Terminal Complement Activation in Preeclampsia

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OBJECTIVE: To evaluate whether C5b-9 concentrations in blood and urine are increased in preeclampsia with severe features.

METHODS: The Complement and Preeclampsia in the Americas study is a prospective, multicenter case–control study performed at six centers in Colombia from November 2015 to July 2016. The case group included women with preeclampsia with severe features, and the control group included women who were healthy or had chronic hypertension, gestational hypertension, or preeclampsia without severe features. We enrolled two women in the control group for every woman in the case group. Soluble C5b-9 concentrations were measured by enzyme-linked immunosorbent assays in blood and urine. The primary outcome was C5b-9 concentrations in women in the case group compared with all women in the control group, and the secondary outcome was C5b-9 levels in women in the case group compared with individual control subgroups. Differences were assessed by test of medians, and associations were further evaluated by receiver operating characteristic curve analysis and logistic regression with $\alpha=0.05$.

RESULTS: Three hundred fifty-two patients were enrolled. Plasma C5b-9 concentrations did not differ significantly between women in the case group and those in the control group, but urine C5b-9 concentrations were higher in women in the case group (median [interquartile range] 9.9 [1.6–43.7] vs 1.8 [0.54–4.1] ng/mL, $P<.001$). In subgroup analysis, plasma C5b-9 concentrations were increased in women in the case group compared with healthy women in the control group (median [interquartile range] 2,778 [1,633–4,230] vs 1,374 [1,064–2,332] ng/mL, $P<.001$), and urine C5b-9 concentrations were increased in women in the case group compared with all control subgroups ($P<.001$). Using receiver operating characteristic analysis, urine C5b-9 concentrations differentiated preeclampsia with severe features from hypertensive women in the control group (area under the receiver operating characteristic curve 0.74, 95% CI 0.68–0.80). Urine C5b-9 22 ng/mL or greater (range 0–158.4 ng/mL) was the optimal cut point for diagnosis of preeclampsia with severe features with adjusted odds ratio of 10.0 (95% CI 3.5–28.8, $P<.001$).

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Supported by Colciencias, Administrative Department of Science, Technology and Innovation, Colombia, Código #111565740967; FUNDARED-MATERNA, and Oregon Health & Science University, Department of Obstetrics & Gynecology, Mission Award.

Presented at the 38th Annual Meeting of the Society for Maternal-Fetal Medicine, January 29–February 3, 2018, Dallas, Texas.

For a list of contributors to Complement Activation in Preeclampsia in the Americas (COPA) study, see Appendix 1 online at http://links.lww.com/AOG/B196.

Each author has indicated that he or she has met the journal's requirements for authorship.

Received July 12, 2018. Received in revised form September 8, 2018. Accepted September 20, 2018. Peer review history is available at http://links.lww.com/AOG/B197.

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Financial Disclosure
Dr. Burwick has served on the speaker's bureau for Alexion Pharmaceuticals. Dr. Burwick's role with Alexion was distinct from this research study, and Alexion was not involved in the concept, design, analysis, writing, or funding of this work in any manner. The other authors did not report any potential conflicts of interest.

ISSN: 0029-7844/18

CONCLUSION: Urinary excretion of terminal complement effector C5b-9 is higher in women with preeclampsia with severe features compared with women with other hypertensive disorders of pregnancy and women without hypertension.

(Obstet Gynecol 2018;132:1477–85)
DOI: 10.1097/AOG.0000000000002980

Preeclampsia, defined by hypertension with proteinuria or end-organ injury, affects 2–4% of pregnancies.[1–3] It may arise from placental inflammation or ischemia with systemic activation of leukocytes and endothelial cells.[4,5] Fulminant disease, termed preeclampsia with severe features, is a leading cause of maternal death globally.[3,6,7] Definitive treatment is delivery, which often results in a premature neonate at risk for long-term developmental impairment and death.[8]

Complement protein activation, critical to host defense, may propagate disease in preeclampsia and hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome.[9–13] Complement activation increases in pregnancy[14] in response to semiallogenic material such as placental apoptotic debris,[15] fetal DNA and RNA,[16,17] immune complexes,[18] and spontaneous, antibody-independent mechanisms (alternative pathway).[19] Activated complement proteins converge to the terminal pathway, whereby C5 is cleaved to C5a and C5b.[20] C5a polarizes macrophages to the antiangiogenic phenotype,[21,22] whereas C5b combines with C6-9 to form C5b-9, which propagates cell lysis and microvascular thrombosis.[23,24] Placental trophoblast cells express complement regulatory proteins that mitigate complement activation (Fig. 1).[10,25] However, activation of C5 may exceed regulatory capacity in preeclampsia as a result of increased fetoplacental debris,[26] comorbid conditions,[27] or complement gene mutations.[12,28]

Concentrations of terminal complement effector C5b-9 appear to be increased in severe forms of preeclampsia, but data are limited.[11] Thus, our primary aim is to compare blood and urine concentrations of C5b-9 in preeclampsia with severe features to women in a control group with a healthy pregnancy, chronic hypertension, gestational hypertension, or preeclampsia without severe features. We hypothesize that C5b-9 concentrations are increased in preeclampsia with severe features.

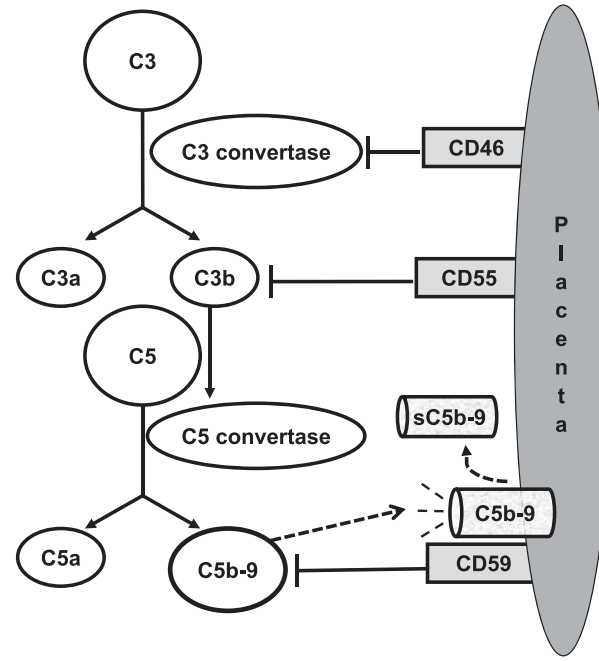

Fig. 1. Schematic of complement activation and regulation at the placental interface. The complement cascade may be activated through classic, lectin, or alternative pathways (not pictured) that converge to generate C3 convertases, which cleave C3 to generate activation products C3a (anaphylatoxin) and C3b (opsonin). Accumulation of C3b leads to generation of C5 convertases, which cleave C5 to generate C5a (anaphylatoxin) and C5b, which combine with complement proteins C6-9 to form C5b-9 (membrane attack complex). C5b-9 may incorporate into cell membranes (*mottled gray cylinder on placental surface*) with sublytic or lytic effects. C5b-9 may also be released in an active soluble form (sC5b-9) (*mottled gray cylinder released from placental surface*). However, the complement cascade may be inhibited by complement regulatory proteins expressed on the syncytiotrophoblast membrane (*gray rectangles on placental surface*: CD46, CD55, CD59). CD46 blocks activation of C3 into C3a and C3b; CD55 inhibits the actions of C3b and reduces C5 activation; CD59 blocks the actions of C5b-9, the membrane attack complex.
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MATERIALS AND METHODS

The Complement and Preeclampsia in the Americas study is a prospective, multicenter case–control study performed at six centers in Colombia from November 2015 to July 2016. Institutional review board approval was obtained at Universidad de Antioquia and all study sites: Clínica Reina Sofía–Sanitas and Hospital San Ignacio (Bogotá), Clínica Universitaria Bolivariana, Hospital Universitario San Vicente Fundación and Hospital General de Medellín (Medellín), and ESE Clinica de Maternidad Rafael Calvo (Cartagena). Participants signed informed consent before study entry and all procedures were followed in accordance with institutional guidelines and the study protocol.

Eligible participants were enrolled sequentially by trained research coordinators during available

work hours. The case group included women with preeclampsia with severe features; women in the control group could have healthy pregnancies or chronic hypertension, gestational hypertension, or preeclampsia without severe features. Women in the control group were enrolled in a two-to-one ratio with women in the case group. The Complement and Preeclampsia in the Americas study targeted enrollment of 100 cases of preeclampsia with severe features, including 50 women at less than 34 weeks of gestation and 50 women at 34 weeks of gestation or greater. Individual sites were given a target of 50 participants to enroll in the study, and coordinators were instructed to enroll two women in a control group for every woman in the case group (with women in the control group matching the gestational age category of the case, less than 34 weeks of gestation or 34 weeks of gestation or greater). Diagnoses were made in accordance with the 2013 American College of Obstetricians and Gynecologists' criteria for hypertension in pregnancy.[3] Teleconferences were held monthly during the study period to gauge study progress and enrollment numbers at each site.

Participants were enrolled from outpatient clinics, labor and delivery floors, antepartum units, and triage or emergency departments. Clinical diagnoses were confirmed within the first 24 hours after enrollment once blood pressure, laboratory values, and symptoms were clarified. The normal reference range for standard blood tests at study sites were aspartate transaminase (15–46 units/L); creatinine (0.5–1.1 mg/dL); lactate dehydrogenase (125–243 units/L); and platelet count (150–450,000/microliter). Exclusions were gestational age less than 24 weeks, uncertain dates, multifetal gestation (two or greater), major chromosomal abnormality, fetal demise at entry, preexisting diabetes mellitus or insulin-dependent gestational diabetes mellitus, chronic kidney disease, systemic lupus erythematosus, immunodeficiency, untreated bacterial or viral infection (including suspected Zika virus), active use of heparin, eculizumab or immunosuppressive agents, or inability to sign informed consent. Study data, including participant demographics, clinical history, laboratory data, and delivery and neonatal outcomes, were recorded through standardized data collection forms and entered into a centralized electronic database.

The primary outcome was C5b-9 concentrations in women in the case group compared with women in the control group, and the secondary outcome was C5b-9 concentrations in women in the case group compared with individual control subgroups. Blood and urine were collected on the day of enrollment. Blood was collected in ethylenediaminetetraacetic acid tubes and urine through a random clean-catch specimen or indwelling catheter. Blood and urine samples were centrifuged at 4° C with supernatant aliquoted and stored in cryovials at each site at −70 to −80° C. At completion of study enrollment, sample aliquots were shipped on dry ice to a central laboratory in Bogotá (Clínica ColSanitas) for analysis. Soluble C5b-9 (C5b-9) was measured in plasma and urine by human C5b-9 enzyme-linked immunosorbent assay. Assays were performed using a DSX automated four-plate enzyme-linked immunosorbent assay. Samples were run in duplicate with negative (blank) and positive controls (pooled plasma or urine). Intra- and interassay coefficients of variation were 3.4% and 10.0% (plasma C5b-9) and 5.3% and 13.8% (urine C5b-9), respectively. Plasma samples were run after 1:200 dilution and urine samples at 1:2–1:20 dilution to obtain concentrations in the linear region of the standard curve. Urine C5b-9 values within 2 SDs of the blank (less than 0.20 ng/mL) were considered below the lower limit of detection. Plasma C5b-9 values were above the limit of detection in all participants. Protein and creatinine concentrations were determined in urine samples by colorimetric assays.

The Complement and Preeclampsia in the Americas study was designed and powered to test the hypothesis that soluble C5b-9 concentrations are increased in plasma and urine in women with preeclampsia with severe features (cases) compared with women in a control group divided into four groups (healthy, chronic hypertension, gestational hypertension, preeclampsia without severe features). Based on prior findings,[12] we determined that 100 women in a case group and 200 women in a control group (50/subgroup) were required to demonstrate a 50% difference in plasma C5b-9 concentrations and a 200% difference in urinary C5b-9 concentrations between women in the case group and those in the control group with $\alpha = 0.05$ and power = 0.80. We anticipated that a smaller difference in plasma C5b-9 concentrations could be detected between groups as a result of a lower SD of C5b-9 concentrations in plasma as compared with urine. Cases of preeclampsia with severe features are more likely to present at an earlier gestational age compared with controls. Therefore, study sites were instructed to enroll women in a case group and those in a control group into stratified groups by gestational age (less than 34 weeks of gestation or 34 weeks of gestation or greater) and diagnosis until recruitment targets were met.

Baseline characteristics of the Complement and Preeclampsia in the Americas study participants are presented with descriptive statistics. Differences between study groups were assessed by χ^2 test for dichotomous data, *t* test or analysis of variance for normal continuous data, and nonparametric equality of medians test for nonnormal continuous data. Data normality was determined based on tests of skewness and kurtosis with nonnormal data displayed as medians (interquartile range). Receiver operating characteristic curve analysis was used to determine the optimal C5b-9 concentration for diagnosis of preeclampsia with severe features. The optimal cut point was determined by the C5b-9 concentration that correctly classified the most participants. Multivariable logistic regression was used to adjust for confounders. Unadjusted and adjusted odds ratios (ORs) were calculated with 95% CIs. Data analysis was performed with Stata, and statistical significance was determined by $\alpha=0.05$.

RESULTS

We enrolled 352 participants in the Complement and Preeclampsia in the Americas study with the following distribution by study site: Hospital Universitario San Vicente Fundación (n=85); Clínica Reina Sofía–Sanitas (n=60); Clínica Universitaria Bolivariana (n=58); ESE Clinica de Maternidad Rafael Calvo (n=53); Hospital Universitario San Ignacio (n=49); and Hospital General de Medellín (n=47). Baseline characteristics of study participants, stratified by enrollment group, are shown in Table 1. Gestational age was similar between groups, consistent with stratified enrollment; 124 participants (35%) were enrolled at less than 34 weeks of gestation and 228 participants (65%) were enrolled at 34 weeks of gestation or greater. Groups varied by maternal age, body mass index (BMI, calculated as weight (kg)/[height (m)]^2), parity, race–ethnicity, blood pressure, and urine protein at enrollment.

Plasma C5b-9 concentrations were not significantly different between cases of preeclampsia with severe features and women in the control group (healthy, chronic hypertension, gestational hypertension, or preeclampsia without severe features) (median [interquartile range] 2,778 [1,633–4,230] vs 2,451 [1,360–3,927] ng/mL, *P*=.29). In subgroup analysis, plasma C5b-9 concentrations were increased in women in the case group compared with healthy women in the control group (median [interquartile range] 2,778 [1,633–4,230] vs 1,374 [1,064–2,332] ng/mL, *P*<.001) (Fig. 2A; Appendix 2 [Appendix 2 is available online at http://links.lww.com/AOG/B196]) and were similarly increased in other hypertensive disorders of pregnancy compared with healthy women in the control group (*P*<.001). There was no significant difference in plasma C5b-9 concentrations between individual hypertensive disorders. However, in participants enrolled at less than 34 weeks of gestation, plasma C5b-9 concentrations were significantly increased in those with early-onset preeclampsia

Table 1. Baseline Characteristics of Complement and Preeclampsia in the Americas Study Participants, Stratified by Enrollment Group

Characteristic	Healthy (n=54)	Chronic Hypertension (n=50)	Gestational Hypertension (n=87)	Preeclampsia Without Severe Features (n=57)	Preeclampsia With Severe Features (n=104)	*P**
Gestational age (wk)	34.2±4.2	34.3±4.2	35.5±4.2	35.4±3.7	33.2±4.2	N/A†
Age (y)	30.2±6.2	29.4±6.8	26.5±6.1	25.9±6.8	25.7±6.5	<.001
BMI (kg/m^2)	23.8±3.5	28.1±5.5	25.4±4.6	25.7±5.0	24.7±4.3	<.001
Systolic BP (mm Hg)	114±13	139±12	142±11	141±11	150±16	<.001
Diastolic BP (mm Hg)	67.0±9.4	85.4±12	89.0±7.7	88.0±9.4	95.8±13	<.001
Urine protein/creatinine (mg/mg)	0.10 (0.07–0.12)	0.12 (0.09–0.14)	0.12 (0.09–0.16)	0.37 (0.16–0.76)	0.91 (0.33–3.7)	<.001
Nulliparous	32/52 (61.5)	26/50 (52.0)	57/83 (68.7)	46/57 (80.7)	65/103 (63.1)	.03
African descent	1/49 (2.0)	5/49 (10.2)	19/83 (22.9)	9/56 (16.1)	19/103 (18.5)	.02

N/A, not applicable; BMI, body mass index; BP, blood pressure.
Data are mean±SD, median (interquartile range), or n/N (%) unless otherwise specified.
* Analysis of variance (continuous data), χ^2 test (dichotomous data), test of medians (nonparametric data).
† Enrollment in blocks by gestational age.

compared with those without preeclampsia (median [interquartile range] 2,953 [1,622–4,472] ng/mL vs 1,965 [1,078–2,986] ng/mL, P=.003) (Fig. 2B; Appendix 2 [Appendix 2, http://links.lww.com/AOG/B196]). Among participants with early-onset preeclampsia, plasma C5b-9 concentrations did not differ between those with or without severe features.

In contrast to plasma, urine C5b-9 concentrations were significantly increased in cases of preeclampsia with severe features compared with women in the control group (healthy, chronic hypertension, gestational hypertension, preeclampsia without severe features) (median [interquartile range] 9.88 [1.6–44] vs 1.8 [0.54–4.1] ng/mL, P<.001) (Fig. 3A; Appendix 2 [Appendix 2, http://links.lww.com/AOG/B196]). In subgroup analysis, urine C5b-9 concentrations were increased in preeclampsia with severe features compared with all other groups, including preeclampsia without severe features. There was no difference in urine C5b-9 concentrations between any of the control groups. Results were similar in participants enrolled at less than 34 weeks of gestation (Fig. 3B;

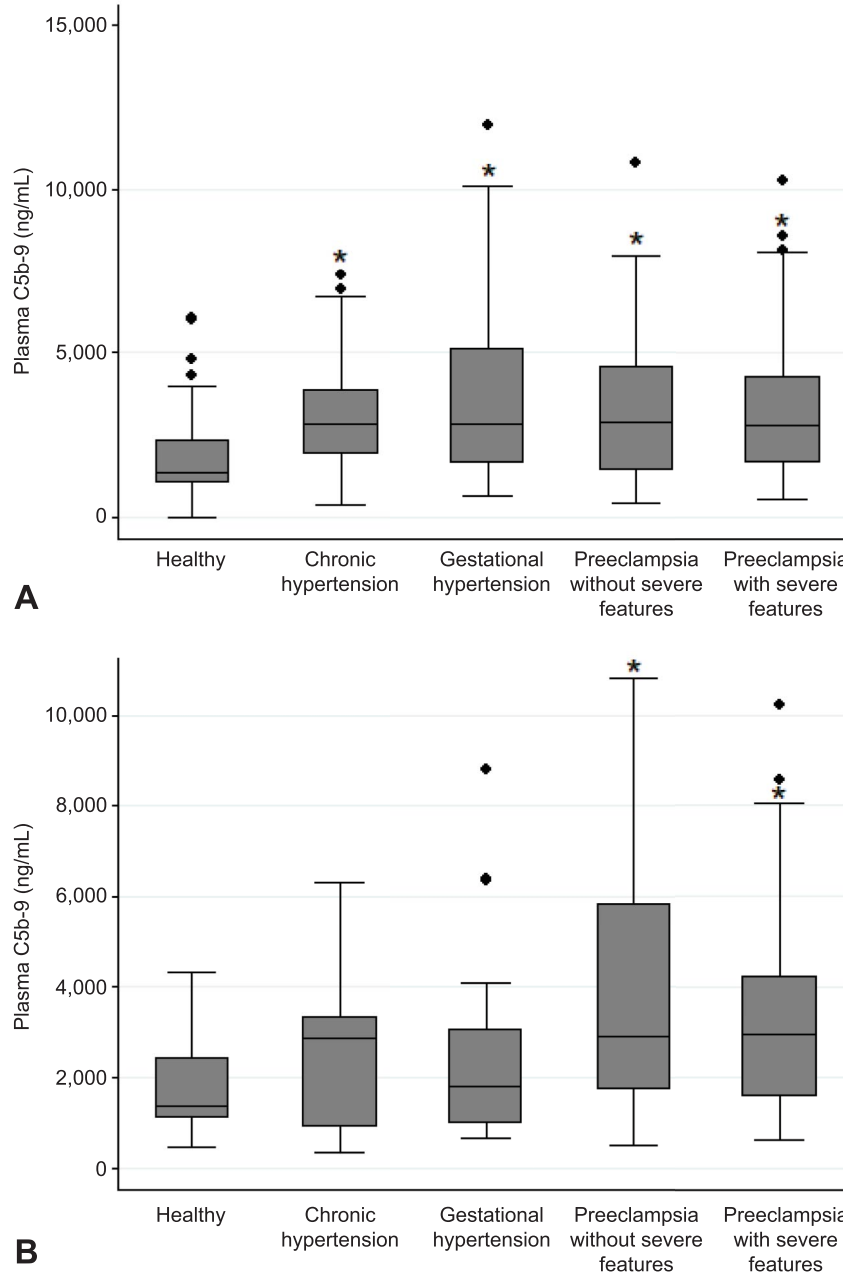

Fig. 2. Median (interquartile range [IQR]) levels of C5b-9 in plasma by study group. C5b-9 levels are displayed as median (*horizontal black line*), IQR (*vertical gray rectangle*), 10th and 90th percentiles (*horizontal whiskers*), and outside values (*black dots*). **A**. Results from the entire cohort of Complement Activation in Preeclampsia in the Americas study participants (n=352). Plasma C5b-9 levels were increased in chronic hypertension, gestational hypertension, preeclampsia without severe features, and preeclampsia with severe features vs healthy women in a control group (*P<.001). **B**. Results from only those patients enrolled at less than 34 weeks of gestation (n=124). Plasma C5b-9 levels were increased in patients with preeclampsia (with or without severe features) vs women in a control group (healthy, chronic hypertension, gestational hypertension) without preeclampsia (*P=.003).

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Appendix 2 [Appendix 2, http://links.lww.com/AOG/B196]). Urine C5b-9 concentrations were significantly increased among participants with early-onset preeclampsia compared with those without preeclampsia (median [interquartile range] 5.50 [1.5–41.0] ng/mL vs 1.40 [0.40–2.5] ng/mL, $P<.001$). Concentrations were most specifically increased in early-onset preeclampsia with severe features compared with early-onset preeclampsia without severe features (median [interquartile range] 7.14 [1.7–47] ng/mL vs 2.25 [0.64–9.5] ng/mL, $P=.04$). There was no difference in urine C5b-9 concentrations between participants with early- and late-onset preeclampsia.

Using receiver operating characteristic curve analysis, we found that urine C5b-9 concentrations differentiated preeclampsia with severe features from women in the control group (healthy, chronic hypertension, gestational hypertension, or preeclampsia without severe features) with area under the receiver operating characteristic curve (0.74, 95% CI 0.68–0.80) (Appendix 3, available online at http://links.lww.com/AOG/B196). Urine C5b-9 concentration

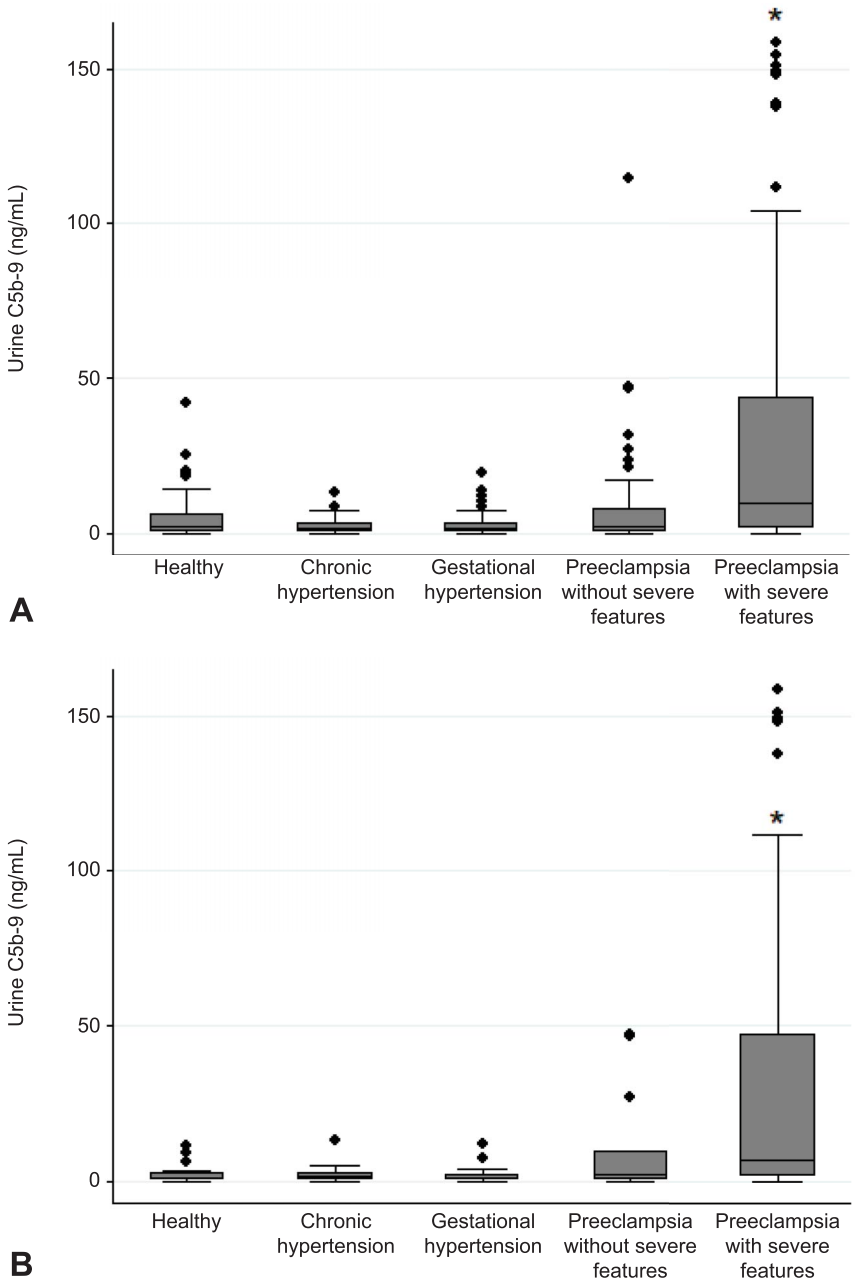

Fig. 3. Median (interquartile range [IQR]) levels of C5b-9 in urine by study group. C5b-9 levels are displayed as median (*horizontal black line*), IQR (*vertical gray rectangle*), 10th and 90th percentiles (*horizontal whiskers*), and outside values (*black dots*). **A**. Results from the entire cohort of Complement Activation in Preeclampsia in the Americas study participants (n=352). Urine C5b-9 levels were increased in preeclampsia with severe features vs all other control groups (*$P<.001$). **B**. Results from only those participants enrolled at less than 34 weeks of gestation (n=124). Urine C5b-9 levels were increased in participants with preeclampsia with severe features vs all other control groups (*$P<.001$).

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Table 2. Odds of Preeclampsia With Severe Features, By Univariable and Multivariable Logistic Regression

Variable	OR for Preeclampsia With Severe Features (n=104)	95% CI	*P*
Urine C5b-9 22 ng/mL or greater	20.3	9.1–45.5	<.001
Urine C5b-9 22 ng/mL or greater +adjustment for maternal age, parity, race, prepregnancy BMI, and gestational age at enrollment	18.8	7.6–46.6	<.001
Additional adjustment for urine protein (mg/dL)	11.1	3.9–31.4	<.001
Additional adjustment urine creatinine (mg/dL)	10.0	3.5–28.8	<.001

OR, odds ratio; BMI, body mass index.

22 ng/mL or greater was the optimal cut point for diagnosis of preeclampsia with severe features with a positive likelihood ratio of 12.5, specificity of 96.8% (95% CI 93.5–98.4%), and sensitivity of 40.4% (95% CI 31.0–50.5%). Notably, among participants with preeclampsia with severe features, 40.4% (42/104) had urine C5b-9 concentration 22 ng/mL or greater compared with 0% (0/137) of participants with chronic or gestational hypertension ($P<.001$) and 10.5% (6/57) of those with preeclampsia without severe features ($P<.001$).

In univariable logistic regression, the odds of preeclampsia with severe features were markedly increased in participants with urine C5b-9 22 ng/mL or greater (OR 20.3, 95% CI 9.1–45.5, $P<.001$) (Table 2). After stepwise multivariable adjustment for maternal age, BMI, race–ethnicity, nulliparity, gestational age at enrollment, urine protein, and urine creatinine, the association between urine C5b-9 22 ng/mL or greater and preeclampsia with severe features remained significant (adjusted OR 10.0, 95% CI 3.5–28.8, $P<.001$). Adjustment for urine protein led to the greatest attenuation in the OR as a result of the correlation between urine protein and urine C5b-9 concentrations ($r=0.57$, $P<.001$). Finally, participants with urine C5b-9 22 ng/mL or greater, compared with those with urine C5b-9 concentrations less than 22 ng/mL, were also more likely to have laboratory evidence of end-organ injury such as serum creatinine 1.0 mg/dL or greater (14.3% vs 4.9%, $P=.02$), lactate dehydrogenase 500 units/L or greater (20.5% vs 7.9%, $P=.02$), and platelet count less than 150,000/microliter (22.2% vs 8.3%, $P=.003$).

DISCUSSION

We found that plasma concentrations of terminal complement effector C5b-9 are not specifically increased in women with preeclampsia with severe features compared with women with other hypertensive disorders of pregnancy or women without hypertension. However, plasma C5b-9 concentrations are broadly increased in all women with a hypertensive disorder of pregnancy compared with women without hypertension. Increased activation of the terminal complement pathway in hypertensive disorders of pregnancy may reflect endothelial dysfunction and systemic inflammation common to these disorders. In contrast to the findings in plasma, urinary excretion of terminal complement effector C5b-9 is increased specifically in women with preeclampsia with severe features compared with women with other hypertensive disorders of pregnancy or women without hypertension. This finding may reflect more profound activation of the terminal complement pathway in severe disease with renal involvement.

Urinary excretion of C5b-9 is not expected as a result of its large molecular weight (greater than 1,000,000 Daltons).[29] However, activated complement proteins may mediate kidney injury directly with urinary excretion of C5b-9 secondary to glomerular or tubular impairment.[30–32] C5b-9 may form at the glomerular membrane with shedding into the urine or C5b-9 may be generated after inflammation and cellular injury at the proximal tubule.[30–34] Plasma and urine C5b-9 concentrations do not correlate well, arguing against simple renal clearance of complement proteins. Moreover, the association between urine C5b-9 concentrations and preeclampsia is independent of total urine protein, arguing against generalized proteinuria as an explanation for our results.

Although terminal complement effector C5b-9 is associated with active clinical disease, upstream complement pathways are likely strained from early pregnancy. In women with high blood concentrations of upstream complement split products C3a or Bb, preeclampsia is three to four times more likely[19,35]

and 8–10 times more likely if obesity is present.[27] Concentrations of C3a and Bb are also increased in amniotic fluid among women who eventually develop preeclampsia.[36] Germline mutations in complement regulatory genes may also predispose to preeclampsia and HELLP syndrome.[12,28] In such cases, complement activation is increased as a result of loss-of-function mutations in complement regulators or gain-of-function mutations in complement activators.

Persistent upstream activation of either alternative or classic complement pathways ultimately leads to activation of the terminal pathway. C5b-9, in membrane-bound or soluble form, propagates inflammation, cell lysis, and villous trophoblast injury.[23,24] The placenta may upregulate expression of CD59, a membrane-bound inhibitor of C5b-9, to combat terminal complement activation in preeclampsia,[10] yet we have shown here and previously that maternal C5b-9 concentrations are increased in preeclampsia with severe features.[11] Moreover, urinary excretion of C5b-9 occurs in both early- and late-onset preeclampsia, suggesting that terminal complement activation is a key feature of disease regardless of gestational age. The kidney is most vulnerable to complement activation, likely as a result of decreased expression of complement regulators compared with other end organs such as the brain.[37] However, end-organ effects are not limited to the kidney. Participants with marked urinary excretion of C5b-9 were also more likely to have hemolysis and thrombocytopenia.

End-organ effects such as acute kidney injury, hemolysis, and thrombocytopenia are common to other disorders in which C5b-9 is a key mediator, notably atypical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria.[38,39] Terminal complement blockade is effective for treatment of atypical hemolytic uremic syndrome[37] and paroxysmal nocturnal hemoglobinuria[38] and is a putative treatment for preeclampsia and HELLP syndrome.[13] Specifically, we have previously shown that eculizumab (C5 inhibitory antibody) is effective in treating severe preeclampsia and HELLP syndrome arising at 26 weeks of gestation with resolution of hemolysis and thrombocytopenia and prolongation of pregnancy by 17 days. Concentrations of C5b-9 in blood and urine decreased together with disease remission.[40]

Our study is not without limitations. As a result of the observational design, we are unable to draw definitive conclusions regarding causal relationships. For example, it remains unknown whether C5b-9 is present in urine before the onset of preeclampsia or if concentrations rise or fall with progression of disease (eg, preeclampsia to HELLP syndrome). Although C5b-9 concentrations correlated with some features of HELLP (ie, hemolysis and thrombocytopenia), we had too few patients with HELLP for detailed analysis. We also did not measure other upstream complement split products of classic or alternative complement pathways, but instead focused solely on their shared terminal effector. Urinary measurement of C5b-9 does not have immediate clinical applicability because it is not validated for use in patient samples. Although plasma C5b-9 concentrations can be measured from patient samples, we did not detect a discriminatory level for clinical use. The strengths of our study include its multicenter case–control design, large number of participants with severe disease, and inclusion of participants with the full range of hypertensive phenotypes.

Terminal complement activation may be a central factor in severe forms of preeclampsia and HELLP syndrome, and urinary excretion of C5b-9 may identify those women who would stand to benefit from therapeutic complement blockade. Although there is increasing evidence that C5 blockade with eculizumab is safe in pregnancy,[41] without neonatal harm,[42] such use remains off-label and clinical trials are needed to assess whether such an approach is safe and effective for preeclampsia and HELLP syndrome.

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