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THE ANTIBIOTIC TREATMENT OF PRETERM PROM STUDY: SYSTEMIC MATERNAL AND FETAL MARKERS AND PERINATAL OUTCOMES

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Abstract

Objectives—To correlate maternal and cord blood cytokine and ICAM-1 levels with antibiotic exposure and perinatal outcomes after conservatively managed preterm PROM

Methods—Conservatively managed women with preterm PROM at 24–32 weeks had blood sampling at randomization (N=222) and delivery (N=121). Plasma from these, and umbilical cord blood (N=196), was stored at –70C. IL-6, IL-10, G-CSF, TNF-alpha and ICAM-1 levels were assessed for associations with antibiotic treatment, latency, amnionitis, neonatal sepsis, pneumonia, and composite neonatal morbidity.

Results—Cord blood IL-6 and G-CSF were higher than maternal levels. Antibiotic treatment lowered only maternal G-CSF ($p=0.01$). Elevated maternal cytokine levels were associated with delivery within seven days and with development of chorioamnionitis. All umbilical cord blood markers were increased with amnionitis ($p\leq 0.01$ for each). No maternal marker was associated with neonatal morbidities. Cord G-CSF and IL-6 were increased with neonatal sepsis within 72 hours of birth ($p=0.004$ for both), and with composite neonatal morbidity; ($p=0.001$ and 0.002 , respectively). Maternal and umbilical cord cytokine levels demonstrated low predictive values for perinatal outcomes.

Conclusions—Umbilical cord blood cytokine values are higher than maternal levels, suggesting significant fetal/placental contribution. Maternal and umbilical cord cytokine levels are not adequately predictive to be used clinically.

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Keywords

cytokines; tumor necrosis factor-alpha; granulocyte colony stimulating factor; intercellular adhesion molecule-1; premature rupture of membranes; PROM

Introduction

Preterm premature rupture of the membranes (preterm PROM) is associated with brief latency from membrane rupture to delivery, infectious complications, and adverse neonatal outcomes related to preterm birth.(1,2) The availability of markers that could predict these adverse outcomes would be helpful in guiding management when preterm PROM occurs remote from term. Prior knowledge of these outcomes could heighten awareness, and might lead to a decision to proceed with early delivery if conservative management were destined to fail.

Elevated maternal plasma and amniotic fluid cytokines in the second trimester and at delivery have been associated with adverse pregnancy and neonatal outcomes in symptomatic and asymptomatic gravidas.(3–16) Elevated cytokine levels in neonatal cord blood have been associated with perinatal infectious complications in the mother and newborn. (17–20) Adhesion molecules play an important role in the interaction between neutrophils and vascular endothelium in response to inflammatory stimuli. Circulating intercellular adhesion molecule-1 (ICAM-1) levels are also increased in both adults and neonates with sepsis. However, studies of inflammatory markers with respect to preterm PROM have generally included women already experiencing labor and/or amnionitis, or have studied heterogeneous groups with both preterm PROM and preterm labor with intact membranes. Further, the impact of antibiotic therapy on maternal cytokine levels has not been prospectively studied in this setting.

To be useful clinically, predictive markers of perinatal complications should discriminate between those who will and will not have the adverse outcome, and these differences should be evident before the outcome occurs. Optimally, such markers would be obtained non-invasively. Herein, we evaluate maternal and umbilical cord plasma cytokine (granulocyte-colony stimulating factor, interleukin-6, interleukin-10, tumor necrosis factor-alpha) and ICAM-1 levels during conservative management of preterm PROM remote from term to evaluate their change during conservative management, the relative distributions of these in the fetal and maternal circulation, the impact of antibiotic treatment on their levels, and their predictive value for adverse perinatal outcomes.

Materials and Methods

This study includes women, with singleton gestations, who participated in a multicenter double-masked trial of antibiotic treatment of preterm PROM at 24–32 weeks' gestation. Details of the study have previously been reported.(21) Briefly, this trial was performed at 11 clinical centers between 1992 and 1995. A total of 614 non-laboring gravidas with preterm PROM, were randomized to antibiotic therapy or a matching placebo regimen during conservative management. The antibiotic regimen consisted of intravenous ampicillin (2g q6h) and erythromycin (250 mg q6h) for 48 hours, followed by oral amoxicillin (250 mg q8h) and erythromycin-base (333 mg q8h) for 5 days unless delivery occurred. Group B streptococcus (GBS) carriers were identified and treated prior to and during labor. Consistent with prevalent practice at that time, tocolysis and corticosteroid administration were prohibited subsequent to randomization. Elective labor induction prior to 34 weeks was prohibited. Neonatal management was left to the discretion of the neonatal care-giver. Head

sonograms were performed routinely on newborns weighing less than 1,750 grams, and on larger infants when clinically indicated. The protocol was reviewed and approved by the Human Research committees of all participating institutions prior to initiation of randomization.

Seven of eleven participating clinical centers undertook to collect maternal blood at randomization and at the time of delivery, and umbilical cord blood at the time of delivery. Specimens were collected into pre-chilled 10 ml EDTA-treated glass tubes, and underwent refrigerated centrifugation (4°C) at 200 G. The plasma supernatant was then stored in aliquots at -70°C until they were transported in dry ice to a central laboratory at the University of Tennessee, Memphis, for analysis (D.T.C.). Interleukin-6 (IL-6; sensitivity: 1 pg/ml, Endogen Inc.), Interleukin-10 (IL-10; sensitivity: 3 pg/ml, Endogen Inc.), granulocyte-colony stimulating factor (G-CSF; sensitivity: 39 pg/ml, R & D Systems), tumor necrosis factor-alpha (TNF-alpha; sensitivity: 5 pg/ml, Endogen Inc.), and circulating ICAM-1 (ICAM-1; sensitivity: 7 ng/ml, R&D Systems) levels were determined in batch fashion by personnel masked to study group assignment and clinical course.

Evaluated perinatal outcomes included: delivery within 7 days after randomization, clinical amnionitis, confirmed neonatal sepsis within 72 hours of birth or before discharge, neonatal pneumonia before discharge, and composite neonatal morbidity (one or more of: fetal/neonatal death, neonatal sepsis within 72 hours of birth, grade 3–4 IVH, stage 2–3 necrotizing enterocolitis, or respiratory distress syndrome). The diagnosis of clinical amnionitis required at least two of: antepartum temperature $\geq 100.4^{\circ}$ F, uterine tenderness, foul smelling vaginal discharge or amniotic fluid, maternal tachycardia (>100 bpm), fetal tachycardia (>160 bpm), or maternal white blood cell count $>20,000$. Respiratory distress syndrome (RDS) was diagnosed in symptomatic infants who required ventilatory support for at least 24 hours and had radiographically confirmed hyaline membrane disease or a diagnosis of respiratory insufficiency of prematurity. Confirmed sepsis was diagnosed when an infant had suspicious clinical findings and a positive blood or cerebrospinal fluid culture or evidence of cardiovascular collapse requiring volume expansion or pressor agents. Pneumonia was diagnosed if there were compatible symptoms with diagnostic X-ray findings and positive blood cultures, or persistent leukopenia ($<4,000$) or a band count $>15\%$.

Statistical analyses were performed using SAS® statistical software. Cytokine (pg/ml) and ICAM-1 (ng/ml) levels are presented as medians [25th – 75th percentile]. Multivariable logistic regression controlled for gestational age at randomization, GBS culture status, and antibiotic study group assignment. Biomarker values for patients with all three blood samples available were compared in a pair-wise fashion using Wilcoxon matched pairs signed rank test. For this, the Bonferroni corrected p-value of 0.017 was used to declare significance. Biomarker values for groups with and without outcomes were compared using the Wilcoxon test. Where significant associations were identified, receiver operator characteristic analyses were performed to determine the area under the curve, optimal cutoff points, and predictive values of maternal and umbilical cord markers for brief latency, amnionitis, and adverse neonatal outcomes.

Results

The seven participating centers contributed 417 patients to the overall study (68% of eligible patients), and blood samples were collected from 277 of these patients (66%, range: 10–75 subjects per center, Figure 1.) IL-6, IL-10, G-CSF, TNF-alpha and ICAM-1 levels were analyzed from 222 maternal samples obtained at randomization and 121 at delivery, and from 196 umbilical cord blood samples. Eighty-two patients had all three samples collected.

Clinical characteristics of the pregnancies evaluated in this analysis were similar for those with samples available at randomization, delivery, and from umbilical cord blood (Table 1). The study population was 61% African American with a mean gestation of 29 weeks at randomization. Perinatal outcomes included: clinical amnionitis (31%), neonatal sepsis (14%), pneumonia (4%), and composite morbidity (48%).

Plasma marker distribution in maternal and umbilical cord blood

Evaluation of subjects with all three samples available, and who were assigned to placebo therapy (N=42), revealed umbilical cord blood levels to be higher than maternal levels obtained at randomization and at delivery for IL-6 (median [25–75th percentile]: 185 [41–908] vs. 4 [2–8] and 46 [25–87] pg/ml respectively, $p \leq 0.0001$ for each), and for G-CSF (1,359 [545–5,353] vs. 69 [54–97] and 461 [280–818] pg/ml respectively, $p \leq 0.0001$ for each). Maternal ICAM-1 levels from blood obtained at randomization and at delivery were higher than those obtained from umbilical cord blood (214 [150–269] and 271 [206–341] vs. 163 [117–227] ng/ml, $p < 0.0001$ for each). IL-10 levels were similar between umbilical cord blood (10 [7–20] pg/ml), maternal randomization (3 [3–5] pg/ml) and maternal delivery (27 [16–62] pg/ml) samples. TNF-alpha levels were rarely positive, with over 99, 95, and 90% of samples having undetectable levels at randomization, delivery and in cord blood.

Impact of antibiotic administration on plasma markers

No differences were identified in initial maternal systemic markers between the antibiotic and placebo groups, other than for ICAM-1 which was lower in the placebo group at randomization than the antibiotic group ($p=0.02$). (Table 2) After controlling for gestational age at entry and group B streptococcus carriage, antibiotic treatment resulted in significantly lower maternal G-CSF levels at delivery than in the placebo group (284 vs. 439 pg/ml, $p=0.01$), and statistically insignificant trend toward lower IL-6 levels (28 vs. 35 pg/ml, $p=0.08$), but no other differences in maternal or umbilical cord blood were seen.

Associations between plasma markers and subsequent perinatal outcomes

Maternal blood at randomization—Initial maternal G-CSF ($p=0.008$), IL-6 ($p=0.003$), and IL-10 ($p=0.01$) levels were significantly higher among patients delivering within 7 days after randomization compared with those delivering thereafter (Table 3). However, marked overlap was seen between groups for each marker. Initial TNF-alpha and ICAM-1 levels were not associated with subsequent early delivery. Though initial maternal plasma IL-10 and TNF-alpha levels were higher with subsequent amnionitis ($p=0.05$ and 0.03 respectively), no other marker evaluated at randomization was associated with subsequent maternal amnionitis (Table 3), or adverse neonatal outcomes (neonatal sepsis within 72 hours and before discharge, neonatal pneumonia, composite morbidity) (Table 4).

Maternal blood at delivery—At delivery, maternal G-CSF ($p=0.008$), IL-6 ($p=0.003$), IL-10 ($p=0.02$) and TNF-alpha ($p=0.02$) levels were each higher in the presence of clinical amnionitis, but no marker was significantly associated with brief latency (Tables 3,4). Neither TNF-alpha nor ICAM-1 was associated with brief latency or clinical amnionitis.

Regarding neonatal morbidities, maternal blood levels at delivery were not generally associated with adverse outcomes (Table 4). Maternal plasma TNF-alpha ($p=0.001$) was statistically associated with confirmed neonatal sepsis before discharge.

Umbilical cord blood—Reflecting fetal status at delivery, G-CSF, IL-6, and IL-10 were higher among umbilical cord samples from newborns delivered within 7 days after randomization compared with those delivering thereafter ($p < 0.02$ for each, Table 3). All marker levels were higher in the presence of clinical amnionitis ($p \leq 0.01$ for each). G-CSF,

IL-6, and IL-10 were higher in infants developing confirmed neonatal sepsis within 72 hours of birth ($p \leq 0.04$ for each, Table 4). Similarly, G-CSF, IL-6 and IL-10 levels were significantly associated with neonatal sepsis before discharge ($p \leq 0.03$ for each). G-CSF and IL-6 were also elevated with neonatal composite morbidity ($p \leq 0.002$ for each). However, TNF-alpha and IL-10 levels in cord blood were not significantly increased with any adverse outcomes, and no cord blood marker was significantly associated with neonatal pneumonia.

Results regarding associations between plasma markers and subsequent perinatal outcomes for the 82 subjects from whom all three samples were available (maternal plasma at admission and delivery, umbilical cord blood) were similar to the overall cohort (data not shown).

Prediction of brief latency and adverse perinatal outcomes

For markers that were significantly associated with adverse outcomes, receiver operator characteristic analyses revealed low areas under the curves (<0.70) for maternal plasma cytokines and delivery within 7 days and clinical amnionitis. (Table 5, Figure 2.) Regarding newborn outcomes, umbilical cord blood G-CSF demonstrated an area under the curve >0.70 for prediction of neonatal sepsis within 72 hours of birth (Table 5, Figure 2), but in all other analyses the area under the curve was <0.70 . None of the measured cytokines demonstrated high sensitivity, specificity or discriminative predictive values for any outcome, reflecting the significant overlap in plasma levels for each marker.

Comment

The associations between preterm birth and both maternal and fetal infections have been well established, and cross-sectional studies have identified associations between intra-amniotic infection and elevated intra-amniotic cytokines. (22–24) Similarly, associations between elevated neonatal cytokine levels and concurrent infections have been established. However, prospective studies of asymptomatic conservatively managed women with PROM remote from term for adverse outcomes have been lacking.

We prospectively evaluated women who were considered candidates for conservative management of preterm PROM remote from term, and evaluated potential systemic markers of subsequent brief latency, intrauterine infection and neonatal complications. We also prospectively evaluated the impact of antibiotic treatment for latency prolongation on these levels. We found that, though maternal plasma IL-6, IL-10 and G-CSF levels obtained at the initiation of conservative management of preterm PROM are statistically associated with brief latency, and IL-10 levels are associated with subsequent clinical amnionitis, there is marked variation in levels within groups and overlap between those destined or not to suffer these complications. This overlap limits the potential of these markers to predict perinatal outcomes and guide initial treatment.

Initial maternal plasma markers are not associated with adverse neonatal outcomes, and maternal plasma markers evaluated at delivery are less closely associated with neonatal complications than umbilical cord blood markers which can be evaluated from discarded placentas immediately after delivery. Because umbilical cord blood reflects the intrauterine fetal environment and is associated with subsequent infectious and composite morbidity, cord blood evaluation could potentially be useful in guiding initial newborn care after preterm PROM. However, the highly variable levels of these markers among infants with and without subsequent morbidities limits the predictive value of such testing. It is possible that the combination of levels of these markers and clinical findings could further refine the predictive value of these tests, but our sample size was inadequate to evaluate this.

Despite the demonstrated clinical benefits antibiotic treatment in the randomized trial that provided samples for this analysis, we found only marginal effects of antibiotic treatment on cytokine levels at delivery; specifically, decreased maternal but not fetal G-CSF levels. Though this may reflect an inability of antibiotic treatment to adequately suppress the maternal inflammatory response to subclinical intrauterine infection already present on admission or to prevent an increasing maternal inflammatory response during conservative management, it is also plausible that maternal inflammation becomes evident with labor and delivery regardless of prior events during conservative management of preterm PROM, and that intrapartum events overshadow any effects of antibiotics before delivery. Because neonatal infection and inflammation has been shown to be associated with an increased risk of long-term sequelae, it is disappointing that maternally administered antibiotics did not affect umbilical cord blood levels of these inflammatory markers.

We chose to study inflammatory markers that could be implicated in the infectious process through several mechanisms. TNF-alpha is a key acute phase pro-inflammatory mediator that stimulates the host's inflammatory response to infectious stimuli and injury. In addition stimulating to immune cell function, TNF-alpha leads to increased pro-inflammatory cytokine production. Peak TNF-alpha levels can be achieved within hours of noxious stimuli, with levels returning to baseline thereafter. Thus, rather than being unresponsive to the ongoing processes occurring during conservative management of preterm PROM, it is plausible that TNF-alpha levels in our cohort had already increased and returned to baseline levels before presentation for care. ICAM-1 plays an important role in the interaction between neutrophils and vascular endothelium in response to inflammatory stimuli, including sepsis. It is plausible that infection related endothelial damage could alter ICAM-1 levels in the mother and fetus. However, we found no consistent findings in this regard. Inhibitory cytokines such as IL-10 may play a role in the maintenance of pregnancy. Produced mainly by T-lymphocytes and macrophages, IL-10 inhibits IL-6, TNF-alpha, and IL-8 levels and is increased in active intrauterine infection. We postulated that low maternal IL-10 levels at initial treatment might be associated with an increased inflammatory response and worse pregnancy outcomes. While these findings were evident, strong associations were not identified. Among the most well studied pro-inflammatory cytokines in the setting of preterm birth are IL-6 and G-CSF. We found maternal levels of these cytokines to increase during conservative management, confirming that continued pregnancy increases the potential exposure to a pro-inflammatory environment. However, the higher levels IL-6 and G-CSF seen in umbilical cord blood than in maternal blood suggests strong fetal/placental participation in the inflammatory response related to intrauterine infection and preterm parturition. We did find these markers to be elevated in the setting of maternal and neonatal infection, and also to be associated with brief latency when obtained at initiation of treatment. These elevations likely reflect a subclinical inflammatory process that is present at the time of initial evaluation of these women. Though statistically associated, the wide variation of values among those with good and poor outcomes mitigates against the utility of these cytokines as predictive markers before delivery.

This study is limited by the lack of available fetal blood and amniotic fluid samples at the onset of conservative management. While the availability of such information would be of interest regarding the biologic mechanisms related to our findings, they are not generally available to guide clinical management. Our study is further limited by the lack of available histopathologic placental studies in many cases. Regardless, clinical decision making occurs before delivery and before the availability of placental evaluation. Consistent with our findings regarding maternal systemic inflammatory markers, we have previously found that antibiotic treatment during conservative management of preterm PROM does not significantly impact histological findings in the placenta and membranes.⁽²⁵⁾ It is plausible

that the inflammatory cascade cannot be stopped once initiated. Alternatively it may also be possible that intrapartum events obscure the effects of antibiotics on placental and cytokine findings regardless of extended latency and absence of overt intrauterine infection until near the time of delivery.

In this study, we have found that the evaluated maternal systemic inflammatory markers are marginally affected by antibiotic treatment during conservative management of preterm PROM remote from term, and that evaluation of these systemic inflammatory markers at initiation of treatment is not likely to provide adequately predictive information regarding subsequent adverse maternal and neonatal outcomes to be clinically useful. Similarly, while increased levels of the evaluated umbilical cord blood pro-inflammatory cytokines are associated with subsequent adverse neonatal outcomes, significant overlap in values between newborns with and without subsequent complications limits the predictive and clinical value of these markers for use in clinical practice.

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Listed in the appendix

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Condensation

Umbilical cord G-CSF and IL-6, but not maternal cytokine levels, are associated with adverse neonatal outcomes after conservatively managed preterm PROM.

Figure 1.
Flow diagram of patient recruitment and specimen collection for this biologic fluids ancillary study.

Figure 2.

Receiver operator characteristic curves for selected outcomes according to cytokine levels from maternal blood at randomization (2a) and umbilical cord blood (2b, 2c) among conservatively managed women with PROM before 32 weeks gestation.

Table 1

Baseline characteristics and perinatal outcomes for those with maternal and newborn plasma samples obtained at randomization, delivery, and from umbilical cord blood.

N	Maternal Plasma at Randomization 222	Maternal Plasma at Delivery 121	Umbilical Cord Plasma 196
Baseline characteristics			
Maternal age (years)*	24.8 (5.9)	25.2 (6.1)	25.1 (5.8)
Race (% African American)	61	60	61
Married (%)	29	27	28
Prior preterm delivery (%)	36	37	36
Gestation at randomization (weeks)*	28.9 (2.3)	29.1 (2.2)	29.2 (2.2)
Cervical dilation (median cm)	0	0.5	0
Perinatal outcomes			
Delivery within 7 days (%)	66	61	63
Clinical amnionitis (%)	31	28	27
Confirmed neonatal sepsis within 72 hours (%)	8	10	8
Neonatal sepsis before discharge(%)	14	16	14
Neonatal pneumonia before discharge(%)	4	4	3
Composite neonatal morbidity [#] (%)	52	53	45

* mean (standard deviation)

[#] one or more of: fetal/neonatal death, neonatal sepsis within 72 hours of birth, grade 3–4 intraventricular hemorrhage, stage 2–3 necrotizing enterocolitis, or respiratory distress syndrome

Table 2

Antibiotic treatment during conservative management of preterm PROM and maternal plasma and umbilical cord marker levels.

Maternal Plasma at Randomization	Antibiotics N=111	Placebo N=111	P-value
G-CSF (pg/ml)	64 (49–88)	66(48–97)	0.88
ICAM-1 (ng/ml)	264 (212–320)	235 (178–299)	0.02
IL-10 (pg/ml)	3 (2–5)	3 (2–6)	0.43
IL-6 (pg/ml)	3 (2–6)	3 (2–7)	0.72
TNF-alpha (pg/ml)	4 (4–4)	4 (4–4)	1.00

Maternal Plasma at Delivery	N=60	N=60	
G-CSF (pg/ml)	284 (154–452)	439 (213–831)	0.01
ICAM-1 (ng/ml)	284 (232–343)	261 (196–332)	0.13
IL-10 (pg/ml)	16 (10–40)	22 (12–53)	0.26
IL-6 (pg/ml)	28 (15–48)	35 (21–85)	0.08
TNF-alpha (pg/ml)	4 (4–4)	4 (4–4)	1.00

Umbilical Cord Plasma	N=96	N=94	
G-CSF (pg/ml)	1199 (272–5895)	1476 (517–5748)	0.31
ICAM-1 (ng/ml)	159 (112–221)	167 (118–227)	0.69
IL-10 (pg/ml)	12 (5–24)	13 (6–28)	0.17
IL-6 (pg/ml)	146 (26–438)	209 (38–1090)	0.12
TNF-alpha (pg/ml)	4 (4–4)	4 (4–4)	0.55

Values presented as median (25th–75thpercentiles)

Table 3

Maternal plasma and umbilical cord marker levels and pregnancy outcomes latency (delivery within 7 days, clinical chorioamnionitis) after conservative management of preterm PROM.

		Maternal Plasma at Randomization Median (25 th –75 th %ile)		Maternal Plasma at Delivery Median (25 th –75 th %ile)		Umbilical Cord Plasma Median (25 th –75 th %ile)	
Delivery within 7 days after randomization		<7 days	>= 7 days	p-value	<7 days	>= 7 days	p-value
N		146	76		74	47	73
G-CSF (pg/ml)		71 (49–103)	59 (49–76)	p=0.008	335 (186–666)	332 (188–622)	794 (339–2023)
ICAM-1 (ng/ml)		250 (186–312)	250 (214–309)	p=0.43	271 (197–332)	281 (223–344)	160 (114–206)
IL-10 (pg/ml)		3 (2–7)	2 (2–4)	p=0.01	18 (7–40)	21 (14–51)	15 (8–32)
IL-6 (pg/ml)		4 (2–8)	3 (2–4)	p=0.003	31 (15–64)	34 (19–55)	99 (23–319)
TNF-alpha (pg/ml)		4 (4–4)	4 (4–4)	p=0.31	4 (4–4)	4 (4–4)	4 (4–4)
Clinical amnionitis							
		Amnionitis	No amnionitis	p-value	Amnionitis	No amnionitis	p-value
N		68	152		34	87	144
G-CSF (pg/ml)		76 (48–104)	64 (49–85)	p=0.13	570 (285–967)	298 (181–550)	6070 (1600–13670)
ICAM-1 (ng/ml)		257 (173–323)	245 (203–310)	p=0.67	285 (213–350)	264 (211–329)	158 (111–214)
IL-10 (pg/ml)		4 (2–7)	3 (2–5)	p=0.05	33 (15–66)	16 (10–37)	10 (4–18)
IL-6 (pg/ml)		4 (2–10)	3 (2–5)	p=0.10	46 (24–126)	29 (13–48)	100 (26–400)
TNF-alpha (pg/ml)		4 (4–4)	4 (4–4)	p=0.03	4 (4–4)	4 (4–4)	4 (4–4)

Table 4

Maternal plasma and umbilical cord marker levels and adverse newborn outcomes sepsis after conservative management of preterm PROM.

		Maternal Plasma at Randomization Median (25 th –75 th %ile)		Maternal Plasma at Delivery Median (25 th –75 th %ile)		Umbilical Cord Plasma Median (25 th –75 th %ile)	
Confirmed neonatal sepsis before discharge		Present	Absent	p-value	Present	Absent	p-value
N		31	190		19	101	
G-CSF (pg/ml)		71 (52–101)	65 (49–94)	p=0.19	357 (280–1079)	314 (183–611)	p=0.06
ICAM-1 (ng/ml)		238 (216–315)	254 (190–310)	p=0.50	290 (223–350)	270 (213–332)	p=0.46
IL-10 (pg/ml)		3 (2–10)	3 (2–5)	p=0.15	29 (12–72)	18 (10–39)	p=0.23
IL-6 (pg/ml)		5 (2–10)	3 (2–6)	p=0.42	47 (18–131)	30 (16–50)	p=0.08
TNF-alpha (pg/ml)		4 (4–4)	4 (4–4)	p=0.15	4 (4–4)	4 (4–4)	p=0.001
Confirmed neonatal sepsis within 72 hours of birth		Present	Absent	p-value	Present	Absent	p-value
N		18	203		12	108	
G-CSF (pg/ml)		69 (56–88)	65 (49–95)	p=0.57	393 (281–1198)	325 (185–619)	p=0.10
ICAM-1 (ng/ml)		254 (221–328)	250 (193–310)	p=0.34	303 (240–374)	271 (212–336)	p=0.29
IL-10 (pg/ml)		4 (2–10)	3 (2–5)	p=0.42	35 (14–123)	18 (10–41)	p=0.07
IL-6 (pg/ml)		3 (1–5)	3 (2–6)	p=0.32	59 (29–146)	31 (16–55)	p=0.05
TNF-alpha (pg/ml)		4 (4–4)	4 (4–4)	p=0.67	4 (4–4)	4 (4–4)	p=0.06
Neonatal pneumonia before discharge		Present	Absent	p-value	Present	Absent	p-value
N		9	212		5	115	
G-CSF (pg/ml)		79 (56–97)	65 (49–94)	p=0.43	335 (281–349)	329 (186–645)	p=0.99
ICAM-1 (ng/ml)		240 (227–425)	250 (193–310)	p=0.27	256 (244–421)	274 (213–340)	p=0.56
IL-10 (pg/ml)		3 (2–4)	3 (2–6)	p=0.68	13 (8–18)	19 (11–43)	p=0.34
IL-6 (pg/ml)		4 (2–7)	3 (2–6)	p=0.75	10 (8–35)	32 (17–61)	p=0.17
TNF-alpha (pg/ml)		4 (4–4)	4 (4–4)	p=0.77	4 (4–4)	4 (4–4)	p=0.77
Composite neonatal morbidity		Present	Absent	p-value	Present	Absent	p-value
N		116	106		64	57	
G-CSF (pg/ml)		9251 (1499–26415)	1315 (362–4420)	p=0.004	2279 (504–9319)	1366 (385–5523)	p=0.51
ICAM-1 (ng/ml)		207 (145–232)	162 (112–224)	p=0.18	153 (117–251)	167 (113–224)	p=0.93
IL-10 (pg/ml)		20 (13–45)	12 (5–25)	p=0.04	11 (4–19)	13 (5–27)	p=0.59
IL-6 (pg/ml)		1041 (412–7033)	161 (32–666)	p=0.004	298 (26–1090)	182 (34–935)	p=0.89
TNF-alpha (pg/ml)		4 (4–4)	4 (4–4)	p=0.25	4 (4–4)	4 (4–4)	p=0.41

	Maternal Plasma at Randomization Median (25 th –75 th %ile)			Maternal Plasma at Delivery Median (25 th –75 th %ile)			Umbilical Cord Plasma Median (25 th –75 th %ile)		
G-CSF (pg/ml)	67 (49–99)	65 (49–84)	p=0.30	344 (206–846)	286 (181–554)	p=0.17	2204 (729–8722)	785 (253–2242)	p=0.001
ICAM-1 (ng/ml)	250 (192–314)	252 (194–309)	p=0.82	269 (210–337)	281 (218–340)	p=0.57	167 (111–241)	162 (114–221)	p=0.57
IL-10 (pg/ml)	3 (2–6)	3 (2–5)	p=0.74	20 (13–53)	16 (7–40)	p=0.13	13 (6–32)	11 (5–21)	p=0.13
IL-6 (pg/ml)	3 (2–8)	3 (2–6)	p=0.86	37 (20–73)	24 (13–48)	p=0.06	398 (54–2401)	136 (26–390)	p=0.002
TNF-alpha (pg/ml)	4 (4–4)	4 (4–4)	p=0.18	4 (4–4)	4 (4–4)	p=0.18	4 (4–4)	4 (4–4)	p=0.13

Table 5

Predictive values of maternal plasma and umbilical cord markers for adverse perinatal outcomes.

	Area under curve	Cut-off (pg/ml)	Positive predictive value (%)	Negative predictive value (%)	Sensitivity (%)	Specificity (%)
Maternal plasma at randomization and delivery within 7 days (cohort risk 34%)						
G-CSF (pg/ml)	0.602	89	14.8	58.4	11.8	64.4
IL-6 (pg/ml)	0.620	5	18.9	58.1	18.4	58.9
IL-10 (pg/ml)	0.595	4	25.3	59.1	31.6	51.4
Maternal plasma at randomization and clinical amnionitis (cohort risk 31%)						
IL-10 (pg/ml)	0.580	5	42.0	74.2	42.6	73.7
Umbilical cord plasma and neonatal sepsis within 72 hours of birth (cohort risk 8%)						
G-CSF (pg/ml)	0.711	2950	19.3	96.3	68.8	73.9
IL-6 (pg/ml)	0.686	477	719.7	97.0	75.0	72.8
IL-10 (pg/ml)	0.651	18	14.3	96.0	66.7	66.3
Umbilical cord plasma and neonatal sepsis before discharge (cohort risk 14%)						
G-CSF (pg/ml)	0.674	9183	36.4	90.6	44.4	87.3
IL-6 (pg/ml)	0.629	436	23.9	90.7	57.1	69.6
IL-10 (pg/ml)	0.627	34	31.4	90.5	79.8	57.7
Umbilical cord plasma and composite neonatal morbidity (cohort risk 45%)						
G-CSF (pg/ml)	0.637	2665	66.1	66.2	48.2	80.4
IL-6 (pg/ml)	0.636	418	64.7	65.6	50.0	77.8