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Cord Blood Biomarkers of the Fetal Inflammatory Response

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Abstract

In current neonatal practice, clinical signs of intrauterine infection (IUI) are often non-specific. From a large panel of immune biomarkers, we seek to identify cord blood markers that are most strongly associated with the fetal inflammatory response (FIR), a specific placental lesion associated with serious neonatal complications. We used multiplex immunoassay to measure 27 biomarkers, selected as part of an NIH-funded study of preterm birth, according to gestational age(GA) and extent of placental inflammation: involvement of chorion, amnion, decidua (maternal inflammatory response, MIR); extension to umbilical cord or chorionic plate (FIR). We used false-discovery rate (FDR<5%, P<0.001) to account for multiple comparisons. Among 506 births (GA 23-42wks), IL-1β increased with FIR among preterm subgroups (P=0.0001 for <32wks; P=0.0009 for 33-36wks). IL-6 and IL-8 increased with FIR among preterm and full-term infants (P<0.0001). P-trend for IL-6 and IL-8 with MIR versus FIR was <0.0001. Comparison with respect to clinical IUI yielded persistent elevation with FIR even when clinical signs were absent. The remaining 24 markers were not significantly associated. We conclude that among 27 cord blood biomarkers,

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IL-1β, IL-6 and IL-8 are selectively associated with FIR. These markers may be clinically useful indicators of extensive IUI associated with poor neonatal outcome.

Keywords

Placenta; chorioamnionitis; cytokines; intrauterine infection

INTRODUCTION

Infants born to mothers with clinical evidence of intrauterine infection (IUI) are often treated empirically for presumed neonatal sepsis, in an effort to minimize the adverse outcomes associated with clinical sepsis syndrome. Despite the use of broad spectrum antibiotics, both in the antenatal and postnatal periods, the occurrence of maternal chorioamnionitis and its consequences remains problematic. A sensitive marker of IUI that is linked to adverse neonatal outcome is FIR, a histopathologic finding of inflammatory infiltration to the fetal component of the placental unit. [1] A less extensive form is MIR, in which neutrophilic infiltration is confined to the chorion, amnion, and decidua. [2] The incidence of histologic chorioamnionitis, as defined by the presence of MIR or FIR, has been reported as high as 33% among preterm infants. [3] FIR is associated with increased risk of neonatal sepsis, chronic lung disease, brain injury, and death. [4] More recent epidemiological studies have shown that the incidence of neonatal morbidity is substantially higher with FIR as compared with MIR alone. [5] Better identification of high-risk infants is needed for more targeted management and prevention of these adverse outcomes. Therefore, early biochemical markers that reliably detect the presence of FIR are needed.

Numerous cytokines and related inflammatory biomarkers have been studied to better understand the pathophysiology of preterm birth and the role of IUI. Few have been studied for their association with FIR. The proinflammatory markers IL-6 and IL-8 are the most extensively reported in association with clinical and histologic chorioamnionitis. [6] IL-1β and TNF-α have also been identified as markers of infection. [7] The relative sensitivity of these markers in detecting placental inflammatory changes as compared to alternative immunologic markers has not been reported. Other potential markers of FIR include enzymes and receptors which act as chemokines and regulatory proteins in the cytokine cascade. [8-10] These include degradation enzymes involved in membrane rupture such as matrix metalloproteinase (MMP-9), growth factors related to neuronal maturation such as brain-derived neurotrophic factor (BDNF) and neurotrophins (NT-3, NT-4), and receptors that mediate these immune processes. Many of these have been reported as being either significantly elevated or decreased with preterm birth, [11,12] however, it remains unclear whether intrauterine infection/inflammation is an underlying mechanism of these markers.

In this report, we simultaneously measured the levels of 27 cytokines, chemokines, and neurotrophin markers in the cord blood of 506 births with GA ranging from 23 to 42 weeks. Using corresponding placental data, we analyzed the associations of these 27 markers with the presence and extent of histologic inflammation, accounting for GA. Our objective was to identify from a large panel of immune biomarkers those which are most strongly associated

with a placental inflammatory response, and in particular, with FIR. We hypothesized that previously well-established markers of clinical and histologic chorioamnionitis (IL-1β, IL-6, IL-8, and TNF-α) would be selectively elevated with the presence of placental inflammation, and that the strength of association would increase with the extent of placental inflammatory response (MIR versus FIR). Identification of cord blood markers of FIR will enhance our understanding of the pathways of preterm birth that are mediated by intrauterine infection/inflammation. In addition, our findings may potentially lead to the identification of important clinical prognostic markers.

METHODS

Study population

The population was drawn from a larger case-control study evaluating environmental and genetic determinants of preterm delivery and low birthweight. [13] The parent study (1998-present) is being conducted at Boston Medical Center, a large urban hospital with a predominantly minority, inner-city patient population. Eligible cases were singleton live preterm births under 37 weeks gestation with low birthweight (<2500g). Corresponding healthy term controls (≥37 weeks gestation) with normal birthweight (≥2500g) were also enrolled. Multiple pregnancies, births that resulted from maternal trauma, and newborns with major birth defects were excluded. Cord blood was collected from all births, and placentas were sent for pathology based upon routine clinical indications, including prematurity. The study was approved by the Institutional Review Boards at Boston University Medical Center, Children's Hospital Boston, Beth Israel Deaconess Medical Center, and Children's Memorial Hospital.

Clinical and demographic information

Maternal interview was conducted using a structured questionnaire that included demographic characteristics, medical and reproductive history. In addition, medical record review was conducted using a standardized abstraction form that included data on prenatal care, clinical presentation, intrapartum management, pregnancy complications, and birth outcomes. With regard to clinical signs of intrauterine infection, we examined six variables known to be associated with intrauterine infection syndrome:[14] intrapartum fever >38°C; elevated maternal white blood cell count (WBC) >15,000/mL; maternal heart rate >100bpm; fetal heart rate >160bpm; uterine tenderness; and foul-smelling amniotic fluid or vaginal discharge.

Determination of gestational age

GA was assessed with an algorithm based upon last menstrual period and early ultrasound before 20 weeks gestation. [15] Births were distributed among 3 groups according to increasing GA: 1) *Very preterm group* included births occurring at ≤32wks gestation. 2) *Moderately preterm group* included births at 33-36wks gestation. 3) *Full-term group* included births at ≥37wks.

Cord blood biomarker assays

Cord blood was obtained by trained nursing staff of the labor and delivery service. Blood samples were kept on ice and subsequently centrifuged for 10 minutes in a tabletop refrigerated centrifuge at 2500rpm. Plasma was removed from the cell pellet by pipetting. Each subject's plasma sample was split into 3 aliquots and stored in a −80°C freezer. The following panel of 27 markers was pre-selected based upon an ongoing NIH-funded study to understand biomarkers of intrauterine infection/inflammation related to preterm birth: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, soluble IL-6 receptor-α (sIL-6rα), IFN-γ, TNF-α, TNF-β, granulocyte/macrophage colony-stimulating factor (GM-CSF), triggering receptor expressed on myeloid cells-1 (TREM-1), TGF-β, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein(MIP)-1α, MIP-1β, MMP-9, soluble tumor necrosis factor receptor I (sTNFRI), macrophage migration inhibitory factor (MIF), RANTES (regulated upon activation, normal T cell expressed and presumably secreted), BDNF, NT-3, and NT-4.

Development of Multiplexed Assay—Simultaneous measurement of the 27 biomarkers in cord blood plasma was performed from sandwich immunoassays using bead-coupled capture antibodies, biotinylated detection antibodies, and phycoerythrin-labeled streptavidin according to the techniques described by Skogstrand, et al. [16] Briefly, coupling of capture antibodies to carboxylated beads (Luminex Corp., Austin, TX) was performed using 2.5×10^6 beads washed, sonicated, and activated (activation buffer, 0.1 mol/L sodium phosphate, pH 6.2), then incubated and subsequently mixed with capture antibody solution (R&D Systems, BD Biosciences Pharmingen, MBL, BioSource, Dako). The multiplexed assay containing the 27 biomarkers was developed from sequential addition of analytes while observing interactions among antibodies and cross-reactions to other analytes. After preparation of calibrators using a 1:1 mixture of pig/guinea pig serum (Dako, Jackson ImmunoResearch), the assay was set up for measurement of the calibrators and samples: A suspension of capture antibody-conjugated beads was added to each of the samples prepared on 96-well filter plates. The beads were washed, captured antigens were subsequently reacted with a mixture of biotinylated detection antibodies, and streptavidin-phycoerythrin in assay buffer (Molecular Probes) was added to each well. After further incubation, the beads were washed and resuspended. The samples were analyzed on the Luminex™ 100, according to manufacturer's instructions. All samples were run in duplicate with standard curves and spiked controls on each plate.

Characterization of Assays—The characteristics of the 27-plex xMAP assay are reported by Skogstrand, et al. [16] Measurements were performed on a pool of human serum, and the intra- and interassay coefficients of variance (CV) were determined by repeated measurements. The working range was defined as the range of concentrations for which the CV (standard deviation/mean ×100) was <20%. [17] These were determined by repeated measurements of a mixture of animal serum enriched with different concentrations of the analytes.

Placental histolopathology

All placentas were reviewed by a designated perinatal pathologist, and a subset was independently reviewed by a second pathologist to confirm reliability. According to our previously published protocol the presence and location of inflammation was reported using well-established algorithms. [18] Using a standardized abstraction form, the histologic examination status was classified according to the following definitions: 1) *FIR* included births with placental evidence of inflammation extending to the fetal side of the placental unit (documented funisitis or chorionic plate vasculitis). 2) *MIR* included births with associated subchorionitis, chorioamnionitis, deciduitis, or free membranitis (and the absence of funisitis or chorionic plate vasculitis). 3) *No MIR/FIR* included births without any of the placental findings listed above for MIR or FIR. All cases of FIR had findings of MIR with the exception of ten cases.

Statistical methods

Patient demographics, characteristics and biomarker levels were compared using F-test for continuous variables and Chi-squared tests for categorical data among the three GA subgroups. The biomarker levels were compared between the three groups of placental inflammatory response status stratified by the GA subgroups, using multiple linear regression models after the adjustment of the following potential confounders: maternal age, parity, self-reported maternal ethnicity, presentation with medical induction delivery, corticosteroids, tocolysis, intrapartum antibiotic use, and any clinical sign of intrauterine infection. Because the distributions of biomarker levels were skewed, natural log-transformed biomarker measures were used in the regression analyses. All p-values were from two-sided tests and all statistical analyses were performed using SAS software version 9.1 (SAS Institute Inc, Cary, NC). Since 27 biomarkers were tested for the associations with preterm birth and placental histology status, false-positive rates were a critical concern for the statistical inference. To account for multiple comparisons, we used an FDR-corrected threshold to determine the statistical significance of individual tests. FDR controls for the expected proportion of false positives, rather than the chance of any false positive, and takes into account the observed p-value distribution. [19] Based on the methods by Benjamini and Hochberg, $P<0.001$ was considered statistically significant for FDR <5% (less than 5% of reported results are type I errors).

RESULTS

A total of 506 births (105 very preterm, 237 moderately preterm, and 164 full-term) were included in the analysis. GA ranged from 23 to 42 weeks. Demographics and clinical characteristics of the mother-infant pairs are shown in Table I. Overall, there were 63 (12.5%) births with FIR, 90 (17.8%) births with MIR, and 353 (69.8%) births with no MIR/FIR. As expected, there was a higher incidence of FIR in the preterm subgroups.

Comparison of biomarker levels among GA subgroups

Since our sample was quite heterogenous due to the wide range of GA and the influence of several covariates related to prematurity (Table I), we first analyzed the 27 biomarkers according to GA subgroups to determine the impact of gestational prematurity on cord blood

levels. Table II shows the median levels of the 27 markers according to GA subgroups, regardless of MIR/FIR status. Levels of IL-4, IL-6, IL-8, IL-18, sIL-6Rα, MCP-1, MIP-1β, MMP-9, sTNFRI, BDNF, and NT-3 were significantly different among the 3 subgroups. Of these, median levels were highest in the very preterm group with IL-6, IL-8, MCP-1, MIP-1β, and sTNFRI. Conversely, MMP-9, BDNF, and NT-3 were decreased with GA.

Markers associated with MIR and FIR

Since associations with MIR and FIR could potentially be influenced by GA, we analyzed the 27 markers according to MIR/FIR status and further stratified each by GA subgroups. Using the group without evidence of MIR or FIR as the reference (No MIR/FIR) and an FDR-corrected significance threshold of $P<0.001$, only IL-1β, IL-6, and IL-8 showed statistically significant associations with FIR (Table III). IL-1β was increased with FIR in both moderate ($P=0.0001$) and very preterm ($P=0.0009$) subgroups. IL-6 and IL-8 were increased with FIR in all three GA subgroups ($P<0.0001$). None of the associations with MIR reached statistical significance. However, for IL-6 and IL-8 there was a significant step-wise elevation of beta-coefficients with MIR versus FIR (p-trend<0.0001) in all GA subgroups.

The remaining 24 biomarkers were neither significantly elevated nor decreased. Only MCP-1 was marginally significant with FIR in the ≤32wks group, with a beta-coefficient of 1.11 ($P=0.0033$). Contrary to our hypothesis, TNF-α was not significantly elevated with either MIR or FIR. To illustrate the marked difference in associations from the linear regression model as compared with IL-1β, IL-6, and IL-8, results for marginally significant MCP-1 and non-significant TNF-α are shown in Table IV. Data for all other non-significant markers are not shown.

Associations with signs of intrauterine infection

The incidence of clinical chorioamnionitis, as defined by intrapartum fever plus any two additional signs of IUI [14], was only 6.9% among all births, even though the rates of individual signs of IUI were much higher overall (Table 1). To determine how the above significant associations correlated with clinically apparent infection, we compared IL-1β, IL-6, and IL-8 levels according to MIR/FIR status in the presence and absence of any clinical sign of IUI (intrapartum fever, elevated maternal WBC, maternal or fetal tachycardia, uterine tenderness, foul-smelling amniotic fluid or vaginal discharge). Among births with no clinical signs of infection present, all 3 markers were still significantly elevated with FIR (Table V). Similar but more substantial associations were seen among the births with any clinical sign present, with beta- coefficients roughly 3-fold higher with FIR for both IL-6 and IL-8.

DISCUSSION

Our study distinguishes three specific cord blood cytokines (IL-1β, IL-6, and IL-8) from a large panel of immune biomarkers as being strongly associated with FIR. In addition, the associations of IL-6 and IL-8 with FIR were independent of GA, and were significantly

stronger than with MIR alone. Furthermore, all three markers remained significantly associated with FIR even when common clinical signs of IUI were not present.

The most notable findings in our study were with IL-6 and IL-8, in which we demonstrated a greater than two-fold difference in the beta-coefficients with FIR in both the full term (≥37 weeks) and very preterm (≤32 weeks) subgroups. For moderately preterm births (33-36 weeks), beta-coefficients were roughly 2-fold higher with FIR as compared to the reference group with no evidence of MIR or FIR. The strength of these associations with IL-1β was less pronounced, and statistically significant with preterm birth only. With the exception of MCP-1, all remaining 24 biomarkers had beta-coefficients that were <1.0. This was despite the finding that several markers were either elevated or decreased with GA (Table II). We speculate that cord blood biomarkers within our panel may be markers of gestational prematurity, but not necessarily markers of preterm birth mediated by intrauterine infection/inflammation.

In 1998, Gomez and Romero, et al described the association between elevated fetal plasma IL-6 with a systemic fetal inflammatory response and severe neonatal morbidity. [4] In a more recent study of 70 births, Tasci and colleagues measured cord blood IL-6 with extent of placental inflammation and reported a 100% sensitivity and 81% specificity for predicting FIR and culture-positive newborn sepsis. [20] Other studies have reported similar associations with cord blood IL-1β and IL-8. [21] These studies support the utilization of IL-1β, IL-6, and IL-8 to detect severe intrauterine infection/inflammation that is associated with poor neonatal outcome. Our study provides further convincing evidence. Firstly, our study illustrates that even among a host of other candidate markers that have previously been studied, IL-1β, IL-6, and IL-8 are the most sensitive markers of FIR. Secondly, our results showed that among these three markers, IL-6 and IL-8 are elevated with FIR regardless of gestational maturity. Thirdly, these markers are more sensitive than clinical signs of infection in detecting the presence of FIR (Table V).

Although previous studies have identified IL-1β, IL-6 and IL-8 as markers of IUI, our study is the first to employ multiplex immunoassay techniques in conjunction with placental histopathology to determine how these cytokines compare to other candidate biochemical markers. Our panel included markers with immune functions ranging from pro-inflammatory (IL-1β, IL-2, IL-5, IL-6, IL-12, IL-17, IL-18, sIL-6Rα, IFN-γ, TNF-α, TNF-β, GM-CSF, and TREM-1); anti-inflammatory (IL-4, IL-10, and TGF-β); chemokines (IL-8, MCP-1, MIP-1α, MIP-1β, RANTES), enzyme/receptors (MMP-9, sTNFRI, MIF); and neurotrophic agents (BDNF, NT-3, and NT-4). Furthermore, while most studies have focused on either term or preterm births, our study included births ranging from very preterm to full term gestation in order to account for the influence of GA. In order to account for the testing of multiple hypotheses among groups of placental inflammatory status and GA subgroups, we utilized the FDR method which set the threshold for statistical significance such that fewer than 5% of reported results are expected to be type I false-positive errors.

We hypothesized that TNF-α would also be elevated with FIR, due to its role in overwhelming infection and reported elevation with chorioamnionitis. [22,23] There was a lack of association in our study (Table IV). Among very preterm infants, umbilical vein IL-6 has

been shown to be elevated with both early neonatal sepsis and histologic chorioamnionitis, while TNF-α is not. [24] In vitro studies have shown that TNF-α and IL-1β enhance IL-8 expression in term decidual cells. [25] This suggests that TNF-α, in conjunction with IL-1β, may be an important regulator of chorioamnionitis-related disease but is not as prominent at the cord blood level as IL-8. Timing of measurement, therefore, is important in the interpretation of our results. Although our sample size was relatively large as compared to previous studies, a much larger population is needed to more thoroughly explore the degree of independence and extent of interaction among specific markers. For example, although our study was not powered to test the significance of intra-group associations given the small sample sizes of some subgroups, there was a trend towards step-wise increase in IL-6 and IL-8 associations with extent of placental inflammatory response within each GA subgroup. For example, beta-coefficients for IL-6 increased from 1.62 with MIR to 2.77 with FIR (p-trend<0.0001) in the very preterm subgroup. Similarly, the strength of association roughly doubled for IL-8 (1.38 with MIR to 2.69 with FIR, p-trend<0.0001). Further interpretation of our results will require more targeted studies that are powered to detect interactions among these important biomarkers.

In clinical neonatal practice, serious complications such as chronic lung disease and neurodevelopmental impairment are difficult to predict in premature infants, particularly in the immediate postnatal period. A growing body of evidence supports the fetal inflammatory response and proinflammatory cytokines as early markers of these outcomes.[5,26-29] Severe intrauterine infection, or other in utero events, may trigger an immune response in the fetus, leading to inflammation and organ damage that may have lasting impact in the developing neonate. Accompanying this immune response of the fetus, cord blood cytokines are elevated, suggesting that inflammatory reactions occurring at the placental level and involving the fetus are mediated by these cytokines. Our study demonstrates that IL-6 and IL-8 are important mediators of FIR and therefore may be useful clinical predictors of neonatal morbidity.

In summary, IL-1β, IL-6 and IL-8 are selectively elevated with FIR when comparing among a large panel of biomarkers with a wide range of immune functions. Even after adjustment for important covariates and consideration of GA, these markers remained significantly associated with FIR. Further investigation should be focused on determining diagnostic levels via ROC analysis, as well as combinations of these markers to better understand the timing, extent, and interaction with other inflammatory markers in the pathophysiology of preterm birth. While placental histopathology has been shown to be a more sensitive marker of poor outcome due to infection/inflammation than clinical signs, [30,31] pathology results are not readily available or cost-effective in the majority of cases. Evaluation of cord blood IL-6 or IL-8 at the time of birth may serve as a proxy for placental histopathology. With further investigation of associated clinical outcomes, combinations of these markers may potentially be used as a screening tool that guides the medical management of high-risk deliveries.

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Table I

Maternal demographics and clinical characteristics of the study population.

	Full Term (≥ 37 weeks)n=164	Moderate Preterm (33-36 weeks) n=237	Very Preterm (≤ 32 weeks) n=105	P*
Maternal age, years	28.9 ± 6.6	28.6 ± 6.8	29.0 ± 6.8	0.819
Birth weight, g	3172.3 ± 673.9	2452.0 ± 508.7	1520.4 ± 507.5	<0.001
Gestational age, weeks	39.3 ± 1.3	35.2 ± 1.1	30.1 ± 2.6	<0.001
Race				
Black	113(68.9)	130(54.9)	76(72.4)	<0.001
White	9(5.5)	41(17.3)	8(7.6)	
Hispanic	18(11.0)	43(18.1)	12(11.4)	
Other	24(14.6)	23(9.7)	9(8.6)	
Parity (>1)	92(56.1)	131(55.3)	70(66.7)	0.122
Married	108(65.9)	161(67.9)	67(63.8)	0.746
Medical induction	54(32.9)	89(37.6)	30(28.6)	0.249
Tocolysis	3(1.8)	20(8.4)	20(19.0)	<0.001
Antenatal steroids	3(1.8)	28(11.8)	56(53.3)	<0.001
Antepartum antibiotics	64(43.5)	74(38.5)	48(60.8)	0.004
Signs of Intrauterine Infection (IUI):				
Intrapartum fever (>38°C)	20(12.2)	13(5.5)	12(11.4)	0.040
Elevated WBC	43(26.2)	57(24.1)	43(41.0)	0.005
Maternal or fetal tachycardia	26(15.9)	13(5.5)	11(10.5)	0.003
Uterine tenderness	4(2.4)	3(1.3)	5(4.8)	0.146
Foul-smelling AF or vaginal discharge	0(0.0)	3(1.3)	4(3.8)	0.032
Any above sign	70(42.7)	81(34.2)	53(50.5)	0.014
Placental Inflammatory Response Status:				
No MIR/FIR	123(75.0)	175(73.8)	55(52.4)	<0.001
MIR	32(19.5)	38(16.0)	20(19.0)	
FIR	9(5.5)	24(10.1)	30(28.6)	

*P-value is based upon F-test for continuous variables and chi-square for categorical variables.

Table II

Comparison of 27 biomarker levels among gestational age subgroups[†]

	Full Term (≥37 weeks) (n=164)	Moderately Preterm (33-36 weeks) (n=237)	Very Preterm (≤32 weeks) (n=105)	P
IL-1β	54.7 (23.1-125.5)	46.8(12.2-101.7)	45.6(12.6-149.2)	0.011
IL-2	14.6 (2.0-68.5)	23.5(2.0-74.7)	27.4(2.0-76.7)	0.004
IL-4	4.2 (2.0-9.7)	5.4(2.0-10.3)	5.3(2.0-12.7)	<0.001 *
IL-5	5.2 (2.0-12.8)	6.1(2.0-12.1)	6.8(3.5-15.1)	0.001
IL-6	36.1 (9.2-218.7)	27.1(8.4-138.0)	45.3(10.4-4000.0)	<0.001 *
IL-8	30.1 (2.0-227.3)	26.1(4.0-141.4)	41.0(8.5-1143.6)	<0.001 *
IL-10	239.3 (42.0-567.4)	373.5(4.7-800.7)	502.9(2.0-1163.8)	0.191
IL-12	30.9 (9.3-80.6)	29.8(8.1-73.5)	28.1(4.8-67.7)	0.301
IL-17	132.8 (2.0-488.2)	125.4(2.0-514.0)	166.8(2.0-819.6)	0.024
IL-18	708.4 (255.4-2504.0)	649.9(266.7-1829.8)	443.7(192.4-1035.3)	<0.001 *
sIL-6Rα	[42.0 (25.7-79.2)] × 10^3	[51.4(30.6-99.7)] × 10^3	[49.6(27.9-106.3)] × 10^3	<0.001 *
IFN-γ	36.4 (14.5-88.9)	39.1(10.4-85.6)	39.7(10.0-91.0)	0.800
TNF-α	16.3(6.4-36.3)	23.0(5.9-45.7)	22.6(7.5-6.3)	0.012
TNF-β	120.3 (31.5-364.4)	117.2(27.0-364.0)	161.7(36.1-432.0)	0.146
GM-CSF	30.6 (5.0-101.2)	33.0(5.0-79.4)	42.0(5.0-84.3)	0.079
TREM-1	[1.1 (0.3-4.3)] × 10^3	[1.7(0.3-4.7)] × 10^3	[1.8(0.3-5.2)] × 10^3	0.002
TGF-β	146.4 (20.0-369.3)	113.0(20.0-292.3)	119.3(20.0-269.9)	0.130
MCP-1	346.3 (97.1-790.7)	675.8(239.5-1202.9)	684.8(115.9-1968.7)	<0.001 *
MIP-1α	536.6 (215.1-1778.1)	615.0(291.6-2329.6)	808.6(223.4-2183.7)	0.031
MIP-1β	827.3 (364.8-2132.5)	955.2(459.7-2560.9)	1116.2(454.9-3201.5)	<0.001 *
MMP-9	[607.9 (325.8-1238.9)] × 10^3	[546.4(260.8-1215.1)] × 10^3	[465.6(94.9-857.3)] × 10^3	<0.001 *
sTNFRI	[4.0 (2.3-7.9)] × 10^3	[5.9(2.9-17.2)] × 10^3	[10.9(4.1-23.5)] × 10^3	<0.001 *
MIF	[0.5(0.1-1.3)] × 10^6	[0.6(0.2-1.7)] × 10^6	[0.4(0.1-1.7)] × 10^6	0.002
RANTES	[52.3 (27.6-111.1)] × 10^3	[53.7(31.9-94.8)] × 10^3	[49.3(30.7-83.7)] × 10^3	0.224
BDNF	[2.8 (0.8-9.6)] × 10^3	[1.9(0.4-6.4)] × 10^3	[1.4(0.3-5.1)] × 10^3	<0.001 *
NT-3	169.9 (41.4-543.7)	89.2(4.2-256.0)	75.9(2.0-224.8)	<0.001 *
NT-4	21.9 (6.4-45.7)	23.7(7.8-52.3)	23.1(5.2-37.7)	0.185

[†]Biomarker levels are expressed as median (10-90th percentile range). Units are in ng/L.

*P-values based upon F-test. Statistical significance based upon false-discovery rate (FDR)-corrected threshold of P<0.001.

Table III

Cord blood biomarkers significantly associated with FIR, according to gestational age subgroups.

	Gestational age subgroup (weeks)	Placental inflammatory response status	n	Mean ± SD†	beta§	se	P
IL-1β	≥ 37	No MIR/FIR	123	3.90 ± 0.80	Ref	--	--
		MIR	32	4.07 ± 0.79	0.18	0.16	0.2398
		FIR	9	4.68 ± 0.60	0.60	0.29	0.0369
	33-36	No MIR/FIR	175	3.57 ± 0.98	Ref	--	--
		MIR	38	3.67 ± 1.01	0.02	0.18	0.9027
		FIR	24	4.56 ± 1.16	0.87	0.22	0.0001*
	≤ 32	No MIR/FIR	55	3.36 ± 0.90	Ref	--	--
		MIR	20	3.80 ± 0.95	0.32	0.29	0.2806
		FIR	30	4.32 ± 1.30	0.94	0.28	0.0009*
IL-6	≥ 37	No MIR/FIR	123	3.49 ± 1.19	Ref	--	--
		MIR	32	3.88 ± 1.31	0.56	0.24	0.0186
		FIR	9	6.60 ± 1.70	2.78	0.44	<0.0001*,‡
	33-36	No MIR/FIR	175	3.21 ± 1.18	Ref	--	--
		MIR	38	3.55 ± 1.59	0.30	0.26	0.2415
		FIR	24	5.38 ± 2.31	1.97	0.31	<0.0001*,‡
	≤ 32	No MIR/FIR	55	3.36 ± 1.26	Ref	--	--
		MIR	20	4.86 ± 2.29	1.62	0.51	0.0014
		FIR	30	6.15 ± 2.31	2.77	0.49	<0.0001*,‡
IL-8	≥ 37	No MIR/FIR	123	3.20 ± 1.60	Ref	--	--
		MIR	32	3.76 ± 1.46	0.63	0.31	0.0395
		FIR	9	5.80 ± 1.27	2.33	0.57	<0.0001*,‡
	33-36	No MIR/FIR	175	3.06 ± 1.43	Ref	--	--
		MIR	38	3.38 ± 1.27	0.28	0.27	0.2901
		FIR	24	5.10 ± 1.89	1.95	0.32	<0.0001*,‡
	≤ 32	No MIR/FIR	55	3.13 ± 1.24	Ref	--	--
		MIR	20	4.37 ± 1.96	1.38	0.45	0.0021
		FIR	30	5.73 ± 1.92	2.69	0.43	<0.0001*,‡

† Mean values are expressed using natural log-transformation.

§ Beta-coefficients are adjusted for maternal age, parity, race, presentation with medical induction, corticosteroids, tocolysis, intrapartum antibiotics, and any clinical sign of intrauterine infection.

* P <0.001 indicates statistical significance based upon false-discovery rate (FDR) correction for multiple testing.

‡ P-trend (No MIR/FIR vs. MIR vs. FIR) for IL-6 and IL-8 within each GA subgroup was <0.0001.

Table IV

Linear regression parameters of selected biomarkers not significantly associated with MIR or FIR[**]

	Gestational Age subgroup (weeks)	Placental inflammatory response status	n	Mean ± SD[†]	beta[§]	se	P[*]
MCP-1	≥ 37	No MIR/FIR	123	5.52 ± 1.22	Ref	--	--
		MIR	32	5.73 ± 1.35	0.31	0.24	0.1913
		FIR	9	6.55 ± 1.05	0.75	0.44	0.0869
	33-36	No MIR/FIR	175	6.18 ± 1.25	Ref	--	--
		MIR	38	6.35 ± 1.34	0.06	0.24	0.7945
		FIR	24	6.08 ± 1.80	−0.18	0.29	0.5365
	≤ 32	No MIR/FIR	55	6.00 ± 1.51	Ref	--	--
		MIR	20	6.49 ± 1.44	0.83	0.39	0.0338
		FIR	30	6.75 ± 1.28	1.11	0.38	0.0033
TNF-α	≥ 37	No MIR/FIR	123	2.65 ± 0.68	Ref	--	--
		MIR	32	2.92 ± 0.82	0.29	0.14	0.0407
		FIR	9	3.05 ± 0.59	0.31	0.26	0.2254
	33-36	No MIR/FIR	175	2.92 ± 0.84	Ref	--	--
		MIR	38	2.78 ± 0.96	−0.13	0.16	0.4072
		FIR	24	3.09 ± 1.03	0.12	0.20	0.5386
	≤ 32	No MIR/FIR	55	2.82 ± 0.88	Ref	--	--
		MIR	20	3.20 ± 0.65	0.27	0.23	0.2432
		FIR	30	3.25 ± 0.84	0.39	0.22	0.0837

[†] Mean values are expressed using log-transformation.

[§] Beta-coefficients are adjusted for maternal age, parity, race, presentation with medical induction, corticosteroids, tocolysis, intrapartum antibiotics, and any clinical sign of intrauterine infection.

[*] P<0.001 indicates statistical significance based upon false-discovery rate (FDR)-correction for multiple testing.

[**] Data for all remaining non-significantly associated biomarkers are not shown (beta-coefficients <1.0, P>0.0033 for all comparisons).

Table V

Cytokines associated with MIR and FIR, stratified according to presence versus absence of clinical signs of intrauterine infection

	IL-1β			IL-6			IL-8		
	n	**beta(se)***	**P**	**n**	**beta(se)***	**P**	**n**	**beta(se)***	**P**
No clinical signs present									
No MIR/FIR	226	Ref	--	226	Ref	--	226	Ref	--
MIR	50	0.11(0.15)	0.457	50	0.59(0.23)	0.009	50	0.45(0.24)	0.057
FIR	26	0.65(0.20)	0.001	26	1.59(0.30)	<0.0001	26	1.55(0.31)	<0.0001
Any clinical sign present									
No MIR/FIR	127	Ref	--	127	Ref	--	127	Ref	--
MIR	40	0.08(0.16)	0.613	40	0.47(0.28)	0.090	40	0.75(0.30)	0.013
FIR	37	0.87(0.19)	<0.0001	37	3.09(0.33)	<0.0001	37	2.97(0.36)	<0.0001

* Beta-coefficients adjusted for maternal age, parity, race, presentation with medical induction, corticosteroids, tocolysis, intrapartum antibiotics.