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Early Elevations of the Complement Activation Fragment C3a and Adverse Pregnancy Outcomes

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Abstract

OBJECTIVE—To estimate whether elevations of complement C3a early in pregnancy are predictive of the subsequent development of adverse pregnancy outcomes.

METHODS—A plasma sample was obtained from each enrolled pregnant woman before 20 weeks of gestation. The cohort (n=1,002) was evaluated for the development of adverse pregnancy outcomes defined as hypertensive diseases of pregnancy (gestational hypertension or preeclampsia), preterm birth (before 37 weeks of gestation), premature rupture of the membranes, pregnancy loss (during the embryonic and fetal period), intrauterine growth restriction, and the composite outcome of any adverse outcome.

RESULTS—One or more adverse pregnancy outcomes occurred in 211 (21%) of the cohort. The mean levels (ng/mL) of C3a in early pregnancy were significantly ($P<.001$) higher among women with one or more adverse outcomes (858 ± 435) compared with women with an uncomplicated pregnancy (741 ± 407). Adjusted for parity and prepregnancy body mass index, women with levels of C3a in the upper quartile in early pregnancy were three times more likely to have an adverse outcome later in pregnancy compared with women in the lowest quartile (95% confidence interval, 1.8–4.8; $P<.001$). The link between early elevated C3a levels and adverse pregnancy outcomes was driven primarily by individual significant ($P<.05$) associations of C3a with hypertensive diseases of pregnancy, preterm birth, and premature rupture of the membranes.

CONCLUSION—Elevated C3a as early as the first trimester of pregnancy is an independent predictive factor for adverse pregnancy outcomes, suggesting that complement-related inflammatory events in pregnancy contribute to the subsequent development of poor outcomes at later stages of pregnancy.

The complement system has functions critical to the innate immune response. Named for its “complementary” activity with antibodies to destroy bacteria, the complement system is a series of more than 30 proteins (soluble and membrane-bound) that provides one of the first

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lines of defense against pathogens. The complement system has three main functions: it defends the host against infections, it bridges innate and adaptive immunity, and it disposes of immune complexes, apoptotic bodies, and the products of inflammatory or traumatic injury.^{1,2} Complement has three initiating mechanisms known as the classical, lectin, and alternative pathways. The pathways and the triggers for activation of the individual pathways are described in Figure 1.^{1,3-6} The biologic functions of the complement system are mediated through the production of activation fragments. For example, the activation fragment C3a is an anaphylatoxin. Activation of the complement system therefore carries benefits for the host. This is especially true in early pregnancy, which is a time of intense tissue turnover, and indeed complement activation both reflects and contributes to the heightened inflammatory state seen in normal pregnancy.⁷

In recent times, attention has focused on the importance of the contribution of dysregulation of the complement system to adverse pregnancy outcomes.^{8,9} Dysregulation can present as either excessive activation or inadequate regulation of this complex system. Studies of the murine model of the antiphospholipid antibody syndrome in the early part of this decade demonstrated that excessive complement activation is the primary effector mechanism for intrauterine loss and intrauterine growth restriction (IUGR). Inhibition of the complement cascade blocked the antiphospholipid antibody-related fetal loss and IUGR.^{10,11} Our group translated these results into a study in human pregnancy and found that women with elevated levels of the alternative complement pathway Bb (Fig. 1) in early pregnancy were at risk of developing hypertensive diseases of pregnancy and spontaneous preterm birth later in pregnancy.^{12,13} The last decade has also seen groundbreaking perinatal research on the contribution of mutations in complement regulatory proteins to adverse outcomes,⁵ including pregnancy-associated hemolytic uremic syndrome¹⁴ and the variant of preeclampsia characterized by hemolysis, elevated liver enzymes, and low platelet count and known as HELLP syndrome.^{15,16} All of this recent novel research underscores the important contribution of this understudied area of innate immunity to adverse pregnancy outcomes.

The purpose of the current study was to expand our previous research to another activation fragment namely, C3a (Fig. 1). The aim of the current study was to estimate the role of C3a in adverse pregnancy outcomes to include hypertensive diseases of pregnancy, preterm birth, premature rupture of the membranes, pregnancy loss, and IUGR.

MATERIALS AND METHODS

This prospective study (June 2005 to June 2008) was approved by the Colorado Multiple Institutional Review Board. Details of the study have been described in previous publications.^{12,13,17} In brief, women were recruited from the University of Colorado Hospital prenatal clinics and two affiliated sites. Women were referred to the study's research assistant by the prenatal intake nurse if they were in the first half of pregnancy. Informed consent was obtained and additional plasma (for complement activation fragments) obtained before 20 weeks of gestation with the routine prenatal laboratory values. Data were gathered on the maternal medical and obstetric history. The women were followed throughout pregnancy. At or shortly after delivery, outcome data were collected and the gestational age at blood draw (recruitment visit) was assigned based on the best

overall obstetric estimate incorporating assessment at the first visit and in the great majority of the cohort on early ultrasound examination (more than 90% had an ultrasonography at less than 15 weeks of gestation). Women with an uncertain timing of a pregnancy outcome were classified as having “uncertain dates.”

We excluded from the complete cohort (n=1,287) women with: repeat deliveries during the study period (n=6), women who presented after the second half of pregnancy (n=10), multiple gestations (n=47), loss to follow-up (n=49), induced abortions, congenital or chromosomal anomalies (n=20), and chronic medical disease (cardiovascular disease, insulin-dependent diabetes mellitus, autoimmune disease; n=113). Women with a missing plasma sample (resulting from a deviation from study protocol at the initial blood draw, n=40) were also removed from the analysis. After exclusions, 1,002 women remained in the analytic data set.

The main outcome of the study was adverse pregnancy outcome defined as the composite outcome of one or more of the following pregnancy complications: gestational hypertension, preeclampsia, pregnancy loss, IUGR, premature rupture of the membranes, and spontaneous and medically indicated preterm birth (less than 37 weeks of gestation). The definition of gestational hypertension was a systolic blood pressure greater than 140 mm Hg or a diastolic blood pressure greater than 90 mm Hg on two or more occasions at least 6 hours apart after 20 weeks of gestation in a woman known to have been normotensive before pregnancy and before 20 weeks of gestation.¹⁸ Preeclampsia was defined as 1) gestational hypertension with proteinuria (300 mg+ per 24-hour period) or at least 1+ on dipstick; or 2) in the absence of proteinuria gestational hypertension with cerebral symptoms, epigastric or right upper quadrant pain with nausea or vomiting or thrombocytopenia and an abnormal liver function test.¹⁸ Hypertensive disease of pregnancy was defined as the development of either preeclampsia or gestational hypertension in pregnancy. Pregnancy loss was categorized as occurring in the embryonic (less than 10 weeks of gestation) or fetal period (greater than 10 weeks of gestation).^{19,20} Intrauterine growth restriction was defined as a birth weight less than the tenth percentile²¹ for gestational age using the Colorado Intrauterine Growth Charts.²² Preterm rupture of the membranes was defined as membrane rupture before the onset of labor.²³ Preterm birth less than 37 completed weeks of gestation was categorized as: 1) spontaneous preterm birth resulting from preterm premature rupture of the membranes or spontaneous labor; and 2) medically indicated preterm birth defined as a preterm birth that resulted from a medically indicated delivery for maternal or fetal indications. The gestational age for the lower boundary of preterm birth is controversial. For this analysis, we used 20 weeks of gestation as the lower boundary.²⁴ The outcomes data described previously were collected by the perinatal database staff and entered into the complement study data set housed in the Division of Biostatistics and Bioinformatics at National Jewish Health. A secondary review of each completed maternal record was conducted by one investigator (A.M.L.) before the participant's sample was assayed.

The complement activation fragment, C3a, was the primary exposure examined. Maternal risk factors included in the analysis were: age, race or ethnicity (non-Hispanic white, Hispanic white, African American, Asian, and other), parity (nulliparous compared with multiparous), cigarette smoking at conception (yes compared with no), the maternal

prepregnant body mass index (BMI, calculated as weight (kg)/[height (m)]²) (25 to 30 [overweight], higher than 30 [obese], compared with less than 25), calculated from the maternal prepregnant weight and height, and the gestational age at blood draw (less than compared with greater than 12 weeks of gestation).

Each plasma sample was centrifuged and processed within an average time of 11 minutes, aliquoted, and placed in a freezer at -80°C . At the time of the assay, an aliquot of plasma was thawed. The Pharmingen OptEIATM enzyme-linked immunosorbent assay was used to measure C3a. The inter- and intraassay coefficients of variation were 12.1% and 5.5%, respectively. The individuals performing all study assays were blinded to the participant's pregnancy outcome.

The data were analyzed in SAS 9.2. Initially univariable analysis was used. Measures of the complement activation fragments were examined as continuous and categorical (divided into quartiles) variables. Associations for categorical variables were tested using the chi square test ($P<.05$). Means for continuous variables are reported in the tables, but nonparametric methods (analysis of variance [Kruskal-Wallis test]) were used to test for differences in medians among groups. In some parts of the analysis, we used a contrast statement to test for linear trend within the analysis of variance structure. We performed multivariable logistic regression analysis to estimate the adjusted odds ratio (OR) of C3a for the outcome adjusted for covariates identified as being significantly associated with the outcome in the univariable stage of the analysis. The adjusted ORs of C3a examined as a continuous variable was calculated for every 1-standard deviation change in levels of the biomarker. In the final stage of the analysis, we looked at the levels of C3a in cases of the individual outcomes compared with those of participants in the control group. Deliveries at less than 20 weeks of gestation were removed from this stage of the analysis to comply with the definitions of preterm birth and hypertensive disease of pregnancy.

RESULTS

As seen in Table 1, 21% of this diverse cohort had an adverse pregnancy outcome. Eighty-three (8.5%) women in the cohort had a preterm birth and 8.5% developed gestational hypertension or preeclampsia. A small proportion of the women in the cohort had a pregnancy loss or IUGR. The prevalence of reported cigarette smoking at conception was low at 6%. In the relatively young cohort of women, just more than one third were either obese or overweight before they became pregnant.

Concentrations of C3a were significantly elevated in women with an adverse pregnancy outcome compared with women who had an uncomplicated pregnancy (Table 2). Examined as a categorical variable, a significant association was found between C3a levels in the upper most, third, and second quartiles for an adverse outcome compared with the lower quartile. Regarding other maternal risk factors, a significantly higher proportion of adverse outcomes was observed in nulliparous women. Adverse outcomes were also associated with increasing levels of a maternal prepregnant BMI (Table 2).

Using logistic regression analysis, we determined adjusted for BMI and parity that for every standard deviation increase in concentrations of C3a, the OR for an adverse outcome was 1.3 (confidence interval, 1.1–1.5; $P=.001$). Examined as a categorical variable and adjusted for BMI and parity (Table 3), concentrations of C3a in the top three quartiles were significantly associated with adverse outcomes with adjusted ORs of 2.0, 2.7, and 3.0, respectively (model 1). We explored the data further (Table 3) by stratifying the data by time of gestation of blood draw (before or after 12 weeks of gestation; models 2 and 3) by parity (models 4 and 5) and BMI (models 6 and 7). Stratified by gestational age at blood draw, the adjusted OR was significant in the two upper quartiles among women with a blood draw between 4 and 12 weeks of gestation. Levels of C3a in quartile 2 were not significantly associated with the outcome. The relationship was significant across all quartiles among women who had a blood draw between 12 and 20 weeks of gestation. Stratified by parity (models 4 and 5), the adjusted OR of C3a among nulliparous women for an adverse outcome rose significantly from just more than 3 in the second and third quartiles to 4.1 in the upper quartile. The adjusted ORs for an adverse pregnancy outcome were all elevated (significant in the upper two quartiles) among multiparous women but were slightly attenuated compared with nulliparous women. With obese women removed from the cohort, the adjusted OR of quartiles of C3a remained highly significant for a poor pregnancy outcome (model 6). Similarly, among the smaller group of obese women (model 7), the risk was increased across all categories of C3a. This relationship was significant for levels of C3a in the second and upper quartiles.

In the next stage of the analysis, we separated out the outcomes into their individual components to estimate the contribution of elevated levels of C3a to each pregnancy complication (Table 4). Significantly higher levels of C3a were found in women who developed any hypertensive disease of pregnancy ($P<.001$) or gestational hypertension ($P<.001$). However, although the mean C3a levels were higher among women who developed preeclampsia compared with women who remained normotensive during pregnancy, this relationship did not reach statistical significance ($P=.2$). There were significantly higher levels of the activation fragment in early pregnancy among women who subsequently had a spontaneous preterm birth or medically indicated preterm birth compared with women who had a term delivery. We also found a significant relationship with premature rupture of the membranes. Thirty-one of the 48 cases of spontaneous preterm birth were associated with preterm premature rupture of the membranes. Eleven cases of preterm premature rupture of the membranes occurred at 20 to 34 weeks of gestation and 20 were between 34 and 37 weeks of gestation. We found the highest levels of C3a among the group of women who delivered between 20 and 34 weeks of gestation and lowest levels among the term premature ruptures of the membranes. Importantly, we found a significant linear trend in levels of C3a across gestational age categories of premature rupture of the membranes (Table 4). We found no significant difference between levels of C3a in women with a spontaneous ($n=13$) or medically indicated preterm birth ($n=6$) at less than 34 weeks of gestation compared with term deliveries. Although levels of C3a were elevated among women with pregnancy losses in the fetal period compared with women who had a loss in the embryonic period or women who had no loss (Table 4), this difference was not statistically significant. Among the pregnancy losses, we found 12 women who had an uncertain gestational age at the time of

the loss. We repeated the analysis with these cases (75% were losses during the embryonic period) removed. The mean±standard deviation levels of C3a among women with an embryonic (n=4), fetal loss (n=15), or no loss (n=971) were 681±118, 833±232, and 765±420, respectively ($P=.8$). Similarly, higher levels of C3a were found in women who had a pregnancy complicated by IUGR, but this relationship was not statistically significant.

DISCUSSION

In this analysis we found that the activation fragment C3a measured at a single point in early pregnancy is significantly elevated in women who have an adverse event in later pregnancy compared with women who had an uncomplicated pregnancy. Importantly, we demonstrated that significantly higher levels of C3a are associated with poor pregnancy outcome even among women with a sample drawn in the first trimester. The results were especially striking among nulliparous women; adjusted for BMI, nulliparous women with a C3a level in the upper quartile were 4.1 times significantly more likely to have an adverse pregnancy outcome compared with nulliparous women with levels of C3a in the lower quartile, a finding perhaps related to immune maladaptation²⁵ in early pregnancy. Overall, our study suggests an important role for complement-mediated inflammation in early pregnancy in the subsequent development of adverse pregnancy outcomes.

We cautiously approached the use of a composite outcome in the main part of the analysis for the reasons outlined recently by Cordoba et al.²⁶ We believe that we were justified in using the composite outcome approach because of results of our previous analysis in which we demonstrated higher complement levels in early pregnancy in a subset of women destined to have several adverse pregnancy outcomes.^{12,13,17} We were also meticulous in our definitions of outcomes throughout the analysis and listed results of all the components of the composite outcome with P values to present clearly the outcomes having the most effect on the relationship of the exposure with the composite outcome (Table 4).²⁶ In the breakout analysis into the individual components, we found a significant relationship between C3a concentrations in early pregnancy with the development of hypertensive disease later in pregnancy, in line with the findings of other authors.^{27,28} This association was driven by a strong relationship of C3a with gestational hypertension (Table 4). These findings are aligned with the results of our previous research that demonstrated a relationship between another complement activation fragment, Bb, with preeclampsia.¹² In this current analysis, the relationship of C3a with preeclampsia was not so strong but in the same direction, perhaps secondary to the low number of cases in the outcome (Table 4). Another possibility is that the source of the activation fragments may be different. As previously described, Bb is primarily associated with alternative complement pathway activation,¹² whereas C3a can arise from any of the complement pathways (Fig. 1). We speculate that there may be stronger links with alternative complement pathway activation in women with preeclampsia. This observation is consistent with dysregulation of the alternative pathway of complement activation recently described in HELLP syndrome.¹⁶

We found a significant relationship between elevated levels of C3a in early pregnancy with spontaneous preterm birth at less than 37 weeks of gestation. This is an interesting observation because infection, inflammation, or both is the only risk factor shown

consistently to have a strong causal link with preterm birth.²⁹ Given the important connection between the complement system and inflammation, it is remarkable that complement activation as it relates to preterm birth has only received the attention of a few authors.^{13,30–34} It is of great interest that we found significantly higher levels of C3a in early pregnancy in women who subsequently had an early rupture of the fetal membranes at any gestation in later pregnancy compared with women who had no premature rupture of the membranes. When we examined mean levels of C3a in women with premature rupture of the membranes across these categories of gestational age, we found a dose–response with the highest concentrations of C3a among women who had premature rupture of the membranes between 20 and 34 weeks of gestation and the lowest levels among women with a term premature rupture of the membranes (Table 4). This significant linear trend is in agreement with strong evidence that intrauterine infection or inflammation contributes significantly to spontaneous preterm birth, especially spontaneous preterm birth that occurs less than 34 weeks of gestation.^{35,36} These results are also in agreement with the results of our previous research in which we found a significant relationship between elevated levels of the complement activation fragment Bb in early pregnancy and spontaneous preterm birth less than 34 weeks of gestation.¹³ In the current analysis, we also found C3a to be associated with medically indicated preterm birth, an observation that needs to be examined in a larger cohort. Higher levels of C3a were observed in women who have a pregnancy loss in the fetal period compared with women who had an embryonic loss or with women who had no loss. This observation also needs to be pursued in a larger cohort to specifically address the role of complement activation in pregnancy loss after the first trimester.

Notwithstanding these important results, there are limitations to the study. The main issue is sample size with low number of cases of outcomes when examined individually. This mainly pertains to the outcomes preeclampsia and intrauterine loss and IUGR, discussed previously. With larger numbers, and specifically for preeclampsia, we would have had the opportunity to conduct a stratification analysis by severity of the disease and gestational age at onset of the disease. Women were also undoubtedly missed from this cohort with an intrauterine fetal death as the incidence of this outcome was lower than anticipated.³⁷ We suggest that the death was not captured in this cohort because the event occurred at home before prenatal care was initiated. Our exclusion criteria contributed to the low number of neonates with IUGR. We also acknowledge that the Lubchenco growth curves are limited by their age and need reevaluation. However, currently the Lubchenco curves, developed in Denver, are the only growth curves available for our high altitude. The analysis is also limited by the large range of gestational age at blood draw (between 4 and 20 weeks of gestation). A narrower range of gestational age within data points would be beneficial in estimating the correlation of complement markers with one another and in estimating the more predictive biomarker. We also acknowledge that it will be important in the future to study the role of complement activation at data points beyond the first half of pregnancy. A larger data set would also facilitate exploration of the interaction of these activation fragments with other covariates, specifically maternal obesity (Table 3).¹⁷ Despite these limitations, the data presented provide additional support of the significant relationship between complement-mediated inflammation in early pregnancy with the subsequent development of adverse pregnancy outcomes.

The study results relating to preterm premature rupture of the membranes were especially important and speak to the fact that an early inflammatory episode was part of the natural history of an event that occurred later in pregnancy in a subset of women. The disappointing results of the many antibiotic intervention trials in women at risk for infection-related preterm birth³⁸ underscore the fact that it may be important to further explore inflammation^{39,40} in early pregnancy as a trigger for preterm birth^{41,42} and indeed other adverse pregnancy outcomes.^{43,44} We await results from the ongoing, multicentered, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development–sponsored Effects of Aspirin in Gestation and Reproduction randomized control trial in which low-dose aspirin has been given during preconception and throughout pregnancy in women with a history of intrauterine loss.⁴⁵ The results of this study may shed light on the effect of this agent with antiinflammatory effects on the incidence of not only live birth, but also other adverse pregnancy outcomes. The results of this study build on the results of our previous research. We have now demonstrated that elevated levels of two complement activation fragments in early pregnancy are linked with the subsequent development of adverse pregnancy outcomes. The trigger for the upregulation of the complement system remains to be determined but may possibly be linked with inflammatory events in the placenta^{9–11} or in extraplacental tissues (eg, adipose tissue^{12,17}). The link between complement activation and adverse pregnancy outcomes demonstrated in our research identifies a new potential marker to predict patients at risk for poor pregnancy outcomes.

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Fig. 1.

The classic, lectin, and alternative complement pathways and the triggers for activation (described in detail elsewhere^{1,4,5,46}). The three complement pathways converge to generate C3 convertases that cleave C3, creating C3a and C3b. C3b is a major effector molecule of the complement system. C3b binds covalently to the surface of nearby cells, opsonizes the cells for phagocytosis, and initiates the reactions that lead to the formation of the membrane attack complex (C5–9). Bystander host cells are protected from excessive complement activation by cell-bound and circulating complement regulatory proteins. C3a is an anaphylatoxin that recruits and activates inflammatory cells and has an effect on local blood flow and smooth muscle contractility. The alternative pathway also has the unique ability to serve as an amplification system for the classical and lectin pathways.

Lynch. Complement C3a and Adverse Pregnancy Outcomes. *Obstet Gynecol* 2011.

Table 1

Characteristics of the Cohort (n = 1,002)

Maternal Risk Factor	Total Cohort
Maternal age (y)	31 ± 6
Prepregnant BMI (kg/m ²)	24 ± 5
Gestational age at blood draw (wk)	11.5 ± 2.6
Maternal race	
Non-Hispanic white	626 (62)
Hispanic	246 (25)
African American	69 (7)
Asian and other	61 (6.1)
Nulliparity	443 (44)
Prepregnant BMI (kg/m ²)	
Less than 25	659 (66)
25–30	228 (23)
30 or higher	115 (11)
Cigarette smoking at conception	64 (6)
Outcomes	
Any adverse pregnancy outcome *	211 (21)
Any hypertensive disease of pregnancy	85 (8.5)
Gestational hypertension	51 (5.1)
Preeclampsia	34 (3.4)
Preterm birth at less than 37 wk of gestation	83 (8.5)
Medically indicated	35 (3.5)
Spontaneous	48 (5)
Premature rupture of the membranes	50 (5)
Pregnancy loss	31 (3.1)
Less than 10 wk of gestation	13 (1.3)
Greater than 10 wk of gestation	18 (1.8)
Intrauterine growth restriction	29 (2.9)

BMI, body mass index.

Data are mean ± standard deviation or n (%).

* Defined as gestational hypertension, preeclampsia, preterm (less than 37 weeks of gestation) birth, premature rupture of the membranes, pregnancy loss during the embryonic (less than 10 weeks of gestation) and fetal period (more than 10 weeks of gestation), and intrauterine growth restriction.

Table 2

Relationship of C3a and Select Maternal Risk Factors to Adverse Pregnancy Outcomes*

Maternal Risk Factor	Adverse Pregnancy Outcomes		P
	Present (n = 211)	Absent (n = 791)	
C3a	858 ± 435	741 ± 407	<.001
C3a quartile 1 [†]	27 (13)	221 (28)	
C3a quartile 2	50 (24)	200 (25)	.005
C3a quartile 3	65 (31)	189 (24)	<.001
C3a quartile 4	69 (33)	181 (23)	<.001
Maternal age (y)	31 ± 7	31 ± 6	.8
Maternal prepregnant BMI (kg/m ²)	25 ± 5	24 ± 5	.001
Prepregnant BMI (kg/m ²)			
Less than 25 [†]	122 (58)	537 (68)	
25–30	53 (25)	175 (22)	.1
30 or higher	36 (17)	79 (10)	.002
Maternal race			
Non-Hispanic white [†]	129 (61)	497 (63)	
Hispanic	50 (24)	196 (25)	.9
African American	18 (8.5)	51 (6.5)	.3
Asian and other	14 (7)	47 (6)	.9
Nulliparity	117 (55)	326 (41)	<.001
Multiparity [†]	94 (45)	465 (59)	
Blood draw			
4–12 wk of gestation	109 (52)	350 (44)	.06
More than 12 wk of gestation	102 (48)	441 (56)	
Cigarette smoking at conception			
Yes	19 (9)	45 (6)	.08
No [†]	192 (91)	746 (94)	

BMI, body mass index.

Data are mean ± standard deviation or n (%) unless otherwise specified.

* Defined as preeclampsia, gestational hypertension, pregnancy loss (embryonic and fetal), intrauterine growth restriction, medical or spontaneous preterm birth less than 37 weeks of gestation, and premature rupture of the membranes.

[†]Referent group.

Table 3

The Adjusted Odds Ratio of Quartiles of C3a for Adverse Pregnancy Outcomes in the Cohort and Stratified by Gestational Age at Blood Draw, Parity, and Body Mass Index

Model	C3a		
	AOR	95% CI	P
Model 1: full cohort (n=1,002) ^{*,†}			
C3a Q2	2.0	1.2–3.4	.006
C3a Q3	2.7	1.6–4.4	<.001
C3a Q4	3.0	1.8–4.8	<.001
Model 2: blood draw at 4–12 wk (n=459) ^{*,†}			
C3a Q2	1.9	0.9–3.7	.07
C3a Q3	2.2	1.1–4.3	.02
C3a Q4	2.3	1.2–4.7	.01
Model 3: blood draw 12–20 wk (n=543) ^{*,†}			
C3a Q2	2.7	1.3–5.7	.01
C3a Q3	2.9	1.4–6	.005
C3a Q4	4.0	1.9–8	<.001
Model 4: nulliparous women (n=443) ^{†,‡}			
C3a Q2	3.3	1.6–6.9	.002
C3a Q3	3.2	1.5–6.6	.002
C3a Q4	4.1	2–8.5	<.001
Model 5: multiparous women (n=559) ^{†,‡}			
C3a Q2	1.8	0.9–3.7	.1
C3a Q3	2.2	1.1–4.5	.03
C3a Q4	2.4	1.2–5	.01
Model 6: BMI 15–24.9 kg/m ² (n=659) ^{*,†,‡}			
C3a Q2	2.7	1.3–5.6	.006
C3a Q3	4.7	2.3–9.3	<.001
C3a Q4	3.9	1.9–7.7	<.001
Model 7: BMI 30–52 kg/m ² (n=115) ^{*,†,‡}			
C3a Q2	4.0	1–16	.05
C3a Q3	2.8	0.7–11	.1
C3a Q4	4.4	1.1–18	.03

AOR, adjusted odds ratio; CI, confidence interval; Q, quartile; BMI, body mass index.

Q4 is the upper quartile of levels of C3a, Q3 refers to levels of C3a between the 50th and 75th percentiles, Q2 refers to levels of C3a between the 25th and 50th percentiles, Q1 is the lower quartile of levels of C3a.

Proportion of the cohort in each stratum with an adverse outcome is given in Table 2.

* Adjusted for maternal prepregnant BMI and parity.

[†] The lower quartile is the referent group.

[‡] Adjusted for BMI.

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Table 4

Distribution of Levels of the Complement Activation Fragment C3a Among Women With and Without Individual Adverse Pregnancy Outcomes

Type of Outcome	Levels of C3a (ng/mL)	P*
Any hypertensive disease of pregnancy		
Yes (n=85)	849±310	<.001
No (n=887) ^{†‡}	757±429	
Gestational hypertension (n=51)	859±246	<.001
No gestational hypertension (n=921) ^{†‡}	760±427	
Preeclampsia (n=34)	835±390	.2
No preeclampsia (n=938) ^{†‡}	763±421	
Preterm birth at less than 37 wk of gestation (n=83)	863±405	.004
Term delivery (n=889) ^{†‡}	756±421	
Spontaneous PTB at less than 37 wk of gestation (n=48)	858±406	.03
Term deliveries (n=889) ^{†‡§}	756±420	
Medically indicated PTB at less than 37 wk of gestation (n=35)	870±406	.04
Term delivery (n=889) ^{†‡}	756±420	
PROM (n= 48)	923±452	.006
No PROM (n=924) ^{†‡}	757±417	
PROM by gestational age at delivery (wk)		
20–34 (n=11)	1,013±650	.037 [¶]
34–37 (n=20)	939±428	
37–42 (n=17)	847±325	
No PROM (n=926)	757±417	
Pregnancy loss (n=31)	782±231	.3
No pregnancy loss [†] (n=971)	765±420	
Embryonic loss (n=13)	717±215	.7 [¶]
Fetal loss (n= 18)	830±237	
No intrauterine loss (n=971)	765±420	
Intrauterine growth restriction (n=29)	945±822	.3
No intrauterine growth restriction (n=973) [†]	760±397	

PTB, preterm birth; PROM, premature rupture of the membranes. Data are mean±standard deviation unless otherwise specified.

* By analysis of variance.

[†]Referent group.

[‡]Deliveries at less than 20 weeks of gestation removed from the analysis.

[§]Medically indicated PTB removed from the analysis.

^{||}Spontaneous PTB removed from the analysis.

[¶]Using a contrast statement for linear trend within the analysis of variance structure.

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