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Necrotizing Enterocolitis is associated with *Ureaplasma* Colonization in Preterm Infants

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Abstract

The study objective was to determine whether *Ureaplasma* respiratory tract colonization of preterm infants <33 weeks gestation is associated with an increased risk for necrotizing enterocolitis (NEC). One or more tracheal or nasopharyngeal aspirates for *Ureaplasma* culture and PCR were obtained during the first week of life from 368 infants <33 weeks gestation enrolled from 1999-2003 or from 2007-2009. NEC Bell stage ≥2 was confirmed by radiological criteria, and pathology, if available. Cord serum samples were analyzed for IL-6 and IL-1β concentrations and placentas were reviewed for histological chorioamnionitis in the first cohort. NEC was confirmed in 29/368 (7.9%) of the combined cohorts. The incidence of NEC was 2.2-fold higher in *Ureaplasma*-positive (12.3%) than *Ureaplasma*-negative infants (5.5%) <33 wk (OR 2.43, 95%CI 1.13-5.22, P=0.023) and 3.3-fold higher in *Ureaplasma*-positive (14.6%) than *Ureaplasma*-negative (4.4%) infants ≤28 wks (OR 3.67, 95%CI 1.36-9.93, P=0.01). Age of onset, hematologic parameters at onset, and NEC severity were similar between *Ureaplasma*-positive and negative infants. Cord serum IL-6 and IL-1β concentrations were significantly higher in *Ureaplasma*-positive than in *Ureaplasma*-negative NEC-affected infants. *Ureaplasma* may be a factor in NEC pathogenesis in preterm infants by contributing to intestinal mucosal injury and/or altering systemic or local immune responses.

Introduction

Necrotizing enterocolitis (NEC), a gastrointestinal emergency, affects approximately 5 to 10% of very low birth weight (VLBW) infants. It is a devastating disease with mortality as high as 30%. Prematurity is the greatest risk factor for development of NEC (1, 2). Several studies suggest that the initiation of an intense systemic and local inflammatory cascade leads to intestinal necrosis in response to inciting risk factors (3-8).

Ureaplasma parvum and *U. urealyticum* are commensals of the genital tract of 40 – 80% childbearing aged women (9, 10) and are the most common organisms isolated from infected amniotic fluid and placentas (11). Infertility, chorioamnionitis, preterm delivery and morbidity such as bronchopulmonary dysplasia (BPD) have all been associated with

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perinatal *Ureaplasma* infection (12). The organisms elicit both systemic and local host inflammatory responses in humans (13, 14) and in cell (15) and animal models (16-18). The intestinal and respiratory tracts are directly exposed to infected amniotic fluid containing inflammatory mediators, which could enhance the inflammatory response to certain bacteria and their products. In addition to being isolated from the respiratory tract, *Ureaplasma* has been detected in gastric aspirates by culture (19-21) and molecular methods (22) and in rectal cultures (21). The effects of such synergistic inflammatory interactions could be potentially detrimental to the preterm host leading to a compromised intestinal barrier with development of diseases such as NEC and gastrointestinal-related sepsis.

Although preterm respiratory colonization with *Ureaplasma* is a known risk factor for neonatal morbidities, its association with NEC has not been previously determined. We hypothesized that preterm infants exposed to *Ureaplasma* spp. *in utero* or colonized at birth are at increased risk for NEC. To evaluate the relationship of *Ureaplasma* colonization with NEC, we examined the incidence and associated clinical and inflammatory variables of NEC in two prospectively recruited cohorts of preterm infants with *Ureaplasma* colonization status during the first week of life confirmed by culture and PCR who were born at gestational age <33 weeks and birth weight <1,501 grams.

Methods

Sample

Infants born at gestational age <33 weeks and birth weight <1,501 grams admitted to the neonatal intensive care units at the University of Maryland Medical Center and Mercy Medical Center (Baltimore, MD, U.S.A.) were eligible for study participation. We enrolled patients in 2 studies designed to characterize the effects of *Ureaplasma* on preterm infant outcomes from 1999 to 2003 (Cohort 1) (details of this cohort have been previously reported (23, 24) and from 2007 to 2009 (Cohort 2). The objective of the first study was to determine the incidence of invasive disease with *U. parvum* and *U. urealyticum*, and the relationship with adverse outcomes in VLBW infants. The objective for the current study is to analyze potential single nucleotide polymorphisms in relevant toll like receptor genes associated with risk for *Ureaplasma* respiratory tract colonization and BPD. For both studies, infants were excluded if they had confirmed diagnoses of congenital brain/neural tube defects or congenital viral infections. Parental consent was obtained and the institutional review boards of both institutions approved the study protocols.

NEC assessment

Cases of Stage ≥2 NEC according to the modified Bell criteria (1, 25) were confirmed by typical radiological findings (pneumatosis intestinalis, portal venous air, pneumoperitoneum, and/or fixed intestinal loop) and/or pathology, if available and were classified as medical or surgical NEC. A radiologist blinded to *Ureaplasma* culture status reviewed all abdominal radiographs of suspected NEC cases. Cases that were confirmed by pathological examination as spontaneous intestinal perforation (N=2) were excluded. Postnatal age, presence or absence of feeding and white blood cell and absolute neutrophil counts at birth and onset of NEC were recorded.

Ureaplasma detection

One or more tracheal or nasopharyngeal aspirates were obtained during the first week of life from enrolled infants. Samples were processed, 10-fold serially diluted in 10B broth to 10^{-4} and incubated at 37°C in humidified 5% CO_2. Dilutions in which a color change occurred were inoculated on A8 agar and incubated at 37°C in humidified 5% CO_2. Cultures were examined daily for 1 week for color change or colonies typical of *Ureaplasma* (26). DNA

was extracted from original tracheal aspirate or nasopharyngeal samples and culture-positive isolates using QiAmp DNA Blood Mini kits (Qiagen, Valencia, CA) according to the manufacturer's protocol. PCR for Cohort 1 was performed as previously described with primers directed against the 5′ region of the multiple-banded antigen (mba) gene to identify all positive samples and primers targeting urease gene to identify species (24). For Cohort 2, DNA samples were analyzed by multiplex real-time PCR to differentiate the two *Ureaplasma* species simultaneously as previously described using the Roche LightCycler 2.0 (27).

Serum cytokines

For the first cohort, cord serum samples were analyzed for IL-6 and IL-1β in duplicate samples by standard 2 antibody ELISA using commercial antibody pairs and recombinant standards (Endogen, Boston, MA) as previously described (23). A curve fitted to standards was generated using a computer program (Softpro: Molecular Devices) and cytokine concentrations from each sample were calculated from the standard curve. Assay sensitivities were 1.5 and 0.78 pg/ml for IL-6 and IL-1β, respectively.

Placental pathology

Placental studies were performed on 197/232 (85%) subjects with confirmed *Ureaplasma* respiratory status of the first cohort. Sections of umbilical cord, membrane roll, placental disc near the cord insertion site and the midpoint between cord insertion and the periphery of the placental disc were formalin-fixed, paraffin embedded and hematoxylin- and eosin-stained. A pathologist blinded to maternal and infant clinical status reviewed the sections. Histologic chorioamnionitis was separated into maternal and fetal involvement and a stage assigned based on the scheme proposed by Redline *et al.* (28). Fetal vasculitis was defined as polymorphonuclear infiltration of the chorionic vessels or umbilical cord (28).

Statistical analysis

The t test and analysis of variance was used to compare continuous normally distributed data and Mann-Whitney or Kruskal-Wallis test for non-normally distributed data. The χ^2 or Fisher exact test was used to compare categorical variables. Univariate odds ratios and 95% confidence intervals were calculated for all variables for NEC outcome. Analyses comparing *Ureaplasma*-positive and negative infants were stratified by NEC status. Statistical analysis was performed using STATA 7.0 (Stata Corp., College Station, TX, USA). A *P* -value < 0.05 was considered significant.

Results

Study cohort characteristics

For cohort 1, NEC status was confirmed for 308 of 313 subjects and *Ureaplasma* respiratory status was available on 232/308 (75%). For cohort 2, of 324 infants <33 wks gestation who were eligible for the study, 20 were missed due to lack of parental contact, 168 declined consent and parental consent was obtained for the remaining136 infants. NEC and *Ureaplasma* respiratory status were available for all cohort 2 enrolled subjects. The incidence of NEC was similar for both cohorts (Cohort 1, 15/232 (6.5%); Cohort 2, 14/136 (10.3%), P=0.229) and did not differ from the NEC rate for non-enrolled infants during the study periods. The combined NEC rate was 29/368 (7.9%). *Ureaplasma* respiratory tract colonization rate was also similar for both cohorts (Cohort 1, 75/232 (32%); Cohort 2, 57/136 (42%), P=0.064) with an overall colonization rate of 132/368 (36%). *Ureaplasma parvum* was the predominant species (67%) compared with *U. urealyticum* (27%). Both

species were present in 6% specimens. For all subsequent analyses, the cohorts were combined.

Ureaplasma respiratory tract colonization and NEC in VLBW infants

We first analyzed the relationship of demographic, antenatal and early neonatal factors with NEC. In the combined cohorts, none of the factors included in analyses were significantly associated with NEC (Table 1). Specific details concerning feeding such as age when feedings were started, composition of feeds, or time to full feeds were not recorded. However, all infants were fed according to an established feeding protocol.

Ureaplasma colonized infants were less mature and experienced a higher rate of preterm premature rupture of the membranes, maternal antibiotic exposure, and longer duration of mechanical ventilation, but a lower rate of pregnancy-induced hypertension than non-colonized infants regardless of whether they developed NEC (Table 2). The birth weights of *Ureaplasma*-positive infants were lower than the birth weights of the *Ureaplasma*-negative infants in the non-NEC group. However, the incidence of NEC was 2.1-fold higher in *Ureaplasma*-positive (12.3%) than *Ureaplasma*-negative infants (5.5%) <33 wk (OR 2.43, 95%CI 1.13-5.22, P=0.023) (Table 1) and 3.3-fold higher in *Ureaplasma*-positive (14.6%) than *Ureaplasma*-negative (4.4%) infants ≤28 wks (OR 3.67, 95%CI 1.36-9.93). When adjusted for gestational age, the association of *Ureaplasma* colonization and NEC remained significant (OR 2.47, 95% CI 1.13-5.43). Inclusion of other clinical variables in the logistics model did not affect the estimate of the association of *Ureaplasma* colonization and NEC. There were no differences in NEC rates between the *Ureaplasma* species. Age of onset, hematologic parameters at onset, NEC severity, and mortality were similar between *Ureaplasma*-positive and negative NEC infants (Table 3). All *Ureaplasma*-positive NEC infants had been fed prior to onset compared to 92% *Ureaplamsa*-negative NEC infants, but this difference was not statistically significant.

Inflammatory markers associated with NEC in Ureaplasma-colonized VLBW infants

As shown in Table 4, *Ureaplasma*-colonized infants had significantly higher admission peripheral white blood cell counts and absolute neutrophil counts regardless of NEC status. Although histologic chorioamnionitis was present in 92% placentas from *Ureaplasma* non-NEC and 100% *Ureaplasma* NEC infants, inflammation was detected in 1/7 (14%) placentas from *Ureaplasma*-negative NEC infants (p=0.052). Similarly, fetal vasculitis was present in 79% placentas from *Ureaplasma* non-NEC and 67% *Ureaplasma* NEC infants, but absent in all placentas available for review from *Ureaplasma*-negative NEC infants. When restricted to the subset with placental pathology, histologic chorioamnionitis in the absence of *Ureaplasma* colonization tended to reduce the risk for NEC (OR 0.524, 95%CI 0.178-1.44; p=0.202). Since *Ureaplasma* colonization rarely occurred in the absence of histologic chorioamnionitis, it was not possible to distinguish the relative contribution of each variable to NEC.

Cord serum cytokine measurements were available for 101/232 (44%) subjects of cohort 1. Cord serum IL-6 and IL-1β concentrations were similar in NEC and non-NEC groups (Table 1), but were significantly higher in *Ureaplasma*-positive than in *Ureaplasma*-negative infants. The highest cytokine concentrations were detected in cord blood samples of *Ureaplasma*-positive NEC infants (Table 4).

Discussion

The 2 cohorts experienced similar rates of NEC, suggesting that the rate of the disease has been stable in our NICUs over time. The overall rate of 7.9% is within the range of

confirmed NEC rates for VLBW infants reported by the National Institute of Child Health and Human Development Neonatal Research Network (10.1%) (1) and the Vermont Oxford Network (6.9%) (2).

Since necrotizing enterocolitis is primarily a disease of prematurity, immaturity of gut barrier function and local and systemic immune responses have been implicated in susceptibility to the disease. Recently, more attention has focused on the potential role of the intestinal microbiota in initiating mucosal injury and modulating expression of virulence factors and host immune responses (29). Although many bacterial species and enteric viruses have been reported in association with NEC (29) a causal role for these organisms has not been established. The current study is the first to demonstrate an association of *Ureaplasma* respiratory tract colonization and NEC. Although *Ureaplasma* was only cultured from respiratory secretions, *Ureaplasma* spp. are known mucosal organisms that colonize the adult genitourinary tract (10) and have been previously recovered from other mucosal sites such as gastric aspirates and rectum in preterm infants (21, 30). The observed higher rate of NEC in *Ureaplasma*-positive than negative infants ≤28 wks gestation supports the contention that immaturity of intestinal functions increases the susceptibility to NEC in very preterm infants perinatally-exposed to *Ureaplasma* infection/inflammation.

Using culture techniques, *Ureaplasma* spp. have been isolated from blood, cerebrospinal fluid, tracheal aspirates, and lung and brain tissue of newborn infants (11, 31-33). Epidemiologic studies and experimental infection models support an etiologic role for *Ureaplasma* infection or resulting inflammation in preterm birth and several neonatal morbidities. Although the association of *Ureaplasma* respiratory tract colonization with the development of BPD in preterm infants has been debated, a recent meta-analysis of 31 studies supported this association (34). Experimental antenatal infection models in mice (35), and immature sheep (36), rhesus macque (37), and baboon (17), confirm that *in utero* exposure to *Ureaplasma* infection causes fetal/newborn lung inflammation and altered lung development. In our first study of cohort 1, we observed that *Ureaplasma* species not only colonize the respiratory tract, but also invade the bloodstream and cross the immature blood-brain barrier in 23% VLBW infants (24). Detection of *Ureaplasma* by PCR in serum, but not cerebrospinal fluid, increased the risk for severe intraventricular hemorrhage 2-fold (24). In a mouse model of antenatal *Ureaplasma* infection, neuronal injury and microgliosis were evident in *Ureaplasma*-antenatally-infected pups (35).

There is compelling data from human studies and animal models that *Ureaplasma* is pro-inflammatory in multiple compartments (amniotic fluid, placenta, fetal lung, and brain). The stimulatory effect of *Ureaplasma* on cytokine release has been confirmed *in vitro*. In cultured human cord blood preterm monocytes, *Ureaplasma* stimulated release of TNF-α and IL-8, and when co-administered with Gram-negative lipopolysaccharide, *Ureaplasma* greatly augmented generation of pro-inflammatory cytokines while blocking expression of the counter-regulatory cytokines, IL-6 and IL-10 (15). In the current study, *Ureaplasma*-positive infants were more likely exposed to chorioamnionitis and to express a systemic inflammatory response (fetal vasculitis, elevated admission white blood cell and absolute neutrophil counts, and cord blood IL-6 and IL-1β), suggesting that inflammation was initiated *in utero*. This is consistent with recent evidence that in the setting of preterm premature rupture of membranes, intraamniotic infection with the genital mycoplasmas is associated with a more intense inflammatory response compared to the response to infections with other microorganisms (38). Antenatal exposure to infection/inflammation may predispose the developing intestinal mucosa to subsequent injury or dysregulated inflammatory responses. Previous studies have linked presence of amniotic fluid infection/elevated cytokines (39), cord blood cytokines (40, 41), and fetal vasculitis (42) with risk for NEC in preterm infants. In a rat model of NEC, maternal prenatal exposure to

lipopolysaccharide led to increased frequency and severity of intestinal injury (43). Taken together, these observations suggest that intestinal injury may be initiated *in utero*. Hematologic parameters and postnatal age at NEC onset did not differ between *Ureaplasma*-positive and negative infants, suggesting that other postnatal factors are necessary for disease progression such as initiation of enteral feeds, prolonged exposure to antibiotics (44) or H2-blockers (45), or change in the intestinal microbiome.

There are several limitations of this study. *Ureaplasma* respiratory tract colonization in the first week of life was used as a proxy for intestinal mucosal exposure to this organism. Since the primary outcomes of the studies analyzed for this report were BPD and CNS outcomes, cultures were not obtained at the time of NEC onset. We also cannot exclude that infants were exposed antenatally to other microbes that may have altered intestinal permeability or the local immune response (46). Although the duration of ruptured membranes exceeded one hour in the majority of *Ureaplasma*-positive infants, indicating vertical transmission likely occurred via an ascending infection, the duration of exposure to the organism prior to delivery is unknown. Bacterial load of *Ureaplasma* that correlates with severity of intrauterine inflammation (47, 48) may be an important variable that was not measured in the current study.

Whether there is a causal relationship between perinatal *Ureaplasma* colonization/infection and NEC pathogenesis is currently unknown, but this can be addressed in *in vitro* and animal NEC models in future studies. Molecular methods may improve the detection of these organisms in relevant specimens such as gastric aspirates, stool, and surgical specimens (22) to confirm this association.

This study identifies *Ureaplasma* respiratory tract colonization, a marker of *in utero* infection/inflammation exposure, as a possible risk factor for NEC in VLBW infants. Whether *Ureaplasma* directly contributes to intestinal mucosal injury or alters the local immune response is unknown. Future experimental cell and animal models may determine how *Ureaplasma* contributes to NEC pathogenesis.

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Abbreviations

BPD	bronchopulmonary dysplasia
NEC	necrotizing enterocolitis
VLBW	very low birth weight

Table 1
Association of clinical variables and NEC

Variable	No NEC (N=339)	NEC (N=29)	Unadjusted OR (95% CI)	P value
Birth weight (g), mean ± SD	1021 ± 352	943 ± 288	0.999 (0.998-1.00)	0.247
Gestational age (wk), mean ± SD	27.3 ± 3.6	27.1 ± 2.2	0.985 (0.889-1.091)	0.771
Females, N (%)	161 (47.5)	10 (35)	0.58 (0.263-1.28)	0.182
Black race, N (%)	229 (67)	21 (72)	1.61 (0.59-4.413)	0.351
POL*, N (%)	266 (79)	21 (72)	0.711 (0.302-1.671)	0.433
PPROM, N (%)	145 (43)	13 (45)	1.09 (0.507-2.33)	0.830
Maternal antibiotics, N (%)	259 (76)	22 (76)	0.971 (0.399-2.356)	0.948
Cesarean section, N (%)	185 (55)	17 (58)	1.18 (0.546-2.545)	0.674
PDA, N (%)	174 (52)	12 (43)	0.694 (0.318-1.51)	0.358
Indomethacin, N (%)	151 (46)	11 (39)	0.767 (0.348-1.69)	0.510
Hypotension <4 days age, N (%)	92 (27)	12 (41)	1.857 (0.854-4.03)	0.119
Histologic chorioamnionitis**, N (%)	127 (69)	7 (54)	0.524 (0.178-1.44)	0.202
Fetal vasculitis**, N (%)	104 (57)	4 (31)	0.338 (0.100-1.136)	0.079
Ureaplasma colonization, N (%)	114 (34)	16 (55)	2.43 (1.13-5.22)	0.023
Cord serum IL-6 (pg/ml) †, median (IQR)	27.7 (6.9-163.2)	27.8 (5.69-280.8)	1.00 (0.99-1.00)	0.925
Cord serum IL-1β (pg/ml) †, median (IQR)	0.619 (0-4.873)	0 (0-0.977)	0.967 (0.87-1.01)	0.54

*Abbreviations: IQR, interquartile range; POL, pre-term onset labor; PPROM, preterm premature rupture of membranes; and PDA, patent ductus arteriosus

**Placentas were available for review in 197 subjects in cohort 1

†Cord serum samples were available from 101 subjects in cohort 1

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Table 2

Obstetric and neonatal characteristics of study cohort*

	NEC negative N=339			NEC positive N=29		
Variables	***Ureaplasma* (-) N=225**	***Ureaplasma* (+) N=114**	**P-value**	***Ureaplasma* (-) N=13**	***Ureaplasma* (+) N=16**	**P-value**
Birth weight (g), mean ± SD	1059 ± 355	945 ± 334	0.004	1060 ± 363	848 ± 168	0.048
Gestational age (wk), mean ± SD	27.8 ± 3	26.3 ± 4.2	<0.001	28.2 ± 2	26 ± 2	0.005
Males, N (%)	114 (51)	64 (56)	0.340	11 (85)	8 (50)	0.051
Black race, N (%)	153 (68)	76 (67)	0.504	11 (85)	10 (63)	0.396
POL*, N (%)	167 (75)	99 (87)	0.009	7 (54)	14 (88)	0.044
PPROM, N (%)	76 (34)	69 (61)	<0.001	3 (23)	10 (63)	0.034
ROM <1 hr, N (%)	107 (48)	33 (29)	0.001	8 (62)	5 (31)	0.103
PIH, N (%)	35 (16)	4 (4)	0.001	3 (23)	0	0.042
Clinical chorio-amnionitis, N (%)	47 (21)	33 (30)	0.070	1 (8)	6 (43)	0.049
Maternal antibiotics, N (%)	158 (70)	101 (89)	<0.001	7 (54)	15 (94)	0.013
Cesarean section, N (%)	133 (59)	52 (46)	0.018	9 (69)	8 (50)	0.296
PDA, N (%)	119 (54)	55 (48)	0.331	3 (25)	9 (56)	0.098
Indomethacin, N (%)	102 (47)	49 (44)	0.600	3 (25)	8 (50)	0.180
Late-onset sepsis, N (%)	71 (32)	38 (34)	0.761	9 (75)	9 (56)	0.306
Hypotension <4 days age, N (%)	58 (26)	34 (30)	0.502	5 (38)	7 (44)	0.774
IMV (d), median (IQR)	4 (0-21)	12 (0-34)	0.037	9 (5-16)	25 (10-38)	0.087
Supplemental oxygen (d), median (IQR)	30 (5-55)	52 (4-77)	0.0084	34 (23-50)	36 (22-77)	0.661
BPD at 36 wk PMA, N (%)	49 (23)	39 (35)	0.21	4 (31)	6 (43)	0.516
Length of stay (d), median (IQR)	52 (38-74)	73 (36-92)	0.006	87 (67-118)	85 (52-115)	0.443
Survival, N (%)	210 (95)	110 (97)	0.538	11 (85)	11 (69)	0.321
Death age (d), median (IQR)	15 (7-43)	11 (9-12)	0.391	25 (23-26)	21 (19-39)	0.696

*Abbreviations: IMV, intermittent mechanical ventilation; IQR; interquartile range; POL, pre-term onset labor; PPROM, preterm premature rupture of membranes; ROM, rupture of membranes; PIH, pregnancy induced hypertension; PDA, patent ductus arteriosus; and PMA, post-menstrual age.

Table 3

Characteristics of NEC infants with and without *Ureaplasma* respiratory tract colonization*

	Ureaplasma (-) N = 13	*Ureaplasma* (+) N = 16	P value
Age of onset, d, mean ± SD	22.2 ± 10.5	29.3 ± 19.4	0.246
Presence of feeds, N (%)	12 (92)	15 (100)	0.448
Medical NEC, N (%)	2 (15)	7 (44)	0.101
Surgical NEC, N (%)	11 (85)	9 (56)	
WBC* × 10^3 at NEC onset, mean ± SD	8.6 ± 5.3	12.8 ± 7.8	0.122
Platelets × 10^6 at NEC onset, mean ± SD	244 ± 188	286 ± 178	0.557

*Abbreviation: WBC, white blood cell count

Table 4

Markers of inflammation in infants with and without NEC

Variables	*NEC negative* N=340			*NEC positive* N=29*		
	***Ureaplasma* (-) N=225**	***Ureaplasma* (+) N=114**	**P-value**	***Ureaplasma* (-) N=13**	***Ureaplasma* (+) N=16**	**P-value**
Admission WBC** × 10^3, mean ± SD	11 ± 9.0	19.4 ± 16.1	<0.001	5.2 ± 2.4	16.7 ± 9.4	< 0.001
Admission ANC × 10^3, mean ± SD	5.37 ± 6.48	10.53 ± 1.18	<0.001	1.75 ± 1.16	8.73 ± 6.51	0.0007
Histologic chorioamnionitis, N (%)†	68 (56)	58 (92)	<0.001	1 (14)	6 (100)	0.052
Fetal vasculitis, N(%)	55 (45)	49 (79)	<0.001	0	4 (67)	0.009
Cord serum IL-6 (pg/ml), médian (IQR)‡	13.2 (0.73 -1098)	86.8 (5.9 - 1040)	<0.001	7.89 (1 -280.8)	297 (185 -1916)	0.039
Cord serum IL-1β (pg/ml), median (IQR)	0.23 (0 – 47.9)	1.91 (0 – 40.7)	0.003	0 (0 – 0.3)	10.4 (1.7 – 19.2)	0.022

*WBC and ANC data are derived from combined cohorts 1 and 2 ; Placental pathology and cord cytokine data are derived from cohort 1 only.

**Abbreviations: WBC, white blood cell count; ANC, absolute neutrophil count; IQR, interquartile range

†Placentas were available for review in 197 subjects in cohort 1

‡Cord serum samples were available from 101 subjects in cohort 1