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Soluble concentrations of the terminal complement complex C5b-9 correlate with end-organ injury in preeclampsia

Catalina M. Valencia [a,b,c,*], Alyssa R. Hersh [b,d], Richard M. Burwick [b,d,e], Jesús A. Velásquez [b,f,g], Jorge Gutiérrez-Marín [b,h], Francisco Edna [b,i], Jaime L. Silva [b,j,k], Juliana Trujillo-Otálvaro [b,l], Johanna Vargas-Rodríguez [b,m], Yamile Bernal [b,m], Alvaro Quintero [b,l], Mónica Rincón [b,d], Jorge E. Tolosa [b,d,n]

[a] Clínica Reina Sofía Colsanitas SA, Bogotá, Distrito Capital de Bogotá, Colombia
[b] Fundared-Materna, Bogotá, Distrito Capital de Bogotá, Colombia
[c] Universidad CES, Medellín, Antioquia, Colombia
[d] Oregon Health & Science University, Department of Obstetrics and Gynecology, Portland, OR, USA
[e] Cedars-Sinai Medical Center, Department of Obstetrics and Gynecology, Los Angeles, CA, USA
[f] Universidad de Antioquia, Medellín, Antioquia, Colombia
[g] Hospital Universitario San Vicente Fundación, Medellín, Antioquia, Colombia
[h] Universidad Pontificia Bolivariana, Medellín, Antioquia, Colombia
[i] ESE Clínica de Maternidad Rafael Calvo, Cartagena, Bolívar, Colombia
[j] Hospital Universitario San Ignacio, Bogotá, Distrito Capital de Bogotá, Colombia
[k] Pontificia Universidad Javeriana, Bogotá, Distrito Capital de Bogotá, Colombia
[l] Hospital General de Medellín, Medellín, Antioquia, Colombia
[m] Laboratorio Clínico Colsanitas, Bogotá, Distrito Capital de Bogotá, Colombia
[n] St. Luke's University, Bethlehem, PA, USA

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ABSTRACT

Objective: We sought to determine if soluble levels of C5b-9, the terminal complement complex, correlate with end-organ injury in preeclampsia.
Study Design: Project COPA (Complement and Preeclampsia in the Americas), a multi-center observational study in Colombia from 2015 to 2016, enrolled hypertensive pregnant women into four groups: chronic hypertension, gestational hypertension, preeclampsia, and preeclampsia with severe features. Trained coordinators collected clinical data, blood and urine. End-organ injury was defined by serum creatinine $\geq$ 1.0 mg/dl, aspartate transaminase $\geq$ 70U/L, platelet count $<$ 150,000/µl, or lactate dehydrogenase $\geq$ 500 U/L. Data were analyzed by χ^2 or Fisher's exact test with significance at $P < 0.05$.
Main Outcome Measure: C5b-9 concentrations in plasma and urine, using enzyme linked immunosorbent assays.
Results: In total, 298 hypertensive participants were enrolled. Plasma and urine C5b-9 levels were measured in all participants and stratified by quartile (Q1-4), from lowest to highest C5b-9 concentration. Participants with low plasma C5b-9 levels (Q1) were more likely to have end-organ injury compared to those with higher levels (Q2-Q4) [platelet count $<$ 150,000/µl (20.8% vs. 8.4%, $P = 0.01$); elevated serum creatinine $\geq$ 1.0 mg/dl (14.9% vs. 4.5%, $P = 0.009$)]. In contrast, participants with high urinary C5b-9 levels (Q4) were more likely to have end-organ injury compared to those with lower levels (Q1-Q3) [platelet count $<$ 150,000/µl (19.7% vs. 7.4%, $P = 0.003$); elevated serum creatinine $\geq$ 1.0 mg/dl (12.3% vs. 4.4%, $P = 0.025$)].
Conclusion: We identified a pattern of increased urine and low plasma C5b-9 levels in patients with preeclampsia and end-organ injury. Soluble C5b-9 levels may be used to identify complement-mediated end-organ injury in preeclampsia.

* Corresponding author at: Cra.20 N°2 Sur 185 Clínica, El Rosario del Tesoro, Colombia.
E-mail address: catalina.valencia@medicinafetal.com.co (C.M. Valencia).

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1. Introduction

Preeclampsia is a common pregnancy disorder characterized by high blood pressure with proteinuria or end-organ injury. [1] Worldwide, preeclampsia is one of the leading causes of maternal morbidity and mortality, yet its exact cause remains undetermined. [2] In normal pregnancy physiology, it is known that complement activation is increased due to exposure to placental and fetal compounds, such as placental apoptotic debris and fetal genetic material (DNA and RNA). [3–6] However, excessive complement activation has been associated with negative consequences during pregnancy, including its implication in the pathogenesis of preeclampsia. [7–9].

Previous studies have demonstrated that preeclampsia is more common among women with elevated blood levels of upstream complement proteins, including C3a or Bb. [10–11] Persistent activation of upstream pathways, potentially from early pregnancy, leads to downstream activation of the terminal complement pathway. C5b-9, the terminal complement effector, is responsible for carrying out the harmful inflammatory and lytic actions of the activated complement system. [12] Prior work has demonstrated that the concentration of C5b-9 is increased in urine of women with preeclampsia with severe features when compared to other pregnant women. [13] These results suggest that pregnant women with severe hypertensive disease experience more profound complement activation.

C5b-9 has been implicated in the pathophysiology of other complement disorders, such as atypical hemolytic uremic syndrome (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH), which are also characterized by end-organ injury (acute kidney injury, hemolysis, and thrombocytopenia). [14–15] Recent evidence suggests that some women with preeclampsia may also have complement-mediated disease, similar to aHUS and PNH. While C5b-9 concentrations are increased in preeclampsia, it remains unclear if C5b-9 concentrations are increased in association with end-organ injury in preeclampsia. Therefore, we sought to determine if C5b-9 concentrations are increased in women with preeclampsia who have laboratory evidence of end-organ injury.

2. Methods

The Complement and Preeclampsia in the Americas (COPA) study was a multicenter study with a prospective case-control design, performed at six centers in Colombia between November 2015 through July 2016. Institutional review board approval was obtained at all study sites and Universidad de Antioquia; study sites included Clínica Reina Sofía - Sanitas and Hospital San Ignacio (Bogotá), Clínica Universitaria Bolivariana, Hospital Universitario San Vicente Fundación and Hospital General de Medellín, (Medellín), E.S.E. Clínica de Maternidad Rafael Calvo (Cartagena). Prior to enrollment, all participants gave informed consent and all procedures were followed in alignment with the study protocol and institutional guidelines.

Detailed methods have been published previously. [13] In summary, study participants were enrolled sequentially by trained research coordinators. Cases included women with preeclampsia (PE) with severe features and controls included women with healthy pregnancies, or pregnant women with chronic hypertension (CHTN), gestational hypertension (GHTN), or PE without severe features. The 2013 American College of Obstetricians and Gynecologists' criteria for hypertension in pregnancy were used for diagnosis. [1] We enrolled control participants in a two-to-one ratio with cases. The overall study targeted enrollment of 100 cases of PE with severe features, with 50 cases having a gestational age $<$ 34 weeks and 50 cases $\geq$ 34 weeks. Then, controls were matched to the gestational age category of the case ($<$34 or $\geq$ 34 weeks).

Study participants were enrolled from a variety of settings, including labor and delivery units, outpatient clinics, antepartum units, triage, and emergency wards. As soon as blood pressure, laboratory values and symptoms were evaluated, clinical diagnoses were confirmed within 24 h of enrollment. We used a normal reference range for standard blood tests for all study sites: aspartate transaminase, AST (15–46 U/L); creatinine (0.5–1.1 mg/dl); lactate dehydrogenase, LDH (125–243 U/L); and platelet count (150–450,000/μl). We had multiple exclusion criteria: gestational age $<$ 24 weeks, uncertain dates, multifetal gestation ($\geq$2), major chromosomal abnormality, fetal demise at entry, pre-existing 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. All data were recorded through standardized data collection forms and entered into a centralized electronic database.

The present study was a secondary analysis of the COPA I study. Our primary hypothesis was that C5b-9 concentrations were increased in women with preeclampsia and end-organ injury, compared to those without end-organ injury. Since C5b-9 concentrations in blood and urine were not normally distributed, we stratified C5b-9 data into quartiles (Q1-Q4) based on C5b-9 concentrations in our study population from lowest to highest. To assess study participants for end-organ injury, we utilized available laboratory measures, including AST, serum creatinine, LDH, and platelet count. We defined end-organ injury by AST $\geq$ 70 U/L, creatinine $\geq$ 1.0 mg/dl, LDH $\geq$ 500 U/L, and platelet count $<$ 150,000/μl. These laboratory measures differ slightly from severe features of preeclampsia as defined by ACOG but are consistent with the thresholds put forth by the International Society for the Study of Hypertension in Pregnancy (ISSHP). [16] While there is no consistent threshold for LDH as a marker of end-organ injury, we utilized a threshold value $\geq$ 500 U/L, which was 2-times the upper limit of normal.

Participants with severe blood pressure alone or severe symptoms (e.g., headache, right upper quadrant pain) were not considered to have end-organ injury unless laboratory values were also abnormal. Healthy participants without hypertension were excluded because they did not have routine assessment of AST, creatinine, and LDH. Secondary outcomes included a composite of adverse maternal outcomes (eclampsia, placental abruption, acute kidney injury, hepatic dysfunction, or pulmonary edema) and adverse neonatal outcomes (preterm birth, 5-minute Apgar $<$ 7, neonatal intensive care unit admission, or respiratory distress syndrome).

Descriptive statistics were used for baseline characteristics of COPA study participants, and data distribution was tested for normality using tests of skewness and kurtosis. To assess differences between groups we used χ^2 test for dichotomous data, *t* test or analysis of variance (ANOVA) for normal continuous data, and non-parametric equality of medians test or Spearman's correlation coefficient for non-normal continuous data. We performed all analyses with Stata software (StataCorp, College Station, TX), and used an alpha level of 0.05 to determine statistical significance.

3. Results

There were 352 participants enrolled in the COPA study. Of these, 298 had a hypertensive disorder of pregnancy (CHTN, GHTN, and PE with or without severe features) and were included in our final analysis. Baseline participant characteristics are shown in Table 1. Participants were stratified by hypertensive disorder and enrolled in blocks at each study site to achieve similar distribution of gestational age; therefore, gestational age was similar between groups. Groups varied based on other measured baseline characteristics (Table 1). Laboratory evidence of end-organ injury was present in 18% of all study participants and 37% of those with PE with severe features.

Among study participants, plasma C5b-9 levels were stratified into quartiles (Q1-Q4) from lowest to highest C5b-9 concentration [plasma Q1 ($\leq$1443 ng/ml), Q2 (1444–2558 ng/ml), Q3 (2559–4074 ng/ml), and Q4 ($\geq$4075 ng/ml)]. The distribution of participants by plasma C5b-9 quartile (Q1-Q4) did not vary by hypertensive disorder of pregnancy

Table 1
Baseline characteristics of COPA study population.

Characteristic	Chronic Hypertension (n = 50)	Gestational Hypertension (n = 87)	Preeclampsia without Severe Features (n = 57)	Preeclampsia with Severe Features (n = 104)	P*
Gestational age (wk)	34.3 ± 4.2	35.5 ± 4.2	35.4 ± 3.7	33.2 ± 4.2	N/A†
Age (y)	29.4 ± 6.8	26.5 ± 6.1	25.9 ± 6.8	25.7 ± 6.5	<0.001
BMI (kg/m^2)	28.1 ± 5.5	25.4 ± 4.6	25.7 ± 5.0	24.7 ± 4.3	<0.001
Systolic BP (mmHg)	139 ± 12	142 ± 11	141 ± 11	150 ± 16	<0.001
Diastolic BP (mmHg)	85.4 ± 12	89.0 ± 7.7	88.0 ± 9.4	95.8 ± 13	<0.001
Urine protein/ creatinine (mg/mg)	0.12 (0.09–0.14)	0.12 (0.09–0.16)	0.37 (0.16–0.76)	0.91 (0.33–3.7)	<0.001
Nulliparous	26/50 (52.0)	57/83 (68.7)	46/57 (80.7)	65/103 (63.1)	0.03
African descent	5/49 (10.2)	19/83 (22.9)	9/56 (16.1)	19/103 (18.5)	0.02

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

Table 2
Plasma C5b-9 quartile among study participants, stratified by hypertensive disorder.

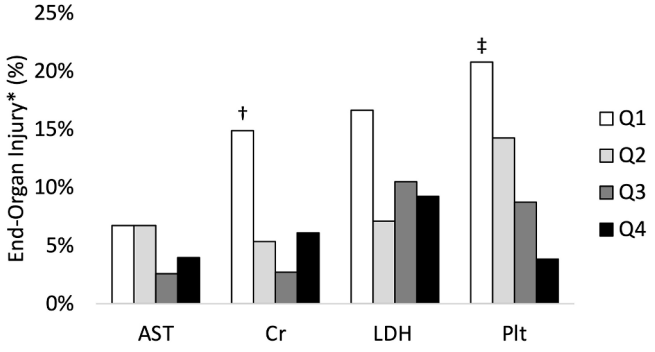
Hypertensive Disorder	Quartile 1 Plasma C5b-9 ≤1443 ng/ml (N = 60)	Quartile 2 Plasma C5b-9 1444–2558 ng/ml (N = 72)	Quartile 3 Plasma C5b-9 2559–4074 ng/ml (N = 83)	Quartile 4 Plasma C5b-9 ≥ 4075 ng/ml (N = 83)	P
CHTN	8 (13)	15 (21)	15 (18)	12 (14)	0.91
GHTN	15 (25)	21(29)	26 (31)	25 (30)	
PE	15 (25)	11 (15)	14 (17)	17 (21)	
PE with SF	22 (37)	25 (35)	28 (34)	29 (35)	

CHTN, chronic hypertension; GHTN, gestational hypertension; PE, preeclampsia; PE with SF, preeclampsia with severe features.
Data are n (%) with P-value by χ^2 test.

(Table 2). Participants with PE with severe features were not more likely to have plasma C5b-9 in the upper quartile (Q4) compared to those with CHTN, GHTN or PE without severe features (27.9% vs. 27.8% P = 0.99). However, in the subgroup of participants with end-organ injury, plasma C5b-9 concentrations were more often in the lowest quartile (Q1). Specifically, compared to participants with plasma C5b-9 levels in Q2-Q4, those with low plasma C5b-9 levels in Q1 were more likely to have low platelet count < 150,000/μl (20.8% vs. 8.4%, P = 0.01) and elevated serum creatinine ≥ 1.0 mg/dl (14.9% vs. 4.5%, P = 0.009), Fig. 1.

Urine C5b-9 levels were also stratified into quartiles (Q1-Q4) for analysis [urine Q1 (<0.7 ng/ml), Q2 (0.70–2.3 ng/ml), Q3 (2.4–8.5 ng/ml), Q4 (>8.5 ng/ml)]. In contrast to the findings in plasma, the distribution of urine C5b-9 concentrations (Q1-Q4) varied significantly by hypertensive disorder (P < 0.001) (Table 3). Compared to those with CHTN, GHTN or PE without severe features, participants with PE with severe features were more likely to have urinary C5b-9 levels in the upper quartile (Q4), (51.9% vs. 11.3%, P < 0.001). Moreover, compared to those with urine C5b-9 levels in Q1-Q3, participants with urinary C5b-9 levels in the upper quartile (Q4) were more likely to have end-organ injury, including low platelet count < 150,000/μl (19.7% vs. 7.4%, P = 0.003) and elevated serum creatinine ≥ 1.0 mg/dl (12.3% vs. 4.4%, P = 0.025), Fig. 2. There was a non-significant increase in LDH ≥ 500 U/L (16.4% vs. 7.7%, P = 0.05).

Consistent with findings above, the composite rate of adverse maternal or neonatal outcomes was highest in those with low plasma C5b-9 levels [adverse maternal outcomes: 18% (plasma C5b-9, Q1) vs.



AST, aspartate transaminase; Cr, creatinine; LDH, lactate dehydrogenase; Plt, platelet count; Q1-4, Plasma C5b-9 Quartile 1-4

* End-organ injury defined by AST ≥70 U/L, Cr ≥1.0 mg/dl, LDH ≥500 U/L, Plt <150,000/μl

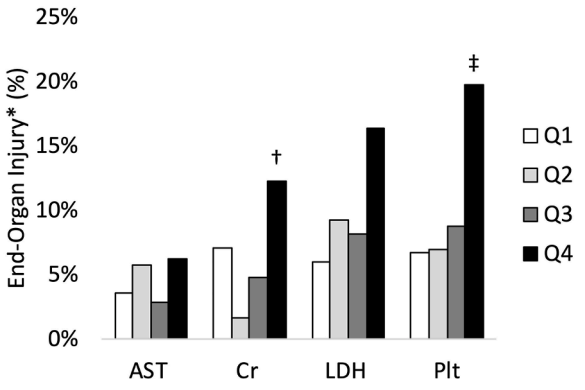
† P=0.009, Q1 vs. Q2-4
‡ P=0.01, Q1 vs. Q2-4

Fig. 1. Plasma C5b-9 Quartiles and End-Organ Injury in Women with Hypertensive Disorders of Pregnancy.

Table 3
Urine C5b-9 quartile among study participants, stratified by hypertensive disorder.

Hypertensive Disorder	Quartile 1 Urine C5b-9 <0.7 ng/ml (N = 72)	Quartile 2 Urine a C5b-9 0.7–2.3 ng/ml (N = 76)	Quartile 3 Urine a C5b-9 2.4–8.5 ng/ml (N = 74)	Quartile 4 Urine C5b-9 > 8.5 ng/ml (N = 76)	P
CHTN	18 (25)	14 (18)	15 (20)	3 (4)	<0.001
GHTN	28 (39)	28 (37)	26 (35)	5 (7)	
PE	14 (19)	14 (18)	15 (20)	14 (18)	
PE with SF	12 (17)	20 (27)	18 (25)	54 (71)	

CHTN, chronic hypertension; GHTN, gestational hypertension; PE, preeclampsia; PE with SF, preeclampsia with severe features.
Data are n (%) with P-value by χ^2 test.
Urine C5b-9 levels were also stratified into quartiles (Q1-Q4) for analysis [urine Q1 (<0.7 ng/ml), Q2 (0.70–2.3 ng/ml), Q3 (2.4–8.5 ng/ml), Q4 (>8.5 ng/ml)]. In contrast to the findings in plasma, the distribution of urine C5b-9 concentrations (Q1.



AST, aspartate transaminase; Cr, creatinine; LDH, lactate dehydrogenase; Plt, platelet count; Q1-4, Urine C5b-9 Quartile 1-4

* End-organ injury defined by AST ≥70 U/L, Cr ≥1.0 mg/dl, LDH ≥500 U/L, Plt <150,000/μl

† P=0.025, Q4 vs. Q1-3
‡ P=0.003, Q4 vs. Q1-3

Fig. 2. Urine C5b-9 Quartiles and End-Organ Injury in Women with Hypertensive Disorders of Pregnancy.

9% (plasma C5b-9, Q3-4), P = 0.05; adverse neonatal outcomes: 55% (plasma C5b-9, Q1) vs. 39% (plasma C5b-9, Q3-4), P = 0.03] or high urinary C5b-9 levels [adverse maternal outcomes: 18% (urine C5b-9, Q4) vs. 8% (urine C5b-9, Q1-2), P = 0.04; adverse neonatal outcomes: 57% (urine C5b-9, Q4) vs. 42% (urine C5b-9, Q1-2), P = 0.04]. Finally, we compared participants with a high-risk C5b-9 profile [low plasma C5b-9 (Q1) and high urine C5b-9 (Q4)], to those with a low-risk C5b-9 profile [plasma C5b-9 (Q2-4) and urine C5b-9 (Q1-3)]. Those with a high-risk profile were significantly more likely to have PE with severe features (70.0% vs. 23.2%, P < 0.001) and end-organ injury (35.0% vs. 14.7%, P = 0.02), Table 4. The high-risk C5b-9 profile was most strongly associated with an increased likelihood of low platelet count < 150,000/μl (19.7% vs. 7.4%, P = 0.003) and elevated serum creatinine ≥ 1.0 mg/dl (12.3% vs. 4.4%, P = 0.025). There was a non-significant increase in LDH ≥ 500 U/L (23.1% vs. 7.6%, P = 0.056).

4. Discussion

In this study, we found that end-organ injury was more common in participants with a hypertensive disorder of pregnancy and either low plasma C5b-9 or high urine C5b-9 concentrations. Those with plasma C5b-9 concentrations in the lowest quartile, or urine C5b-9 concentrations in the highest quartile, were significantly more likely to have low platelet count < 150,000/μl, elevated serum creatinine ≥ 1.0 mg/dl, or other adverse maternal and neonatal outcomes. Moreover, when we categorized participants into low- and high-risk C5b-9 profiles, we found that a high-risk profile conferred an even higher risk for preeclampsia with severe features and end-organ injury. These findings provide further evidence of the role of excessive complement activation in the pathophysiology of preeclampsia and aligns with prior literature demonstrating the role of the terminal complement complex C5b-9 in end-organ injury. [13,17–19].

Table 4
Low-risk and high-risk C5b-9 profiles in study participants and presence of preeclampsia with severe features or end-organ injury.

Diagnosis or laboratory feature	Low-Risk C5b-9 Profile* (N = 177)	High-Risk C5b-9 Profile† (N = 20)	P-value
Preeclampsia with severe features	41 (23)	14 (70)	<0.001
End-organ injury (≥1 of below)	26 (15)	7 (35)	0.02
AST ≥ 70 U/L	8 (4.5)	3 (15)	0.11
Creatinine ≥ 1.0 mg/dL	5 (2.8)	4 (20)	0.003
LDH ≥ 500 U/L	14 (7.9)	5 (25)	0.056
Platelet count < 150,000/μL	11 (6.2)	6 (30)	<0.001

Data are percentages with P-value by χ^2 test. AST, aspartate transaminase; Cr, creatinine.
† High-risk C5b-9 profile: Plasma C5b-9 quartile 1 + Urine C5b-9 quartile 4.
* Low-risk C5b-9 profile: Plasma C5b-9 quartile 2–4 + Urine C5b-9 quartile 1–3.

In a prior study by our group, we demonstrated that soluble C5b-9 are broadly increased in women with preeclampsia with severe features. [13] While all pregnant women with a hypertensive disorder were found to have increased plasma C5b-9 concentrations, those with severe preeclampsia had specific elevations in urine C5b-9 concentrations. In the current study, we identified a subset of hypertensive participants with end-organ injury and adverse outcomes who had paradoxically low plasma C5b-9 levels in conjunction with high urinary C5b-9 levels. This pattern was not present in participants with preeclampsia that did not have end-organ injury, such as those with severe blood pressure or severe symptoms alone in the absence of laboratory abnormalities. While we cannot directly conclude the cause of these findings, it is possible that depletion of complement proteins in the blood combined with excessive urinary loss of complement reflect complement-mediated end-organ injury in preeclampsia.

Diagnostic criteria for preeclampsia severity are variable throughout the world, and do not always correlate well with adverse maternal and perinatal outcomes. [20–22] There may be subtypes of disease that can be identified with specific biomarkers. There is increased recognition for the role of complement dysregulation in preeclampsia, [18] and soluble C5b-9 levels may help to identify a subgroup of patients with complement-mediated disease. Our study provides some evidence on the association of plasma and urinary levels of C5b-9 and end-organ injury, which opens the possibility of considering C5b-9 as a serological marker of complement-mediated end-organ injury in preeclampsia. Studies with greater statistical power and adequate epidemiological design are required to establish the performance of C5b-9 as a serological marker, and establish its role in the clinical context, evaluating levels and risk profiles with maternal and perinatal outcomes of patients with preeclampsia.

The association between complement activation and end-organ injury has been well described in other hematologic disorders, including atypical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria. In these disorders, eculizumab, a monoclonal antibody which blocks C5b and prevents formation of C5b-9, has been effective in mitigating hemolysis, thrombocytopenia and kidney injury. [14–15] Although only tested in individual patients thus far, eculizumab has been proposed for use in treating patients with preeclampsia, as this disorder is complicated by similar severe features. [23–25] Our study provides evidence that there may be a subset of patients with preeclampsia that have increased complement activation in association with end-organ injury, most notably hemolysis, thrombocytopenia and kidney injury. Such patients might stand to benefit from eculizumab in the setting of a clinical trial.

A major strength of this study included its multicenter design with prospective enrollment. We used automated ELISA assays at one central site, ensuring analyses were conducted consistently between centers. Additionally, we were able to stratify participants by hypertensive disorder of pregnancy and disease severity (CHTN, GHTN, PE, PE-SF), enabling us to evaluate differences in complement activation for each of these unique diagnoses. However, due to the observational study design we are unable to determine if there is a direct causal relationship between complement activation and preeclampsia. It remains unclear if C5b-9 levels in blood and urine are altered prior to development of end-organ injury in preeclampsia. We also had relatively limited power for sub-group analysis. For example, we had few women that met traditional ACOG criteria for end-organ injury (i.e., creatinine > 1.1 mg/dl, platelet count < 100,000/μl). However, when we analyzed the

distribution of laboratory values in our study population, we found that the end-organ injury levels were in agreement with the International Society for the Study of Hypertension in Pregnancy (ISSHP) 2018 criteria for renal injury and hematological dysfunction (creatinine $\geq$ 1.0 mg/dl, platelet count $<$ 150,000/µl). [16] It is unclear if our findings can be applied broadly to women with end-organ injury by traditional ACOG or ISSHP criteria. Despite this, we detected significant differences among women with preeclampsia with severe features, suggesting disease severity leads to differing levels of complement activation.

In conclusion, we found that among women with hypertensive disorders of pregnancy, low plasma and high urine concentrations of C5b-9 are associated with preeclampsia with severe features and end-organ injury. This study provides further evidence that the complement system may be involved in the pathophysiology of preeclampsia, and the terminal complement pathway may be a potential target for development of diagnostic or therapeutic innovations.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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