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ANAPHYLATOXINS IN PRETERM AND TERM LABOR

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Abstract

OBJECTIVE—The complement system plays a central role in the first line of defense against invading pathogens, and its activation involves the release of potent pro-inflammatory mediators such as anaphylatoxins C3a, C4a and C5a. The aim of this study was to determine whether differences existed in maternal plasma anaphylatoxin concentrations between patients with term and preterm parturition.

STUDY DESIGN—A cross-sectional study was designed to determine the plasma anaphylatoxin concentrations in 296 pregnant women in the following groups: 1) normal pregnancy between 20–36 6/7 weeks (n=64); 2) term not in labor (n=70); 3) term in labor (n=60); and 4) preterm labor with intact membranes (n=102). Women with preterm labor were classified into: a) term delivery (n=24); b) preterm delivery without intra-amniotic infection (IAI) (n=62); and c) preterm delivery with IAI (n=16). Concentrations of C3a, C4a and C5a were determined by ELISAs. Statistical analysis was conducted with non-parametric methods.

RESULTS—1) The median plasma C5a concentration was lower in women at term in labor than in those not in labor ($p<0.001$). In contrast, there were no differences in plasma C3a and C4a concentrations between the two groups ($p>0.05$). 2) Among patients with preterm labor, those with IAI had a higher median plasma C5a concentration than those without IAI and those who delivered at term (post-hoc tests $p<0.001$ and $p=0.01$, respectively). When comparing the preterm labor subgroups with normal pregnancy, only women with preterm delivery and IAI had a median plasma C5a concentration higher than that of normal pregnant women (Kruskal-Wallis $p<0.001$, post hoc test $p<0.001$) There was no difference in the plasma C4a concentration among patients with preterm labor. The median plasma C3a concentration in patients with preterm labor with IAI was significantly higher than in those without IAI (Kruskal-Wallis $p=0.01$, and post-hoc test $p=0.005$). There was no difference in the plasma C3a concentrations between women with preterm labor who delivered at term and those with preterm delivery, with or without IAI. In addition, no differences were observed in the median plasma C3a concentration between women with normal pregnancy and those in each of the preterm labor subgroups.

CONCLUSIONS—The maternal plasma concentration of anaphylatoxin C5a is increased in women with preterm labor and IAI, but not in spontaneous labor at term.

Keywords

Parturition; human labor; preterm labor; intra-amniotic infection; anaphylatoxin; C3a; C4a; C5a

INTRODUCTION

The complement system is a key component of the innate immunity,[15,56] and is also involved in the regulation of the adaptive immune response.[5] This system is formed by a group of plasma proteins with catalytic properties that react in a sequential manner, yielding active biological mediators and lytic components to clear microorganisms and “non-self” cells.[15, 34,56] Activation of complement through “the classical,” “the alternative,” and “the mannan binding lectin” (MBL) pathways[55,56] leads to the generation of complement split products (C3a, C4a and C5a).[11] These bioactive fragments, known as anaphylatoxins, can induce smooth muscle contraction,[6,9,21] enhance vascular permeability,[6,21,46] and attract white blood cells.[8,24,47] Apart from their role in host defense, uncontrolled or excessive production of anaphylatoxins have been implicated in the pathogenesis of inflammatory diseases including sepsis,[7,36,57] asthma,[28,37] rheumatoid arthritis,[22,35] adult respiratory distress syndrome,[50] ischemia reperfusion injury,[59] and pregnancy loss.[18]

Preterm birth is one of the leading causes of neonatal mortality and morbidity.[14,33,49] A solid body of clinical and epidemiologic evidence implicates systemic and intrauterine infection in the etiology of premature labor and delivery.[4,20] However, other processes associated with the preterm parturition syndrome include utero-placental ischemia, uterine over-distention, allergy, cervical insufficiency, endocrine diseases, and stress disorders.[43]

Phenotypic and metabolic changes of monocytes and granulocytes consistent with intravascular inflammation have been demonstrated in women with preterm labor and intact membranes,[16] as well as in those with preterm premature rupture of membranes.[17] Activated neutrophils and monocytes can generate C5a[26,53,54,58] and this, in turn, may exacerbate the inflammatory response.[57] There is a paucity of information regarding the behavior of anaphylatoxins during human parturition. This study was conducted to determine whether maternal plasma concentrations of anaphylatoxins change during spontaneous preterm and term labor.

PATIENTS AND METHODS

Study design

A cross-sectional study was conducted by searching our clinical database and bank of biological samples. This study included 296 women in the following four groups: 1) normal pregnancy between 20–36^{6/7} weeks (n=64); 2) normal pregnancy at term not in labor (n=70); 3) normal pregnancy at term in labor (n=60); and 4) preterm labor with intact membranes (n=102). The inclusion criteria for normal pregnancy consisted of: 1) no medical, obstetrical or surgical complications; 2) no labor; and 3) delivery of a neonate (≥ 37 weeks) with a birthweight appropriate for gestational age (10th and 90th percentile).[3] Normal pregnant women were enrolled from either a labor/delivery unit (in cases of scheduled cesarean section) or an antenatal clinic. All were followed until delivery. Normal pregnant women at term had a gestational age ≥ 37 weeks. Women with normal pregnancy at term in labor were defined with similar criteria to those of normal pregnant women at term, but with spontaneous labor. Preterm labor was characterized by the presence of regular uterine contractions occurring at a frequency of at least 2 every 10 minutes, with cervical changes that led to delivery at < 37 completed weeks of gestation. This group included only patients who had transabdominal amniocentesis performed within 24 hours of maternal blood sampling. Women with preterm

labor and intact membranes were classified into: a) term delivery without intra-amniotic infection (IAI) (n=24); b) preterm delivery without IAI (n=62); and c) preterm delivery with IAI (n=16). IAI was defined as a positive amniotic fluid culture for microorganisms. All women provided written informed consent prior to the collection of the plasma sample. The collection of samples was approved by the IRBs of both Wayne State University and the National Institute of Child Health and Human Development (NIH). Many of these samples have been used in previous studies.

Blood Collection

Samples of peripheral blood were collected in tubes containing EDTA (ethylene diamine tetraacetic acid). The samples were centrifuged and stored at -70° C. Specific and sensitive enzyme-linked immunoassays (ELISAs) were used to determine the concentrations of complement C3a, C4a and C5a. Immunoassay systems for C3a and C4a were obtained from Assay Designs, Inc. (Ann Arbor, MI). C5a immunoassays were obtained from American Laboratory Products Company (Windham, NH). Complement C3a, C4a and C5a assays were performed following the manufacturers' recommendations. Briefly, maternal plasma samples were incubated in duplicate wells of microtiter plates, which had been pre-coated with antigen specific (C3a, C4a or C5a) antibodies. Complement C3a, C4a or C5a present in the standards or maternal plasma samples were immobilized by their specific pre-coated antibodies (forming antigen antibody complexes) during this incubation. Repeated washing and aspiration were conducted to remove unbound materials from the assay plates. This step was followed by incubation with a specific antibody-enzyme reagent. Following a wash step to remove excess unbound materials, a substrate solution was added to the wells of the microtiter plates, and color developed in proportion to the amount of antigen bound in the initial step of the individual assay. The color development was stopped with the addition of an acid solution, and the intensity of color was read using a programmable microtiter plate spectrophotometer (Ceres 900 Microplate Workstation, Bio-Tek Instruments, Winooski, VT). The concentrations of complement C3a, C4a or C5a in maternal plasma samples were determined by interpolation from individual standard curves composed of purified human C3a, C4a or C5a. The calculated inter-assay coefficients of variation (CV) for C3a, C4a and C5a immunoassays in our laboratory were 5.4%, 6.1% and 4.0%, respectively. Calculated intra-assay CV for C3a, C4a and C5a were 6.6%, 6.9% and 2.3%, respectively. The detection limit (sensitivity) was 0.13 ng/ml for C3a, 0.32 ng/ml for C4a and 0.06 ng/ml for C5a.

Statistical Analysis

Shapiro-Wilk tests were used to test for normal distribution of the data. A Kruskal-Wallis test was utilized for multiple comparisons. Mann-Whitney U tests were used for post-hoc comparisons with Bonferroni correction of the alpha in order to maintain the overall probability of a type I error at 0.05. Chi square was used to compare proportions. The statistical package employed was SPSS 12 (SPSS Inc., Chicago, IL). A p value of <0.05 was considered significant.

RESULTS

The clinical and obstetrical characteristics of women with normal term pregnancies are displayed in Table 1. Normal pregnant women at term in labor (20 – 36 6/7 weeks) had a median gestational age at blood sampling and delivery higher than those without labor ($p<0.001$ and $p=0.008$, respectively). In contrast, maternal age was significantly higher in women at term gestation and no labor than those in labor ($p=0.01$).

Labor did not have a significant effect on maternal plasma concentrations of C3a and C4a in normal women delivering at term ($p=0.8$ and $p=0.1$, respectively; Figures 1A and B). Pregnant

women at term in labor, however, had a lower median plasma C5a concentration than those at term not in labor ($p < 0.001$; Figure 1C).

The clinical and obstetrical characteristics of women with normal pregnancy not in labor and those with preterm labor and intact membranes are displayed in Table 2. According to the study design, gestational age at delivery and neonatal birthweight were significantly different between the two groups ($p < 0.001$ for each). No differences were observed in the median plasma C3a and C4a concentrations between patients with preterm labor and those with normal pregnancy ($p = 0.4$ and $p = 0.08$, respectively; Figures 2A and B). In contrast, women with preterm labor had a median plasma C5a concentration higher than normal pregnant women ($p = 0.003$; Figure 2C).

Among patients with preterm labor, those with IAI had a higher median plasma C5a concentration than those without IAI who delivered preterm, as well as those who delivered at term (Kruskal-Wallis $p < 0.001$; post-hoc tests $p < 0.001$ and $p = 0.01$, respectively; Figure 3C). No difference in the plasma C5a concentration was observed between patients with preterm delivery without IAI and those with preterm labor who delivered at term ($p = 0.4$; Figure 3C). Comparisons between the three subgroups of preterm labor and the group with normal pregnancy showed that only patients with preterm delivery and IAI had a median plasma C5a concentration higher than that of normal pregnant women (Kruskal-Wallis $p < 0.001$, post hoc test $p < 0.001$; Figure not shown). The maternal plasma C4a concentration was similar in all subgroups of patients with preterm labor (Figure 3B). The median plasma C3a concentration of patients with preterm delivery with IAI was higher than that of those with preterm delivery without IAI (Kruskal-Wallis $p = 0.01$ and post-hoc test $p = 0.005$; Figure 3A). No difference in the median plasma C3a concentration was found between women with preterm labor who delivered at term and those who delivered preterm, with or without IAI (all post-hoc tests $p = 0.1$; Figure 3A). Similarly, no differences were observed in the plasma C3a concentrations between women with normal pregnancy and those in each of the preterm labor subgroups.

DISCUSSION

Our study demonstrates that spontaneous human parturition at term is associated with lower median maternal plasma C5a concentrations than term gestation not in labor. In contrast, women with preterm labor and IAI have higher median maternal plasma C5a concentrations than those with preterm labor without IAI.

The median maternal plasma concentrations of the anaphylatoxins C3a, C4a and C5a are higher than that of non-pregnant women in the secretory phase of the menstrual cycle.[41] Moreover, the plasma concentrations of anaphylatoxins do not change with increasing gestational age (20 weeks to term gestation).[41] We have proposed that these findings may represent a normal adaptation during pregnancy, in which the activity of the innate immune system is enhanced to compensate for the reduced adaptive immunity considered necessary for the tolerance of the conceptus.[41]

A previous study reported no changes in maternal plasma C3 and C4 concentrations through the last trimester of pregnancy and labor.[32] Similarly, in our study, maternal plasma C3a and C4a concentrations did not change during labor. On the other hand, maternal plasma C5a concentration at term was significantly lower in women with labor than in those not in labor. Since human parturition has been linked to an inflammatory process[30,31,52] with an increase in the maternal neutrophil count[48] and proinflammatory cytokine concentrations including IL-1 β , IL-6 and IL-8,[25] we expected an increase in the maternal plasma C5a concentration. Surprisingly, the results of the study do not support this view. Further studies will be required

to determine both the clinical significance and the mechanisms responsible for the lower maternal plasma C5a concentrations observed in normal human labor at term.

Huffacker et al.[27] reported that the maternal serum complement hemolytic activity (CH50) in women with preterm labor was similar between those who delivered preterm and those who delivered at term. These results, however, should be interpreted with caution since the CH50 assay is an insensitive marker of complement activation.[1]

The current study shows that the median maternal plasma C5a concentration is higher in patients with preterm labor associated with IAI than in those with preterm labor without infection delivering preterm or at term. This finding suggests that the maternal immune system is responding to either microbial products or pro-inflammatory mediators produced in the uterine cavity, and such response can be detected in the maternal compartment. It is possible that enzymes from activated phagocytic cells can cleave C5 and contribute to the increased maternal concentration of C5a.[26,53,54,58] Furthermore, C5a may induce degranulation of inflammatory cells,[8,23,51] enhance respiratory burst with generation of reactive oxygen species in leukocytes,[10,19,60] delay neutrophil apoptosis,[40] and up-regulate the expression of adhesion molecules from granulocytes[29] and endothelial cells[13] to combat intrauterine infection. Another function of C5a is the induction and/or release of inflammatory cytokines from neutrophils, mononuclear and endothelial cells. [2,12,38,39,42,44,45] Altogether, these properties of C5a have been associated with the induction of a potent inflammatory response and the prolongation of the life span of neutrophils in the presence of antigens. The lack of change in C4a suggests that the classical and MBL pathways may not be the main complement routes activated in women presenting with preterm labor and IAI.[1]

In conclusion, term and preterm labor are associated with different profiles of maternal plasma anaphylatoxin concentrations. At term, maternal plasma C5a concentration is lower in patients with spontaneous labor than in those not in labor. In contrast, patients with preterm labor with IAI had higher plasma C5a concentrations than those without IAI delivering preterm or at term. Our study provides further evidence that maternal intravascular inflammation is present in patients with preterm delivery and IAI.

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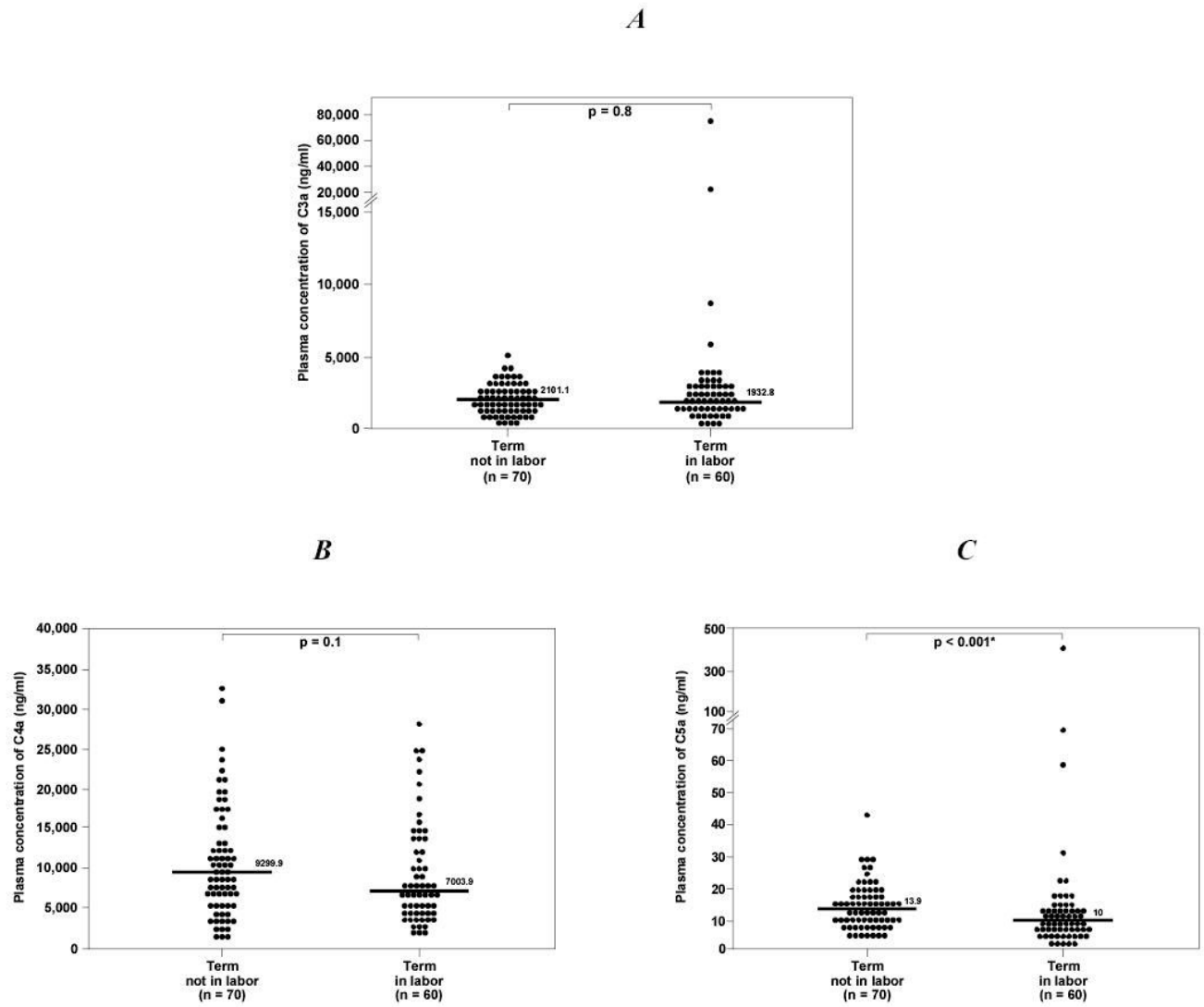
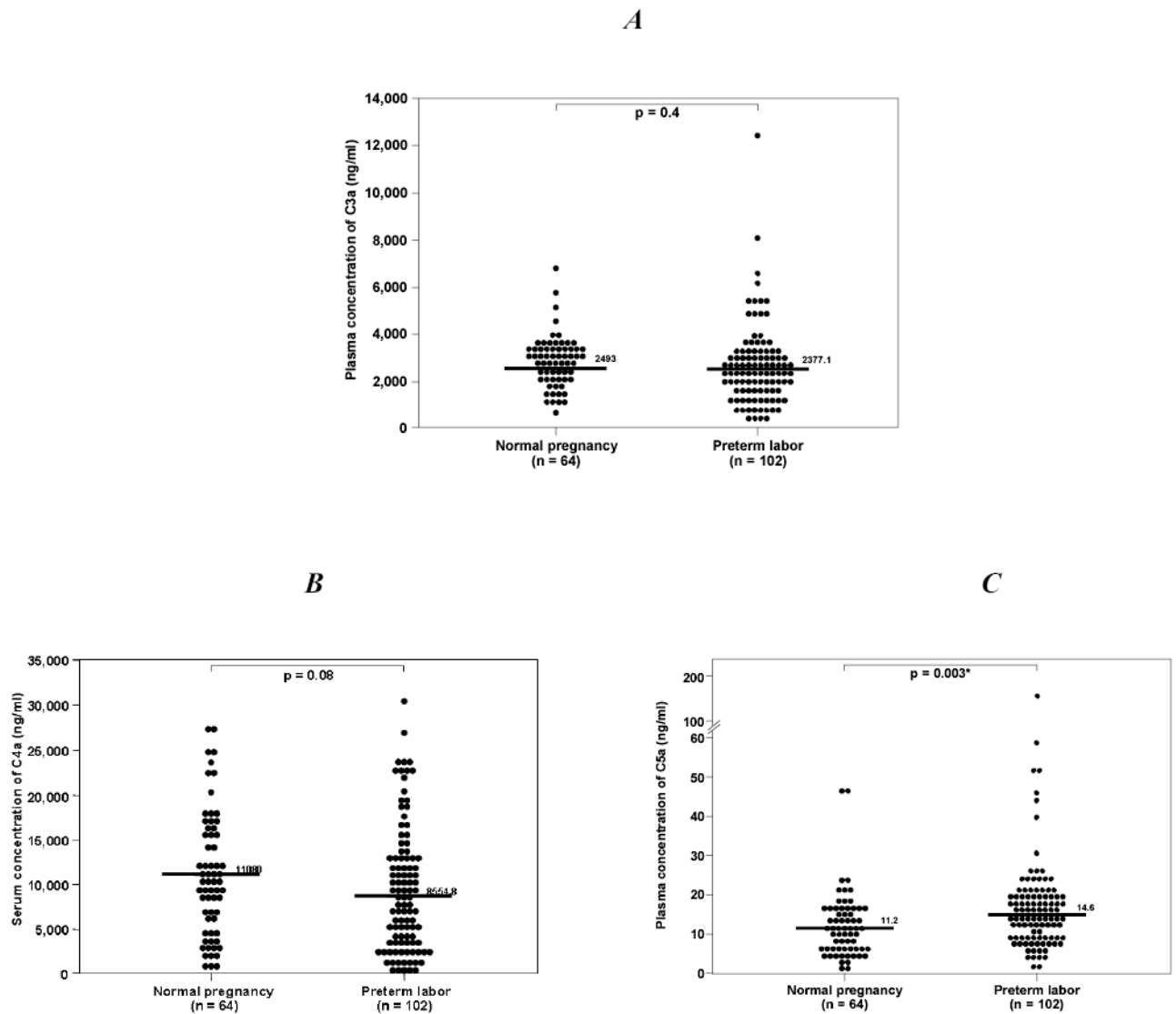


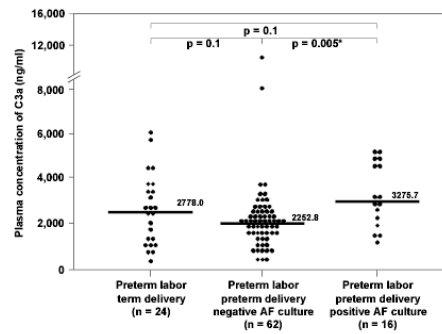
Figure 1.

Plasma anaphylatoxin concentrations of normal pregnant women at term. **A**. There was no difference in the median plasma C3a concentration between women at term in labor and those not in labor [median: 1932.8 ng/ml (range 337.9 – 74300) vs. median: 2101.1 ng/ml (range 582.8 – 5103.6), $p=0.8$]. **B**. Similarly, the plasma C4a concentration did not differ between pregnant women at term in labor and those not in labor [median: 7003.9 ng/ml (range 2038.9 – 27910) vs. median: 9299.9 ng/ml (range 1284.1 – 32640), $p=0.1$]. **C**. In contrast, the median plasma C5a concentration of normal pregnant women at term in labor was lower than those not in labor [median: 10 ng/ml (range 1.9 – 428.3) vs. median: 13.9 ng/ml (range 5.4 – 43), $p<0.001$].

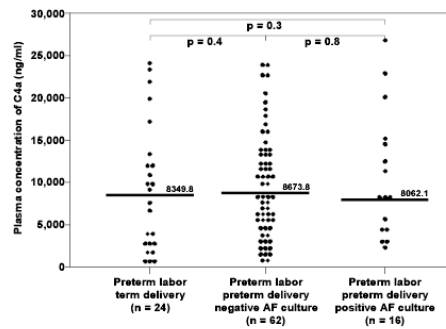
**Figure 2.**

Plasma anaphylatoxin concentrations of normal pregnant women (20–36 6/7 weeks) and patients with preterm labor. **A**, There was no difference in the median plasma C3a concentration of normal pregnant women and those with preterm labor [median: 2493 ng/ml (range 557.9 – 6642.7) vs. median: 2377.1 ng/ml (range 436.1 – 12690), $p=0.4$]. **B**, Similarly, no difference in the median plasma C4a concentration was observed between normal pregnant women and those with preterm labor [median: 11080 ng/ml (range 850.7 – 27850) vs. median: 8554.8 ng/ml (range 436.2 – 30560), $p=0.08$]. **C**, In contrast, the median plasma C5a concentration of patients with preterm labor was higher than that of normal pregnant women [median: 14.6 ng/ml (range 1.9 – 166.8) vs. median: 11.2 ng/ml (range 1.2 – 87.1), $p<0.003$].

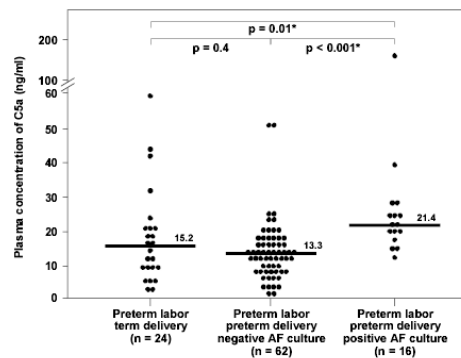
A



B



C

**Figure 3.**

Plasma anaphylatoxin concentrations among patients with preterm labor. **A**, Patients with preterm labor and IAI had a median plasma C3a concentration higher than those without IAI [median: 3275.7 ng/ml (range 1305.8 – 5541.9) vs. median: 2252.8 ng/ml range 436.1 – 12690), Kruskal-Wallis $p=0.01$, post hoc $p=0.005$]. There was no difference in the median C3a concentration between patients with preterm labor who delivered at term and those who delivered preterm, whether or not they had IAI. **B**, There was no difference in the median plasma C4a concentration among patients with preterm labor (Kruskal-Wallis $p=0.6$). **C**, Patients with preterm labor and IAI had a median plasma C5a concentration higher than those without IAI [median: 21.4 ng/ml (range 12.5 – 166.8) vs. median: 13.3 ng/ml (range 1.91 –

51.9) Kruskal-Wallis $p < 0.001$ post hoc $p < 0.001$] and patients with preterm labor who delivered at term [median: 15.2 ng/ml (range 2.2 – 59.1), post hoc $p = 0.01$]. There was no difference in the median plasma C5a concentration between patients with preterm labor who delivered at term and those who delivered preterm without IAI.

Table 1

Clinical and obstetrical characteristics of women at term gestation not in labor and in labor

	Term gestation no labor	Term gestation in labor	<i>P</i>
	n = 70	n = 60	
Maternal age (y)	27 (17–40)	24 (15–36)	0.01 *
Nulliparity	14 (20)	18 (30)	0.2
Smoking	11 (15.7)	15 (25)	0.2
Gestational age at venipuncture (wks)	39.1 (37–41.7)	40 (37–41.8)	<0.001*
Gestational age at delivery (wks)	39.2 (37.1–42.4)	40 (37.1–41.8)	0.008*
Birthweight (g)	3365 (2850–4080)	3335 (2800–3950)	0.2

Values are expressed as median (range) or number (percent)

* Statistically significant, $P < 0.05$

Table 2

Clinical and obstetrical characteristics of normal pregnant women without labor and patients with preterm labor and intact membranes

	Normal pregnancy no labor n = 64	Preterm labor n = 102	P
Maternal age (y)	23 (17–34)	23 (13–39)	0.4
Nulliparity	23 (35.9)	41 (40.2)	0.6
Smoking ^a	12 (19)	30 (29.7)	0.1
Gestational age at venipuncture (wks)	30.7 (20–36.8)	29.8 (20.1–33.7)	0.1
Gestational age at delivery (wks)	39.5 (37–42)	32.8 (21–41.4)	<0.001*
Birthweight (g) ^b	3325 (2610–4030)	1870 (400–3750)	<0.001*

Values are expressed as median (range) or number (percent)

* Statistically significant, $P < 0.05$

^a Normal pregnancy no labor (n=63); preterm labor (n=101)

^b Preterm labor (n=100)