Lipopolysaccharide and soluble CD14 in cord blood plasma are associated with prematurity and chorioamnionitis

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BACKGROUND: Lipopolysaccharide (LPS), an endotoxin of Gram-negative bacteria, causes preterm birth in animals and has been implicated as a factor triggering preterm labor and systemic complications in humans. Little is known regarding LPS in the cord blood (CB) of term and preterm infants and its association with maternal and fetal characteristics.

METHODS: CB was obtained from term ($n = 15$) and preterm infants ($n = 76$) after delivery. Plasma levels of LPS, C-reactive protein (CRP), and soluble CD14 (sCD14) were measured using commercially available kits (limulus amoebocyte lysate and enzyme-linked immunosorbent assay). Four linear regression models were created in order to identify independent variables that predict plasma LPS levels.

RESULTS: The analyte levels were significantly higher in preterm vs. term infant CB: LPS (24.48 vs. 1 pg/ml; $P = 0.0009$), CRP (87.9 vs. 47 ng/ml; $P = 0.01$), and sCD14 (0.32 vs. 0.35 μ g/ml; $P = 0.013$). There was a (significant) positive correlation between CB LPS levels and gestational age, birth weight, CRP levels, sCD14 levels, and association with both clinical and histological chorioamnionitis.

CONCLUSION: Our data suggest that LPS is associated with preterm labor and inflammation (CRP elevation and chorioamnionitis). These findings may be relevant to the understanding of the role of LPS in prematurity and its role in preterm morbidities.

Chorioamnionitis is a major risk factor for preterm birth, especially at earlier gestational ages, and it contributes to prematurity-associated morbidity and mortality (1,2). Chorioamnionitis increases the risk of developing morbidities associated with preterm birth, including necrotizing enterocolitis, bronchopulmonary dysplasia, periventricular leukomalacia, sepsis, retinopathy of prematurity, and persistence of a fetal inflammatory state that predisposes for further post-natal injury (3–8). Several animal and *in vitro* models have been developed to clarify the mechanisms by which chorioamnionitis affects the fetus (9–13). These models mimic the clinical scenario of chorioamnionitis in pregnant women by using antenatal lipopolysaccharide (LPS) infusion into maternal circulation to induce inflammation. LPS, an endotoxin of

Gram-negative bacteria, causes preterm birth in animals and has been implicated as a factor triggering preterm labor in humans. These established models also apply to the induction of neonatal systemic complications such as pulmonary hypertension (12,14), cardiac failure (15,16), bronchopulmonary dysplasia (17,18), alveolar development (19–21), and necrotizing enterocolitis (22–24).

In contrast, limited information is available about LPS in the cord blood (CB) of term and preterm infants and its association with maternal and fetal characteristics. Gram-negative bacteria and soluble CD14 (sCD14) have been identified in the amniotic fluid of women in preterm labor, but only two studies have shown the presence of LPS in CB (25–29). LPS could be an important factor in the initiation of chorioamnionitis and fetal inflammation. In plasma, LPS binds to LPS-binding protein, forming a complex that stimulates macrophages by binding to CD14 and TLR4 (30). In addition, CD14 can be shed from monocytes and macrophages. This sCD14 can activate cells that normally lack CD14 and do not respond to LPS alone. In the presence of this complex, even low levels of LPS can initiate a cytokine cascade (31). This cytokine cascade (interleukin-6, tumor necrosis factor- α , and interleukin-1 β) is associated with morbidities linked to preterm birth (32–34). Little is known about the association between chorioamnionitis and LPS/sCD14 levels in CB of preterm infants.

The goals of the present study were to evaluate and compare CB plasma LPS and sCD14 levels in term and preterm infants and to identify the maternal and fetal characteristics that are associated with the level of CB plasma LPS and sCD14.

RESULTS

Patient Characteristics

Ninety-one infants were enrolled in the present study. The maternal and infant characteristics of the subjects are shown in Table 1. None of the mothers of term infants had any symptoms associated with clinical chorioamnionitis or histological evidence of chorioamnionitis. In the preterm group, histological chorioamnionitis (41%) was more common than clinical chorioamnionitis (12%). Only 36 of 76 preterm infants had a diagnosis of histological chorioamnionitis. Detailed clinical

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Table 1. Maternal and infant characteristics

Characteristics	Term (n = 15)	Preterm (n = 76)	P value
Maternal age (y; mean $\pm$ SD)	27.5 $\pm$ 5.9	28.06 $\pm$ 6.9	0.74
Gravidity (median (IQR))	3 (1–4)	2 (1–5)	0.31
Parity (median (IQR))	1 (0–3)	1 (0–3)	0.26
Gestational age (wk; mean $\pm$ SD)	39.2 $\pm$ 0.7	30.3 $\pm$ 3.4	<0.0001
Birth weight (g; mean $\pm$ SD)	3300 $\pm$ 497	1558 $\pm$ 618	<0.0001
Gender (% male)	60	45	0.39
Race (%)			0.31
AA	6 (40)	24 (31)	
C	3 (20)	31 (41)	
H	6 (40)	21 (28)	
Mode of delivery (%)			0.39
V	7 (46.7)	26 (34.2)	
C/S	8 (53.3)	50 (65.8)	
Group β Streptococcus status (%)			0.11
Positive	5 (33)	12 (16)	
Negative	8 (53)	35 (46)	
Unknown	2 (14)	29 (38)	
Prenatal antibiotics (%)	4 (26.7)	48 (63)	0.01
Prenatal steroids (%)	0	55 (72)	<0.0001
Prenatal magnesium sulfate (%)	0	44 (58)	<0.0001
Maternal white blood cell count at delivery ($\times 10^3$; mean $\pm$ SD)	n/a	13.2 $\pm$ 5.4	
Clinical chorioamnionitis (%)	0	9 (12)	0.35
Histological chorioamnionitis (%)	0	31 (41)	0.01
Stage 1 (%)	n/a	6 (20)	
Stage 2 (%)	n/a	10 (32)	
Stage 3 (%)	n/a	15 (48)	
Fetal involvement (funisitis) (%)	n/a	15 (48)	

AA, African American; C, Caucasian; C/S, cesarean section; H, Hispanic; IQR, interquartile range; V, vaginal.

characteristics of the preterm group are shown in [Table 2](#). There were no statistically significant differences observed between the groups for maternal age, gravidity, parity, sex, race, mode of delivery, and group β Streptococcus status. Preterm infants were stratified by gestational age and birth weight.

CB Plasma LPS and CRP Levels Are Higher in Preterm vs. Term Infants

CB plasma LPS was detected in both term and preterm infants. LPS levels (median: 25th to 75th percentiles) were significantly higher in the CB of preterm infants than that in the CB of term infants (24.48 (7.8–59.7) vs. 1 (1–12.2) pg/ml, respectively; $P = 0.0009$; [Figure 1a](#)). Preterm CB had a wider concentration range than that in term infants (from below detection levels (0.7 pg/ml) to 500 pg/ml vs. below

Table 2. Preterm infants clinical characteristics

Characteristics	N = 76
Classification by gestational age (%)	
22–27 wk	14 (18.4)
28–32 wk	42 (55.3)
33–36 wk	20 (26.3)
Classification by birth weight (%)	
Extremely low birth weight	15 (19.7)
Very low birth weight	26 (34.2)
Low birth weight	35 (46.1)
Cord blood initial white blood cell count ($\times 10^3$; mean $\pm$ SD)	8.6 $\pm$ 4.1
Cord blood C-reactive protein (ng/ml; mean $\pm$ SD)	3098 $\pm$ 8460
Positive blood culture in first 72 h of life (%)	0
Positive blood culture after the first 72 h of life (%)	9 (11.8)
Necrotizing enterocolitis (%)	3 (3.9)
Died (%)	
Before 24 h of life	1 (1.3)
After 24 h of life	2 (2.6)

Figure 1. Increased levels of lipopolysaccharide (LPS) and C-reactive protein (CRP) in cord blood (CB) of preterm infants. (a) Median and confidence intervals of LPS levels in CB of term ($n = 15$) and preterm ($n = 76$) infants. Statistical significance was determined by Mann–Whitney U -test ($*P = 0.0009$). (b) Median and confidence intervals of CRP levels in CB of term ($n = 15$) and preterm ($n = 76$) infants. Statistical significance was determined by independent sample test ($*P = 0.01$). (c) Scatter plot graph showing correlation between CB LPS levels (y-axis, log scale) and CRP (x-axis, log scale) in all infants. Spearman rank correlation test used ($r = 0.361$; $P = 0.002$).

Figure 2. Cord blood (CB) lipopolysaccharide (LPS) and CRP correlate with both gestational age and birth weight. **(a)** Correlation between CB LPS levels (y-axis, log scale) and gestational age (x-axis, wk). White dots represent term infants and black dots represent preterm infants. Spearman rank correlation test used ($r = -0.394$; $P = 0.0001$). **(b)** Correlation between CB LPS levels (y-axis, log scale) and birth weight (x-axis, g). White dots represent term infants and black dots represent preterm infants. Spearman rank correlation test used ($r = -0.403$; $P = 0.0001$). **(c)** Correlation between CB CRP levels (y-axis, log scale) and gestational age (x-axis, wk). White dots represent term infants and black dots represent preterm infants. Spearman rank correlation test used ($r = -0.407$; $P = 0.001$). **(d)** Correlation between CB CRP levels (y-axis, log scale) and birth weight (x-axis, g). White dots represent term infants and black dots represent preterm infants. Spearman rank correlation test used ($r = -0.443$; $P = 0.0001$).

detection to 35.3 pg/ml in term infants). C-reactive protein (CRP) levels were also significantly higher in preterm vs. term CB (87.9 (27.4–518.7) vs. 47 (36.6–74.4) ng/ml; $P = 0.01$; **Figure 1b**). There was a significant positive correlation between CB LPS and CRP levels (Spearman's $\rho = 0.361$; $P = 0.002$; **Figure 1c**).

CB LPS and CRP Levels Correlate With Both Gestational Age and Birth Weight

There was a significant negative correlation between CB LPS and gestational age (Spearman's $\rho = -0.394$; $P = 0.0001$), between CB LPS and birth weight (Spearman's $\rho = -0.403$; $P = 0.0001$; **Figure 2a,b**), between CB CRP and gestational age (Spearman's $\rho = -0.407$; $P = 0.001$), and between CB CRP and birth weight (Spearman's $\rho = -0.443$; $P = 0.0001$; **Figure 2c,d**). There were no significant correlations (term and preterm) between CRP or LPS and sex, mode of delivery, race, and duration of rupture of membranes. In the preterm group, maternal exposure to prenatal steroids, antibiotics, and magnesium sulfate did not correlate with CRP or LPS. In the preterm group, infants in the lowest gestational age and birth weight categories had the highest mean ($\pm$ SD) LPS levels (101.8 ± 174.6 and 110.2 ± 166.4 pg/ml, respectively; **Table 3**).

Table 3. Preterm cord blood LPS by classification

Classification	Mean ($\pm$ SD) LPS (pg/ml)
Classification by gestational age	
22–27 wk	101.8 (± 174.6)
28–32 wk	79.5 (± 143.4)
33–36 wk	29.7 (± 29.7)
Classification by birth weight (%)	
Extremely low birth weight	110.2 (± 166.4)
Very low birth weight	58.8 (± 102.41)
Low birth weight	97.7 (± 181.3)

LPS, lipopolysaccharide.

Chorioamnionitis and Increased LPS Levels in CB

For CB LPS and CRP, median (percentiles) levels were significantly higher in CB of infants from pregnancies that were affected vs. unaffected by chorioamnionitis (52.7 (28.7–76.9) vs. 18.3 (1–41.5) pg/ml, $P = 0.01$ for LPS and 2418.9 (593.9–21735.8) vs. 54.2 (27.6–200.1) ng/ml, $P = 0.0001$ for CRP; **Figure 3a,b**).

There was a statistically significant difference in LPS ($P = 0.02$) and CRP ($P = 0.002$) levels in CB from pregnancies with higher histological chorioamnionitis staging

Figure 3. Cord blood (CB) lipopolysaccharide (LPS) and CRP levels are elevated in both clinical and histological chorioamnionitis. **(a)** Median and confidence intervals of LPS levels (log scale) in CB of infants with no chorioamnionitis ($n = 82$) and clinical chorioamnionitis ($n = 9$). Statistical significance was determined by Mann–Whitney U -test ($*P = 0.01$). **(b)** Median and confidence intervals of CRP levels (log scale) in CB of infants with no chorioamnionitis ($n = 82$) and clinical chorioamnionitis ($n = 9$). Statistical significance was determined by Mann–Whitney U -test ($*P = 0.0001$). **(c)** Median LPS levels in CB of preterm infants ($n = 76$) with different staging of histological chorioamnionitis. Statistical significance between groups ($P = 0.02$) was determined by ANOVA. **(d)** Median CRP levels in CB of preterm infants ($n = 76$) with different staging of histological chorioamnionitis. Statistical significance between groups ($P = 0.002$) was determined by ANOVA.

(Figure 3c,d). Samples from stage 2 and 3 cases had the highest LPS levels (33.4 (22.2–282.7) and 30.3 (1–52.7) pg/ml, respectively), and stage 3 samples had the highest CRP levels (5222.7 (518.7–30528.9) ng/ml).

Predictors of CB LPS Levels

To control for multicollinearity, birth weight was not included in the prediction model for LPS since it was highly correlated with gestational age (Spearman's $\rho = 0.923$; $P < 0.01$). The sequential addition of clinical variables to the model increased its predictive value, with R^2 increasing from 0.163 (gestational age only) to 0.191 (gestational age and CRP) to 0.214 (gestational age, CRP, clinical chorioamnionitis, and any stage of histological chorioamnionitis; Table 4).

CB sCD14 and Its Relationship With LPS, Chorioamnionitis, and Prematurity

sCD14 plasma levels (median and percentiles) were significantly different between samples from term infants (0.32

Table 4. Exploring predictors of cord blood lipopolysaccharide

Predictors	Adjustments			
	Model 1	Model 2	Model 3	Model 4
Gestational age	+	+	+	+
Cord blood C-reactive protein	—	+	+	+
Clinical chorioamnionitis	—	—	+	+
Histological chorioamnionitis	—	—	—	+
R	0.404	0.437	0.446	0.462
R^2	0.163	0.191	0.199	0.214
Significant F change	0.000	0.001	0.002	0.004

(0.2–0.5) $\mu\text{g/ml}$) and the three gestational age categories were the following: (22–27 wk (0.51 (0.4–0.6) $\mu\text{g/ml}$), 28–32 wk (0.25 (0.2–0.3) $\mu\text{g/ml}$), and 33–36 wk (0.27 (0.2–0.3) $\mu\text{g/ml}$); $P = 0.013$; Figure 4a). There was no significant association between sCD14 levels and sex, mode of delivery, race, CB CRP, CB white blood cell count, clinical chorioamnionitis,

Figure 4. Soluble CD14 (sCD14) levels are elevated in preterm infants. (a) Median sCD14 levels in cord blood (CB) of term infants ($n = 13$) and three different gestational age categories ($n = 45$). Statistical significance between groups ($P = 0.013$) was determined by Kruskal–Wallis test. (b) Correlation between CB lipopolysaccharide levels (y-axis, log scale) and sCD14 (x-axis, µg/ml) in preterm infants. Spearman rank correlation test used ($r = -0.378$; $P = 0.011$).

or histological chorioamnionitis. There was a significant correlation between CB LPS and CB sCD14 for all samples ($r = -0.317$; $P = 0.015$; data not shown) and for preterm infant samples ($r = -0.378$; $P = 0.011$; **Figure 4b**).

DISCUSSION

In this study, we determined if LPS is present in CB plasma of term and preterm infants and what factors are associated with the variability in CB LPS levels. While LPS was present in preterm and term infant CB, levels were higher in preterm infants. In addition, lower birth weight and gestational age were associated with higher LPS levels. This association is of clinical relevance since it is known that preterm birth in very early gestational ages is associated with acute or subacute placental infections. Differences in placental inflammation may explain these findings. We hypothesized that the presence of bacterial LPS CB plasma plays a role in promoting an inflammatory state. LPS has a high molecular weight, which makes it difficult to cross the placental barrier. However, during placental inflammation, changes in placental endothelium increase permeability, thus facilitating LPS translocation. We proposed that inflammation (chorioamnionitis) facilitates the translocation of LPS toward fetal circulation as demonstrated by the increased CB LPS of the infants exposed to both clinical and histological chorioamnionitis.

Since we proposed that inflammation may play a substantial role in translocation of LPS to fetal plasma, we used CRP and histological chorioamnionitis as markers of systemic and local inflammation. CRP, an acute-phase reactant, is elevated in mothers with intrauterine infection and inflammation (35). In our study, both CB CRP and histological chorioamnionitis were associated with CB LPS levels. Since CRP takes between 24 and 48 h to peak in plasma in the setting of an inflammatory process, we speculate that infants with higher CRP levels have prolonged exposure to inflammation *in utero*, thus increasing the risk for LPS translocation. Histological chorioamnionitis can be present without maternal symptoms, remaining clinically silent for some time, thus exposing the infant to subacute inflammation. The duration and degree of inflammation may play a role in translocation of LPS to fetal plasma. The combination of these inflammatory factors (e.g., chorioamnionitis

and CRP) is highly associated with CB LPS elevation as demonstrated by our predictive model. The model suggests that 21.4% of the CB LPS variation can be explained by its association with the predictor variables (gestational age, CRP, and clinical and histological chorioamnionitis). The association between inflammation and infection as cause for extreme preterm labor is well described (36,37) and can explain the difference in LPS levels between term and preterm infants, especially among those with extremely low gestational ages and low birth weight. Even though some association is observed with the predicting factors and CB LPS levels, the vast majority of variation could be due to other maternal or fetal factors. Our analysis shows no difference between LPS levels and the mode of delivery, group β Streptococcus status, duration of rupture of membranes, systemic maternal infection, and maternal antibiotic, magnesium sulfate, or steroid exposure.

Another marker in our study that was different between term and preterm infants was CD14. CD14 is a glycoprotein, which mediates the interaction of LPS with cells, thereby signaling the presence of Gram-negative bacteria. It is either soluble or membrane bound. CD14 is expressed primarily on myeloid cells, such as monocytes, macrophages, and neutrophils, the cells most sensitive to LPS. sCD14 appears to mediate LPS stimulation of cells that do not express CD14. The relationship between sCD14 and LPS is complex. CD14 and LPS thus appear to participate in a complex feedback mechanism of immune regulation involving both upregulation and downregulation of the inflammatory process triggered by LPS (38). Other studies have shown that intrauterine infection/inflammation is associated with higher median amniotic fluid sCD14 concentration in both preterm and term parturition (26). We found a negative correlation between sCD14 and CB LPS levels in infants, in contrast to adults, in whom the correlation is positive (39). This different relationship between sCD14 and LPS in preterm infants could be related to other factors causing cleavage of CD14 since the LPS-TLR4 pathway in preterm infants is intact (40). Factors such as the presence of group β Streptococcus may play an important role in the release of sCD14 to plasma (41). Preterm infants, particularly those born at very early gestational ages, had higher sCD14 and LPS levels than term infants and preterm

infants born at later gestational ages. These findings could have clinical implications since endotoxemia has been associated with increased intestinal permeability in experimental models, and sCD14 is associated with immune activation and increased comorbidities in HIV infection. It is not clear about what clinical implications these biomarkers would have in preterm infants.

Various studies have demonstrated elevated concentrations of maternal plasma LPS-binding protein in pregnancies complicated by prematurity and chorioamnionitis (42), but no studies, to our knowledge, have shown LPS in maternal plasma. LPS-binding protein is an acute-phase protein produced mainly by hepatocytes, which binds with high affinity to LPS in blood plasma. Elevated LPS-binding protein levels may reflect increased maternal LPS levels. Further investigation is needed to determine the mechanism of LPS translocation and if maternal LPS is related to preterm labor induction.

Our study has several limitations. We did not measure LPS levels in maternal plasma. It is not known if the elevation of LPS in preterm infants is secondary to elevation of maternal LPS levels or is due to ascending vaginal flora. Additional studies of this question are of great interest. Another limitation is that we did not have enough statistical power to detect differences in LPS values and prenatal comorbidities (such as bronchopulmonary dysplasia and necrotizing enterocolitis). No difference in LPS levels was found between infants who developed late-onset sepsis (defined as positive blood culture after 72 h of life) and those who did not. Infants at higher risk vs. those at lower risk for late-onset sepsis (gestational age: 22–27 wk and histological chorioamnionitis) had higher LPS levels; however, the difference between groups did not achieve statistical significance (see **Supplementary Figure S1** online). We also excluded term infants with chorioamnionitis from our analysis because previous observations have demonstrated that they have a state of immune activation similar to that of preterm infants (43).

We conclude that LPS is present in both preterm and term CB plasma, but lower gestational age, clinical and histological chorioamnionitis, and CRP elevation are associated to increased LPS levels in preterm infants. In light of these findings, it might be interesting to understand the neonatal immunological and clinical consequences of elevated LPS and sCD14 levels in plasma. This question is currently under investigation in our laboratory.

METHODS

Subjects

CB samples were collected from the infants born at Tampa General Hospital after obtaining informed consent from mothers. These studies were performed in accordance with the policies and the approval of the Institutional Review Boards at the University of South Florida and Tampa General Hospital. Subjects included healthy term infants (gestational age: ≥ 37 wk; $n = 15$) and preterm infants (gestational age: $\leq 36^{6/7}$ wk; $n = 76$). Term infants whose mothers had symptoms of chorioamnionitis or histological diagnosis of chorioamnionitis were excluded from the study. Infants with genetical or gastrointestinal disorders (e.g., gastroschisis and omphalocele) were also excluded. Demographical and clinical details for the infants and mothers were obtained from the medical records (**Tables 1** and **2**). Preterm infants were stratified into two of the following additional

categories: gestational age (22–27, 28–32, and 33–36 wk) and birth weight (extremely low birth weight: $<1,000$ g; very low birth weight: $<15,000$ g; and low birth weight: $<2,500$ g).

CB Collection

CB was collected using sterile techniques. After the placenta was delivered, a section of the umbilical cord was cleaned with povidone-iodine or alcohol topical antiseptics. The umbilical vein was identified, and blood was obtained using a 21G needle. Blood was then transferred into EDTA-coated tubes. Samples were analyzed within 6–12 h of collection.

Plasma Separation, LPS Quantification, CRP, and sCD14 Measurement

Plasma was collected from CB samples after two centrifugations to separate cellular components from plasma. Plasma samples were then stored at -80°C for batch analysis. Plasma was diluted to 10 or 20% with endotoxin-free water and then heated to 85°C for 15 min to denature plasma proteins. Plasma levels of LPS were measured using a commercially available kit (Limulus Amebocyte Lysate QCL-1000; Lonza, Walkersville, MD) according to the manufacturer's protocol. Samples were run in triplicate; backgrounds were subtracted, and mean values were reported. The lower level of detection for the assay was 0.7 pg/ml. Plasma levels of CRP were measured using a commercially available sandwich enzyme immunoassay kit (Quantikine ELISA DCRP00; R&D, Minneapolis, MN). Samples were run in duplicate, and mean values are reported. The minimum level of detection was 0.01 ng/ml. Plasma levels of sCD14 were measured using a solid-phase enzyme-linked immunosorbent assay (Quantikine ELISA DC140; R&D). Samples were run in duplicate, and mean values were reported. The minimum detectable level was 125 pg/ml.

Chorioamnionitis Determination and Placenta Examination

The term chorioamnionitis is used to describe an intrauterine status of inflammation in tissues of either mixed fetal-maternal (chorio-decidua space) or fetal origin (chorioamniotic membranes, amniotic fluid, and umbilical cord). Clinical chorioamnionitis was defined as the presence of maternal fever (intrapartum temperature: $>100.4^{\circ}\text{F}$ or $>37.8^{\circ}\text{C}$), significant maternal tachycardia (>120 bpm), fetal tachycardia (>160 – 180 bpm), purulent or foul-smelling amniotic fluid or vaginal discharge, uterine tenderness, and maternal leukocytosis (total blood leukocyte count: $>15,000$ – $18,000$ cells/ μl). Histological chorioamnionitis may present without maternal symptoms such as fever or uterine tenderness and is a manifestation of a more subacute infection. Placental examination is routinely performed for all preterm deliveries at Tampa General Hospital and is used to diagnose histological chorioamnionitis. In most cases, histological chorioamnionitis is accompanied by the evidence of invasion of pathogens in normally sterile tissues. Histological chorioamnionitis was classified into one of the following three stages (44): stage 1, neutrophils in placental chorionic plate only; stage 2, neutrophils throughout chorionic plate and subamniotic connective tissue; and stage 3, necrotizing inflammation or multifocal abscess. Funisitis includes inflammation of the connective tissue of the umbilical cord.

Statistical Analysis

Nominal variables are reported as frequencies, and continuous variables are reported as mean values $\pm$ SD or as medians (25th to 75th percentiles). Parametric data are reported as means and nonparametric data as medians. Data were analyzed by using SPSS statistical software (IBM SPSS for Windows, version 20; SPSS, Armonk, NY). Nominal variables were compared using χ^2 analysis or Fischer's exact test as appropriate. Group medians were compared using a Mann-Whitney U -test or Kruskal-Wallis test. Group means were compared using an independent samples t -test or ANOVA. Spearman rank correlation test was used to measure the strength of association between variables. A P value ≤ 0.05 was considered statistically significant. Four linear regression models were created in order to identify independent variables that predict plasma LPS levels. The first model used gestational age as a predictor. The second model included gestational age and CB CRP. The third model included gestational age, CB CRP,

and clinical chorioamnionitis. The fourth model included all the previous variables and histological chorioamnionitis. A log scale transformation was used for LPS and CRP to reduce positive skewness and to allow a larger range to be displayed without small values being compressed in the bottom of the figure.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

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REFERENCES

- Bersani I, Thomas W, Speer CP. Chorioamnionitis—the good or the evil for neonatal outcome? *J Matern Fetal Neonatal Med* 2012;25:Suppl 1: 12–6.
- Redline RW, Abramowsky CR. Clinical and pathologic aspects of recurrent placental villitis. *Hum Pathol* 1985;16:727–31.
- Duggan PJ, Edwards AD. Placental inflammation and brain injury in preterm infants. *Dev Med Child Neurol Suppl* 2001;86:16–7.
- Lee J, Dammann O. Perinatal infection, inflammation, and retinopathy of prematurity. *Semin Fetal Neonatal Med* 2012;17:26–9.
- Leviton A, Hecht JL, Allred EN, Yamamoto H, Fichorova RN, Dammann O; ELGAN Study Investigators. Persistence after birth of systemic inflammation associated with umbilical cord inflammation. *J Reprod Immunol* 2011;90:235–43.
- Dessardo NS, Mustac E, Dessardo S, et al. Chorioamnionitis and chronic lung disease of prematurity: a path analysis of causality. *Am J Perinatol* 2012;29:133–40.
- Moscuzza F, Belcari F, Nardini V, et al. Correlation between placental histopathology and fetal/neonatal outcome: chorioamnionitis and funisitis are associated to intraventricular haemorrhage and retinopathy of prematurity in preterm newborns. *Gynecol Endocrinol* 2011;27:319–23.
- Chen ML, Allred EN, Hecht JL, et al; ELGAN Study. Placenta microbiology and histology and the risk for severe retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2011;52:7052–8.
- Gantert M, Jellema RK, Heineman H, et al. Lipopolysaccharide-induced chorioamnionitis is confined to one amniotic compartment in twin pregnant sheep. *Neonatology* 2012;102:81–8.
- Abdulkadir AA, Kimimasa T, Bell MJ, Macpherson TA, Keller BB, Yanowitz TD. Placental inflammation and fetal hemodynamics in a rat model of chorioamnionitis. *Pediatr Res* 2010;68:513–8.
- Melville JM, Bischof RJ, Meeusen EN, Westover AJ, Moss TJ. Changes in fetal thymic immune cell populations in a sheep model of intrauterine inflammation. *Reprod Sci* 2012;19:740–7.
- Polglase GR, Hooper SB, Gill AW, et al. Intrauterine inflammation causes pulmonary hypertension and cardiovascular sequelae in preterm lambs. *J Appl Physiol* 2010;108:1757–65.
- Kallapur SG, Jobe AH, Ball MK, et al. Pulmonary and systemic endotoxin tolerance in preterm fetal sheep exposed to chorioamnionitis. *J Immunol* 2007;179:8491–9.
- Huang XL, Ling YQ, Zhu TN, Zhang JL, Ling YL. Multiple factors contributing to lipopolysaccharide-induced reactivity changes in rabbit pulmonary artery. *Sheng Li Xue Bao* 2005;57:737–41.
- Velten M, Hutchinson KR, Gorr MW, Wold LE, Lucchesi PA, Rogers LK. Systemic maternal inflammation and neonatal hyperoxia induces remodeling and left ventricular dysfunction in mice. *PLoS ONE* 2011;6:e24544.
- Rounioja S, Räsänen J, Glumoff V, Ojaniemi M, Mäkilä K, Hallman M. Intra-amniotic lipopolysaccharide leads to fetal cardiac dysfunction. A mouse model for fetal inflammatory response. *Cardiovasc Res* 2003;60:156–64.
- Cheah FC, Pillow JJ, Kramer BW, et al. Airway inflammatory cell responses to intra-amniotic lipopolysaccharide in a sheep model of chorioamnionitis. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L384–93.
- Kunzmann S, Collins JJ, Kuypers E, Kramer BW. Thrown off balance: the effect of antenatal inflammation on the developing lung and immune system. *Am J Obstet Gynecol* 2013;208:429–37.
- Velten M, Heyob KM, Rogers LK, Welty SE. Deficits in lung alveolarization and function after systemic maternal inflammation and neonatal hyperoxia exposure. *J Appl Physiol* 2010;108:1347–56.
- Cao L, Wang J, Tseu I, Luo D, Post M. Maternal exposure to endotoxin delays alveolarization during postnatal rat lung development. *Am J Physiol Lung Cell Mol Physiol* 2009;296:L726–37.
- Ueda K, Cho K, Matsuda T, et al. A rat model for arrest of alveolarization induced by antenatal endotoxin administration. *Pediatr Res* 2006;59:396–400.
- Leapheart CL, Cavallo J, Gribar SC, et al. A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. *J Immunol* 2007;179:4808–20.
- Cilieborg MS, Schmidt M, Skovgaard K, et al. Fetal lipopolysaccharide exposure modulates diet-dependent gut maturation and sensitivity to necrotizing enterocolitis in pre-term pigs. *Br J Nutr* 2011;106:852–61.
- Giannone PJ, Nankervis CA, Richter JM, Schanbacher BL, Reber KM. Prenatal lipopolysaccharide increases postnatal intestinal injury in a rat model of necrotizing enterocolitis. *J Pediatr Gastroenterol Nutr* 2009;48:276–82.
- Scheifele DW, Fussell S, Olsen E. Bacterial endotoxins in umbilical cord blood of neonates. *Biol Neonate* 1984;45:119–24.
- Espinosa J, Chaiworapongsa T, Romero R, et al. Evidence of participation of soluble CD14 in the host response to microbial invasion of the amniotic cavity and intra-amniotic inflammation in term and preterm gestations. *J Matern Fetal Neonatal Med* 2002;12:304–12.
- Hazan Y, Mazor M, Horowitz S, Leiberman JR, Glezerman M. The diagnostic value of amniotic fluid Gram stain examination and limulus amoebocyte lysate assay in patients with preterm birth. *Acta Obstet Gynecol Scand* 1995;74:275–80.
- Gardella C, Hitti J, Martin TR, Ruzinski JT, Eschenbach D. Amniotic fluid lipopolysaccharide-binding protein and soluble CD14 as mediators of the inflammatory response in preterm labor. *Am J Obstet Gynecol* 2001;184:1241–8.
- Nakajima M, Inagaki M, Ando Y, et al. Endotoxin-specific chromogenic assay for plasma in pregnant women, umbilical cords, neonates and children. *Brain Dev* 1988;10:382–4.
- Levy O. Innate immunity of the human newborn: distinct cytokine responses to LPS and other Toll-like receptor agonists. *J Endotoxin Res* 2005;11:113–6.
- Tapping RI, Tobias PS. Soluble CD14-mediated cellular responses to lipopolysaccharide. *Chem Immunol* 2000;74:108–21.
- Takahashi N, Uehara R, Kobayashi M, et al. Cytokine profiles of seventeen cytokines, growth factors and chemokines in cord blood and its relation to perinatal clinical findings. *Cytokine* 2010;49:331–7.
- Goepfert AR, Andrews WW, Carlo W, et al. Umbilical cord plasma interleukin-6 concentrations in preterm infants and risk of neonatal morbidity. *Am J Obstet Gynecol* 2004;191:1375–81.
- Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. *Am J Obstet Gynecol* 1998;179:194–202.
- Bek KM, Nielsen FR, Qvist I, Rasmussen PE, Tobiassen M. C-reactive protein (CRP) and pregnancy. An early indicator of chorioamnionitis. A review. *Eur J Obstet Gynecol Reprod Biol* 1990;35:29–33.
- Himes KP, Simhan HN. Risk of recurrent preterm birth and placental pathology. *Obstet Gynecol* 2008;112:121–6.
- Jacobsson B. Infectious and inflammatory mechanisms in preterm birth and cerebral palsy. *Eur J Obstet Gynecol Reprod Biol* 2004;115:159–60.

38. Kielian TL, Blecha F. CD14 and other recognition molecules for lipopolysaccharide: a review. *Immunopharmacology* 1995;29:187–205.
39. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;12:1365–71.
40. Burl S, Townsend J, Njie-Jobe J, et al. Age-dependent maturation of Toll-like receptor-mediated cytokine responses in Gambian infants. *PLoS ONE* 2011;6:e18185.
41. Henneke P, Takeuchi O, van Strijp JA, et al. Novel engagement of CD14 and multiple toll-like receptors by group B streptococci. *J Immunol* 2001;167:7069–76.
42. Torbé A, Sokolowska M, Kwiatkowski S, Rzepka R, Torbé B, Czajka R. Maternal plasma lipopolysaccharide binding protein (LBP) concentrations in pregnancy complicated by preterm premature rupture of membranes. *Eur J Obstet Gynecol Reprod Biol* 2011;156:153–7.
43. Luciano AA, Yu H, Jackson LW, Wolfe LA, Bernstein HB. Preterm labor and chorioamnionitis are associated with neonatal T cell activation. *PLoS ONE* 2011;6:e16698.
44. Redline RW, Heller D, Keating S, Kingdom J. Placental diagnostic criteria and clinical correlation—a workshop report. *Placenta* 2005;26:Suppl A:S114–7.