were measured from blood on days 1, 3, 7, 14, and 21 after birth. 996 ELBW infants contributed a minimum of 4080 unique measurements for each cytokine during the 5 sampling periods. Infants with BSI had lower levels of the inflammatory cytokines IL-17 (P=0.01), and higher levels of the regulatory cytokines, IL-6 (P=0.01) and IL-10 (P<0.001). Higher levels of regulatory cytokines relative to pro-inflammatory cytokines were associated with increased risk of BSI even after adjusting for confounding variables. In ELBW infants, the ratio of immune regulatory cytokines to inflammatory cytokines was associated with development of BSI. Altered maturation of regulatory and inflammatory cytokines may increase the risk of serious infection in this population.

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Conflicts of interest: None

1. INTRODUCTION

Newborn infants are at high risk for acquiring serious, sometimes fatal bloodstream infections (BSI) with bacteria, fungi and viruses. The risk of both bacterial BSI and BSI mortality increases with lower birth weight and earlier gestational age. [1,2] Some infants never develop BSI, others appear to eradicate blood-borne pathogens efficiently, and still others will die or suffer serious morbidity from BSI. Certain components of newborn innate [3,4] and adaptive [5,6] immunity differ from older subjects, and these differences may contribute to the functional immunodeficiency of infancy. [7]

Central to the function of the adaptive immune arm is the differentiation of naive T cells into distinct effector T helper (Th) cells. Cytokines have emerged as critical inducers of Th subset development. These differentiated T cells were originally categorically designated Th1 and Th2 cells based on distinct functional properties and the cytokines that drive their development. [8] Th1 type cytokines, such as IFN- γ and IL-2 play a key role in initiating early resistance to pathogens, and induction of cell-mediated immunity. Th2 cytokines drive the system toward immune tolerance rather than toward defense from microbial infections. Accumulating evidence suggests that Th1 responses in newborns are compromised at several steps including deficient production of Th1 type cytokines by neonatal CD4⁺ T cells and hyporesponsiveness of neonatal macrophages to stimulation by INF- γ . These deficiencies contribute to the apparently weak cellular immunity in newborns biased towards a Th2 type response. [9,10]

Since the original Th1 and Th2 description, other cytokines have proven to be important regulators of the immune response including IL-10 and IL-17. In addition, a differentiated T cell population, Th17 cells, has been shown to have a pathogenic role in organ-specific autoimmune and chronic inflammatory diseases. Th17 cells have also been shown to play a protective role in immunity to infection, where they take on Th1-like effector functions to promote pathogen clearance by enhancing neutrophil recruitment to sites of infection and activating macrophages. [11,12]

Our objective was to determine if levels of Th2/immune regulatory interleukins (IL-4, IL-5, IL-6 and IL-10) and pro-inflammatory Th1 and Th17 pathway cytokines (INF- TNF-, IL-2 and IL-17) are associated with increased risk of developing culture-proven bloodstream infection in extremely low birth weight (ELBW) infants after controlling for known risk factors for nosocomial infection. Our hypothesis was that infants who do not decrease high levels of immune regulatory interleukins and do not increase low levels of pro-inflammatory cytokines are at greater risk for BSI.

2. PATIENTS AND METHODS

This study was a secondary analysis of data from a prospective cohort study (the Cytokines Study) performed in the 17 centers of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Neonatal Research Network (NRN). Infants born March 2000-December 2001 with birth weight 401–1000 g and <72 hours of age were eligible for inclusion in the cohort study. The 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 1), 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. Samples from day 1 were available for 53% of the infants, from day 3 for 93%, from day 7 for 90%, from day 14 for 89%, and from day 21 for 85%. 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 using a multiplex Luminex assay (Luminex Corp., Austin, TX) as described previously. [13] 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. Storage of the blood spots appropriately does not change cytokine concentrations. [14]

For this secondary analysis, infants with one or more of the following were excluded: death in the first seven days of age, major congenital malformation or syndrome, or early onset sepsis, defined as isolation of a pathogen from blood or cerebrospinal fluid (CSF) culture taken within 72 hours of birth. Blood stream infection (BSI) was defined by a blood and/or CSF culture positive for bacteria or fungi that occurred later than 72 hours after birth. Cultures positive for *Corynebacterium* spp., *Propionibacterium* spp., *Alcaligenes* spp., *Penicillium* spp., and Diptheroids were considered contaminants and excluded. Cultures positive for coagulase negative Staphylococcus (CONS) were reviewed and categorized as a definite infection, possible infection or probable contaminant. [1] Definite CONS infection was defined as multiple CONS positive cultures within five days (counted as one definite episode) or one positive blood culture and elevated c-reactive protein (CRP) >1 mg/L within two days of blood culture or infant died within five days of CONS positive blood culture. Possible CONS infection was defined as one positive blood culture and patient treated with vancomycin, oxacillin, methicillin, or nafcillin for five or more days. Probable CONS contaminant was defined as one positive culture without elevated CRP or antibiotic therapy as outlined above and infant survived. Definite and possible CONS infections were included as BSIs while probable contaminants were not. Thus, infants who had cultures positive only for pathogens considered contaminants were included in the non-infected group.

Serious infection or serious BSI was defined by a blood or CSF culture positive for gram negative bacteria, fungi, or gram positive bacteria excluding all CONS and bacillus. Infants with cultures positive for CONS or bacillus only were not considered to have serious BSI and were included in the no serious BSI group.

2.1 Statistical Analysis

Individual cytokine values, including IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, IFN-, and TNF- were compared between infected and uninfected groups at each sample day. To evaluate the proportional relationship between immune regulatory (Th2) and inflammatory (Th1/Th17) cytokines we utilized the following ratios of representative cytokines: ratio 1 – IL-4+IL-5/ IFN- +IL-17, ratio 2 – IL-6+IL-10/ TNF- +IL-2, and ratio 3 – IL-10 / IL-17. Cytokine and ratio values were ranked at each sample day and linear regression models were fit to the ranks. F tests from these models were used to provide statistical significance for comparisons between infected and uninfected groups at each sample day. Adjusted comparisons were determined from models fit to the ranks which included study center and gestational age. Characteristics of study infants were compared between infants with BSI and no BSI with statistical significance determined by the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables. No adjustment for multiple comparisons was made.

Associations between cytokines and BSI (and serious BSI) were examined using Cox proportional hazards models. Each individual cytokine and each ratio was included in the models as a time dependent covariate in order to insure that the last value taken (baseline, day 7, 14 or 21) prior to diagnosis of BSI or censoring was utilized. Since the day 1 sample was missing for approximately 50% of the infants, the baseline value was estimated as the mean of the day 1 and day 3 samples when day 1 was available; otherwise the day 3 sample

was used. Four separate models were run: all eight individual cytokine measures were included in one model, with each of the cytokine ratios included alone in three separate models. Covariates included in all models were network center, gestational age, small for gestational age (yes/no), race (black, white, other), and gender. Gestational age was entered as a continuous variable. Adjusted hazard ratios (HR) with associated 95% confidence intervals and statistical significance determined by Wald chi-square tests were reported from these models.

3. RESULTS

3.1. Patient characteristics

1,071 infants born at 17 NICHD NRN centers were included in a cytokines study. Of these, 75 were excluded for this analysis: 45 infants who died in the first 7 days, 15 infants who had one or more major congenital anomalies and 15 who had early onset sepsis. Among the 996 infants studied, 436 (44%) developed BSI. Thirty infants had pathogens isolated from both blood and CSF, 383 from blood alone, 23 from CSF alone. 124 (28.4%) infants with BSI had more than one episode of infection. There were 222 infants diagnosed with CONS, 102 (46%) with definite CONS infection and 120 (54%) with possible CONS infection. CONS was the most frequently isolated pathogen on blood and CSF (47% for each). In addition to CONS, pathogens responsible for the first infection in the 413 infants with blood infections included *Staphylococcus aureus* for 8%, *Enterococcus sp.* and other streptococcal organisms 5%, other Gram positive 6%, Gram negative and enteric organisms 17%, *Candida sp.* 11%, and polymicrobial infections 6%. Median age at first BSI was 18 days, (interquartile range [IQR] 11–30 days). More than half (58%) of infants with BSI were diagnosed between day 7 and 21 of age. Infants with any BSI were of lower gestational age and birth weight compared to those without BSI ($p < 0.001$, median=25 versus 26 weeks and median=733 versus 792 grams, respectively (Table 1). No significant differences were found between infected and non-infected infants by gender or race.

3.2. Gestational age differences in cytokine values

Table 2 summarizes the primary activities of each of the immune regulatory and inflammatory cytokines we examined in ELBW infants. To determine the influence of gestational age we compared early, day 7, day 14 and day 21 median cytokine values between infants born at gestational age ≤ 25 weeks, 26 to 27 weeks and ≥ 28 weeks who did not have a bacterial or fungal BSI. For the Th1/Th17 pro-inflammatory cytokines, median values of TNF- and IL-2 increased with greater gestational age on most sampling days (Table 3). Notably, IL-17 values were similar in the three gestational age groupings, suggesting no maturational change with advancing gestational age. For the Th2 regulatory cytokines, only IL-5 demonstrated an increase in cytokine concentration by gestational age for sampling periods on or after 7 days. ($P \leq 0.001$).

3.3. Temporal Cytokine Profiles

Figure 1 demonstrates the median cytokine values for infants with and without BSI for each sampling period. Statistically significant differences in the median values are noted on the graphs.

Generally, cytokine values were highest on day 1 and then rapidly fell on subsequent sampling days. This pattern was similar for the Th1/Th17 as well as for the Th2/regulatory cytokines.

The majority of differences in cytokine values between infants with and without BSI occurred on sample days 14 and 21 or overall; few differences were found for the early

samples. After adjusting for study center and gestational age, there were no differences for cytokines IFN-, TNF-, IL-2, IL-4, and IL-5 at any sample day or overall. While differences were not found on any individual day, IL-17 values were significantly lower for infants with BSI overall ($p=0.01$). Higher values were found in the infected group for IL-6 on day 21 ($p=0.001$) and overall (adjusted $p=0.01$) and for IL-10 on day 7 (adjusted $p=0.02$), day 14 (adjusted $p<0.001$), day 21 (adjusted $p<0.001$) and overall (adjusted $p<0.001$).

Figure 2 shows that significant differences between infected and non-infected infants occurred for the ratios of IL-6+IL-10/TNF- +IL-2 and IL-10/IL-17. After adjusting for study center and gestational age, median values were significantly higher in the infected group for: IL-6+IL-10/TNF- +IL-2 on day 7 ($p=0.04$), day 14 ($p<0.01$), day 21 ($p<0.01$) and overall ($p<0.001$); IL-10/IL-17 on day 7 ($p<0.01$), day 14 ($p<0.001$), 21 ($p<0.001$), and overall ($p<0.001$).

3.4. Risk of BSI

Results from Cox proportional hazard models utilizing time dependent covariates are summarized in Table 4. After adjusting for network center and baseline covariates including gestational age, increased IL-6 values were significantly associated with an increased hazard for BSI ($p<0.001$, adjusted HR 1.02) as were TNF- values ($p=0.01$, adjusted HR 1.04). The ratios, IL-6+IL-10/TNF- +IL-2 and IL-10/IL-17 were significantly associated with any BSI with each unit increase in the ratio associated with an increase in the adjusted hazard ($p<0.03$, adjusted HR=1.003; $p=0.02$, adjusted HR=1.004, respectively). Similarly, IL-6+IL-10/TNF- +IL-2 and IL-10/IL-17 were also significantly associated with serious BSI ($p=0.02$, adjusted HR=1.004; $p=0.01$, adjusted HR=1.005, respectively).

4. DISCUSSION

In this study, we demonstrated that ELBW infants who developed at least one episode of BSI during their hospital stay started with lower serum concentrations of IL-17 and higher concentrations of the anti-inflammatory cytokine IL-10 than their counterparts who never acquired BSI. We also detected differences in Th2 to Th1/Th17 cytokine ratios in these two groups and an increased risk for BSI with increasing values of these ratios after controlling for gestational age and other patient characteristics, suggesting that differential maturation of various T-helper cell lineages can alter the risk of serious infections in ELBW infants. These analyses of the Th2 to Th1/Th17 ratios also suggested a Th2 bias in the infants who developed BSI.

The fetus experiences a constant barrage of “foreign” antigens, mainly derived from the mother, and must down-regulate the immune response to survive. The developing fetus curtails its immune response to these antigens through expression of various immune regulatory cytokines such as IL-4, IL-5 and IL-10 by Th2 cells.[15] After birth, however, the infant is immediately exposed to micro-organisms and the immune system must activate to “contain” these micro-organisms on various cutaneous and mucosal surfaces. This process, when successful, involves a shift in the T-helper cell balance from a fetal Th2-dominance to a pro-inflammatory, Th1/Th17-dominant state in the postnatal period. During sepsis, a robust pro-inflammatory cytokine response, as in mature infants and adults, is essential for the prompt clearance of pathogens [16–19], whereas a blunted/dysregulated inflammatory response seen frequently in ELBW infants may be inadequate to eradicate the infection. Although we did not detect important differences in serum levels of INF- γ , the prototypal Th1 cytokine, we found that infants who acquired BSI had significantly lower levels of Th17 cytokine IL-17 overall [reviewed in [12]] than those who did not. Emerging clinical and experimental evidence emphasizes the role of IL-17 in the recruitment of neutrophils, macrophages, and T-helper cells to sites of infection and shows its critical role in the

clearance of pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Candida albicans*. In our study, the high IL-10 levels and increased IL-10/IL-17 ratios in the BSI infants further indicated a Th2-biased, hypo-inflammatory state. These data show for the first time that developmental deficiencies in the newly-defined Th17 arm (or suppression by IL-10) is associated with increased risk of systemic infection in premature neonates. Proof of causality will require additional laboratory investigation utilizing transgenic IL-17 receptor-deficient animals or IL-17 receptor blockade *in vivo*.

We observed a progressive decline in the T-cell-derived cytokines during the first two postnatal weeks. We speculate that the decline in Th2 cytokine levels can be partially explained by the cessation of placental supply (placenta-derived Th2 cytokines suppress inflammation at the uteroplacental interface) after birth and resolution of perinatal stress. Perinatal factors, such as subclinical chorioamnionitis, may also have dampened the postnatal Th2-to-Th1/Th17 shift in the cytokine ratios, providing a possible explanation for the modest reduction in cytokine ratios. Another possibility for our failure to detect major differences may be related to the combined effect of environmental influences as well as intrinsic developmental events, which would be related to the postmenstrual age and the maturation of T-helper cell lineages. Blood samples were obtained between 26 to 30 weeks postmenstrual age, and using this sampling interval could miss developmental changes during later gestation.

On the basis of altered cytokine profiles, we show here that immune development may be different in infants who develop serious BSI. Strategies to mature the neonatal immune response could include interventions that directly or indirectly alter cytokine levels. While cytokines orchestrate the immune response, it may be naïve to assume that one cytokine has a direct effect on the infection risk. Rather, there appears to be interaction of multiple cytokines that affects the overall balance. This complex interplay and balance of cytokines makes immune modification or therapeutic intervention challenging and not without potential risk. Enhanced immune function might come at the expense of increased self-reactivity or other inflammatory collateral damage. Because neonates with antenatal conditions which produce excessive inflammation (such as chorioamnionitis) may develop associated pathology such as periventricular leukomalacia or bronchopulmonary dysplasia, [20,21] the notion of augmenting the immune system response must be tempered by concerns for potential untoward effects.

The strengths of this study include the relatively large sample of ELBW infants recruited from multiple sites and prospective data collection by trained observers. Additionally, we measured multiple cytokines at different time-points. The determination of the postnatal temporal profile of these cytokines and the associations of some of these cytokines with BSI 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 for clinical prediction models.

Measuring cytokine levels in the circulation only does not identify which cells produce or respond to cytokines, or a specific interaction with innate immune components. Chorioamnionitis may alter or reprogram infant cytokine profiles. We were unable to evaluate the relationship of clinical and histological chorioamnionitis to cytokines and BSI as this data was not collected during the years of this study. There is potentially a genetic component to development of infection, and cytokine gene polymorphisms may influence cytokine concentrations.[22] Cytokine polymorphisms may reveal a genetic rationale for increased susceptibility to infection, although studies to date have not demonstrated strong associations with sepsis risk.[23]

5. CONCLUSIONS

In ELBW infants, postnatal development of the immune system involves a shift from higher levels of immune regulatory cytokines to higher levels of inflammatory cytokines. Infants who appear to incompletely up regulate inflammatory cytokines may be at greater risk for BSI. Our results suggest that serious infection is associated with altered maturation of regulatory and inflammatory cytokines. Higher levels of cytokines favoring immune suppression may play a critical role in the functional immune deficiency of infancy. An imbalance of pro-inflammatory and anti-inflammatory cytokines, particularly IL-10, was associated with an increased risk of infection. Identification of cytokines that contribute to serious infection in ELBW infants may lead to therapeutic strategies directed to accelerate maturity of the immune system and make these infants more able to resist microbial invasion.

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Abbreviations

BSI	Bloodstream infection
ELBW	extremely low birth weight
IL	interleukin
IFN	interferon
TNF	Tumor necrosis factor
CONS	coagulase negative Staphylococcus
Th	T helper
CD	cluster of differentiation
CRP	c-reactive protein

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Data collected at participating NRN sites were transmitted to RTI International, the data coordinating center (DCC) for the NRN, which stored, managed, and analyzed the data for this study. On behalf of the network, Dr. Abhik Das (DCC PI) and Ms. Sarah Taylor (DCC

Statistician) had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

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Figure 1.

Temporal cytokine values for the five sampling periods. A. Shows values for the Th1/Th17 cytokines. B shows values for the Th2 regulatory cytokine group. Gray line-open circles are median values for the no BSI group. Black line and closed boxes are median values for the group of infants with BSI. * $P < 0.05$ and † $P < 0.01$ for unadjusted values. After adjusting for study center and gestational age, significant differences were no longer found at the indicated sample days for IL-17 and IL-5. Differences remained as indicated for IL-6 and IL-10.

Figure 2.

Ratios of Th2/regulatory to Th1/Th17 cytokine values for the five sampling periods after adjustment for center and gestational age. Gray line-open circles are median values for the no BSI group. Black line and closed boxes are median values for the group of infants with BSI. * $P < 0.05$ and † $P < 0.01$.

Table 1

Characteristics of study infants

	BSI (n=436)	No BSI (n=530)	P value*
BW grams, median (IQR)	733 (627–856)	792 (675–895)	p<0.001
GA weeks, median (IQR)	25 (24–27)	26 (25–27)	p<0.001
Male Gender, n (%)	215 (47%)	262 (49%)	0.44
Black Race, n (%)	218 (50%)	268 (48%)	0.75
White Race	211 (48%)	281 (50%)	
Other	7 (2%)	11 (2%)	
Intrapartum antibiotics, n (%)	316 (73%)	373 (67%)	0.04
Antenatal Steroids, n (%)	330 (76%)	435 (78%)	0.54
Cesarean section, n (%)	239 (55%)	349 (62%)	0.02
Surfactant, n (%)	363 (83%)	440 (78%)	0.08
Maternal pre-eclampsia, n (%)	85 (20%)	168 (30%)	<0.0001
Small for gestational age, n (%)	52 (12%)	95 (17%)	0.03

* Statistical significance for a difference between infants with and without BSI was determined by chi-square tests for categorical variables and Kruskal-Wallis tests for continuous variables. IQR refers to the interquartile range.

Table 2

Representative activities of immune regulatory and inflammatory cytokines

<i>Immune Regulatory Th2 cytokines</i>	Representative Activities
IL-4 (24)	Promotes differentiation of Th2 cells, which mediate allergic diseases and control chronic infections
IL-5 (25)	Activates B cells and eosinophils; influences differentiation of B cell subsets, and regulates innate and acquired immune responses
IL-6 (26)	Participates in acute phase responses; stimulates lymphocytes; contributes to resolution of innate immune responses; promotes adaptive immunity
IL-10 (27)	Inhibits activation and effector function of T cells, monocytes, and macrophages
<i>Inflammatory Th1/Th17 cytokines</i>	
TNF-	Activates innate and adaptive immune responses, organizes developing lymphoid tissues
IL-2 (29)	Potent inducer of T-cell proliferation and T-helper cell differentiation; contributes to induction and termination of inflammatory immune responses
IL-17 (12, 30)	Neutrophil recruitment to sites of infection and macrophage activation, promotes chronic inflammatory and organ-specific autoimmune disease
IFN-	Plays a key role in initiating early resistance to pathogens, and induction of cell-mediated immunity; regulates and promotes T-helper cell differentiation.

Table 3

Median cytokine values by gestational age among ELBW infants with no bacterial or fungal blood stream infections

Cytokine	Sample Day	GA (wks)			p-value*
		<= 25	26-27	>=28	
IFN-	≤3	125 (56-236)	139 (57-272)	191 (79-330)	0.02
	7	135 (69-260)	138 (55-236)	158 (49-309)	0.71
	14	123 (56-222)	134 (41-252)	143 (68-259)	0.46
	21	117 (44-227)	115 (45-233)	132 (49-257)	0.70
TNF-	≤3	263 (122-533)	271 (159-516)	279 (144-637)	0.56
	7	195 (80-395)	206 (103-386)	249 (138-459)	0.04
	14	198 (75-365)	207 (116-396)	232 (115-448)	0.23
	21	186 (72-371)	227 (123-409)	229 (111-485)	0.02
IL-2	≤3	47 (23-79)	46 (23-80)	56 (29-94)	0.13
	7	34 (20-65)	41 (21-73)	48 (25-84)	0.04
	14	32 (11-68)	35 (15-67)	48 (20-91)	0.01
	21	34 (13-72)	32 (13-75)	43 (19-75)	0.44
IL-17	≤3	180 (95-357)	174 (89-280)	156 (92-303)	0.69
	7	154 (81-309)	159 (96-278)	141 (93-280)	0.98
	14	162 (78-300)	147 (80-276)	142 (94-301)	0.78
	21	155 (84-287)	156 (95-268)	145 (82-253)	0.80
IL-4	≤3	124 (72-199)	115 (71-187)	123 (75-175)	0.64
	7	107 (52-181)	99 (50-185)	103 (60-174)	0.99
	14	91 (51-175)	95 (46-172)	99 (48-168)	0.99
	21	94 (40-166)	107 (63-194)	92 (46-148)	0.06
IL-5	≤3	93 (52-156)	102 (62-172)	108 (59-200)	0.24
	7	79 (42-121)	100 (55-176)	115 (44-241)	<0.001
	14	68 (36-112)	93 (51-150)	101 (45-193)	<0.0001
	21	57 (32-102)	89 (50-134)	86 (38-151)	<0.0001
IL-6	≤3	114 (63-246)	129 (56-219)	117 (63-207)	0.90
	7	77 (41-133)	74 (42-143)	69 (38-116)	0.59

Cytokine	Sample Day	GA (wks)			p-value*
		<= 25	26-27	>=28	
IL-10	14	65 (42-115)	68 (31-122)	71 (31-111)	0.78
	21	56 (32-91)	64 (31-119)	47 (21-83)	0.05
	≤3	431 (276-631)	397 (252-565)	431 (208-673)	0.52
	7	321 (196-473)	347 (213-510)	348 (177-614)	0.19
	14	301 (169-455)	339 (189-464)	329 (154-490)	0.46
	21	271 (130-457)	332 (198-464)	265 (123-481)	0.29

* Statistical significance for a difference in the distribution of values by gestational age at each time point determined by the Kruskal-Wallis test. Values in parentheses are the interquartile range.

Table 4

Adjusted hazard for blood stream infections in ELBW infants

Model	Cytokine	Any BSI			Serious BSI		
		Adjusted HR	95% CI	P	Adjusted HR	95% CI	P
Individual Cytokines	IL-4	0.99	0.92–1.07	0.82	0.94	0.82–1.08	0.38
	IL-5	0.92	0.81–1.04	0.17	0.93	0.80–1.08	0.36
	IL-6	1.02	1.01–1.03	<0.001	1.02	1.01–1.03	<0.01
	IL-10	1.00	1.00–1.01	0.41	1.00	1.00–1.01	0.52
	IFN-	1.03	0.99–1.08	0.19	1.00	0.98–1.02	0.93
	TNF-	1.04	1.01–1.07	0.01	1.01	0.96–1.06	0.81
	IL-2	0.86	0.71–1.04	0.13	0.96	0.73–1.27	0.80
	IL-17	0.98	0.93–1.03	0.34	0.97	0.91–1.04	0.40
	IL-4+IL-5/IFN- +IL-17	0.99	0.95–1.04	0.70	1.00	0.94–1.06	0.94
	IL-6+IL-10/TNF- +IL-2	1.003	1.000–1.006	0.03	1.004	1.001–1.008	0.02
Ratio 1	IL-10 / IL-17	1.004	1.001–1.007	0.02	1.005	1.001–1.009	0.01

Adjusted hazard ratios (HR), confidence intervals, and p-values from Cox proportional hazards models which included effects for network center, gestational age, small for gestational age, race, and sex as well as all eight individual cytokines or each cytokine ratio alone as shown. Each unit change in the HR represents a change of 100 picograms per milliliter. BSI included bacteria and fungi excluding CONS cultures deemed to be contaminants (see Methods for details). Serious BSI included gram negative bacteria, fungi, and gram positive bacteria excluding all CONS and bacillus infections.