Central to innate immunity, complement activation is heightened in pregnancy, in part, to facilitate normal clearance of fetoplacental material, including apoptotic blebs, circulating fetal DNA, and immune complexes. Under normal pregnancy conditions, the fetus is protected from maternal immune responses through an array of mechanisms, including trophoblast expression of complement regulatory proteins that inhibit complement at different steps of the activation cascade. However, in women who develop preeclampsia, early placental aberrations affect blood flow and oxygenation to the placenta, thereby predisposing to necrotic shedding of syncitiotrophoblast. The burden of fetoplacental debris becomes exaggerated in severe preeclampsia, propagating a systemic inflammatory response and placing strain on both classical and alternative complement signaling pathways (Figure 1) as early as the first trimester. In plasma, upstream complement activation fragments Bb and C3a are associated with future risk of preeclampsia, whereas excess generation of downstream factors C5a and C5b-9 is associated with severe clinical disease.

The kidney is at particular risk of complement-mediated injury. For example, in response to renal ischemia, C3a and C5a induce inflammatory responses that contribute to renal tissue destruction, and C5b-9 mediates direct renal tubular injury. Because kidney injury is fundamental to the diagnosis and pathophysiology of preeclampsia and because both activated complement proteins and complement regulatory proteins have been found in the urine of patients with renal disease, we hypothesized that complement markers in urine, rather than plasma, would better reflect the degree of complement dysregulation in preeclampsia. To investigate this, we measured urine and plasma levels of C3a, C5a, and C5b-9 in subjects with severe preeclampsia compared with controls.

**Methods**

We enrolled 25 cases with severe preeclampsia, 25 controls with chronic hypertension, and 25 healthy controls without hypertension from a cohort of women receiving care at Brigham and Women’s Hospital from March 2012 to March 2013. The approval of institutional review board was obtained through the Partners Human Research Committee, and subjects gave informed consent. All procedures followed were in accordance with institutional guidelines. Pregnancies with multiple gestation or major fetal anomalies were excluded. Cases with preeclampsia were recruited from the labor and delivery ward or antepartum units and defined as severe if ≥1 of the following criteria were present: (1) persistent blood pressure ≥160 mmHg systolic or ≥110 mmHg diastolic, (2) proteinuria ≥5 g in a 24-hour urine specimen
Blood pressure measurements were performed by licensed nurses or medical assistants, using automated digital manometers with the subject in sitting position and the arm at the level of the right atrium. Small, medium, or large blood pressure cuffs were used depending on body mass index (BMI). Control subjects had blood pressure measurements on the day of enrollment and additional measurements at subsequent antenatal visits to confirm proper group assignment. Eight subjects with chronic hypertension and 1 healthy control who developed preeclampsia after enrollment were excluded, being replaced by newly selected matched controls. Subjects with preeclampsia had severe preeclampsia by parity (nulliparous or multiparous) and gestational age (±2 weeks). To minimize selection bias, every eligible case that was severe by blood pressure criteria had ≥160/110 mm Hg systolic or diastolic pressure and clinical laboratory parameters (eg, serum uric acid and creatinine) determined with the Parameter Creatinine Assay Kit (R&D Systems, Minneapolis, MN). Additional clinical variables (eg, BMI and blood pressure) and clinical laboratory parameters (eg, serum uric acid and 24-hour urine protein) were abstracted from medical records. The syndrome of hemolysis, elevated liver enzymes, and low platelets was diagnosed by established criteria.

Results

The baseline characteristics of the 3 study groups are provided in Table 1. Healthy nonhypertensive controls and hypertensive controls were matched to cases with severe preeclampsia by parity and gestational age at enrollment. Subjects with
chronic hypertension or preeclampsia had higher BMI, subjects with chronic hypertension were older, and race was not significantly different between groups. Cases with severe preeclampsia were most commonly characterized by severe range blood pressures (72%), fetal growth restriction (56%), and transaminitis (40%); 5 cases (20%) had hemolysis, elevated liver enzymes and low platelets syndrome superimposed on severe preeclampsia and 15 (60%) required delivery before 34 weeks of gestation. The peak systolic/diastolic blood pressure (mean±SD) among cases on the day of enrollment was 176±20.7/104±11.0 mm Hg, with 24-hour urine protein (median [range]) measuring 1020 (376–7329) mg.

Table 1. Characteristics of Study Subjects by Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy Controls (n=25)</th>
<th>Chronic Hypertension (n=25)</th>
<th>Severe Preeclampsia (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at enrollment,* wk</td>
<td>30.9±4.8</td>
<td>31.3±4.7</td>
<td>32.3±4.2</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>39.5±1.2</td>
<td>38.2±1.6</td>
<td>32.7±4.0†</td>
</tr>
<tr>
<td>Peak SBP at enrollment, mm Hg</td>
<td>111±9.5</td>
<td>133±12.1‡</td>
<td>176±20.7†</td>
</tr>
<tr>
<td>Peak DBP at enrollment, mm Hg</td>
<td>67.0±7.9</td>
<td>82.3±10.6‡</td>
<td>104±11.0†</td>
</tr>
<tr>
<td>Urine total protein/creatinine,§ mg/mg</td>
<td>0.64 (0–1.3)</td>
<td>0.64 (0.3–1.2)</td>
<td>1.9 (1.0–4.0)</td>
</tr>
<tr>
<td>Nulliparous,* %</td>
<td>68.0</td>
<td>68.0</td>
<td>68.0</td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>29.0±5.5</td>
<td>33.6±5.9¶</td>
<td>30.6±5.2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.6±4.1</td>
<td>34.4±9.2#</td>
<td>31.8±6.0**</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>44.0</td>
<td>52.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Black</td>
<td>24.0</td>
<td>32.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>28.0</td>
<td>16.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Other</td>
<td>4.0</td>
<td>0.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Data are mean±SD; comparisons between groups assessed by paired t tests and differences are nonsignificant (P>0.05) unless otherwise specified. CHTN indicates chronic hypertension.

Table 2. Plasma C3a, C5a, and C5b-9 in Cases and Controls

<table>
<thead>
<tr>
<th>Study Measure</th>
<th>Healthy Controls (n=25)</th>
<th>Chronic Hypertension (n=25)</th>
<th>Severe Preeclampsia (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma C3a</td>
<td>3219±548</td>
<td>3038±656</td>
<td>3373±916</td>
</tr>
<tr>
<td>Plasma C5a</td>
<td>31.6±13.5</td>
<td>51.8±9.9*</td>
<td>40.8±15.5†</td>
</tr>
<tr>
<td>Plasma C5b-9</td>
<td>348±172</td>
<td>485±233‡</td>
<td>444±171‡</td>
</tr>
</tbody>
</table>

Data are mean±SD (ng/mL); comparisons between groups assessed by paired t tests and differences are nonsignificant (P>0.05), unless otherwise specified. CHTN indicates chronic hypertension.

*P<0.0001, CHTN vs healthy controls; †P=0.01, CHTN vs preeclampsia.
‡P=0.04, preeclampsia vs healthy controls.
¶P<0.0001, preeclampsia vs healthy controls; P<0.0001, CHTN vs healthy controls.

Urinary levels of complement activation are displayed in Figure 2. Urinary C3a levels were detectable in nearly all subjects but were significantly increased in severe preeclampsia group versus both control groups (P<0.01). These findings were unchanged after adjustment for U-Cr (median urine C3a/U-Cr [interquartile range], preeclampsia: 0.59 [0.17–2.5] ng/mg or chronic hypertension: 0.46 [0.27–0.81] ng/mg versus healthy controls: 0.11 [0–0.38] ng/mg; P<0.01). Urine C5a levels correlated with total urine protein (r=0.43; P=0.0001) and peak systolic blood pressure (r=0.36; P=0.002) but not BMI.

Urinary C5a was detectable in 92% of subjects with severe preeclampsia or chronic hypertension compared with only 52% of healthy controls (P<0.001). C5a levels were not significantly different between preeclampsia and chronic hypertension groups but were greater in both these groups compared with healthy controls (Figure 2). These findings were unchanged after adjustment for U-Cr (median urine C3a/U-Cr [interquartile range], preeclampsia: 0.59 [0.17–2.5] ng/mg or chronic hypertension: 0.46 [0.27–0.81] ng/mg versus healthy controls: 0.11 [0–0.38] ng/mg; P<0.01). Urine C5a levels correlated with total urine protein (r=0.43; P=0.0001) and peak systolic blood pressure (r=0.36; P=0.002) but not BMI.

Urinary C5b-9 was detectable in 96% of subjects with severe preeclampsia compared with only 12% of controls with chronic hypertension and 8% of healthy controls (P<0.0001). Furthermore, urinary C5b-9 levels were markedly elevated in severe preeclampsia group compared with both control groups.
In severe preeclampsia, heightened activation of downstream complement protein C5 leads to excess generation of C5a and C5b-9. C5a propagates a potent proinflammatory response, whereas C5b-9 incorporates into cell membranes, including villous trophoblast, and contributes to platelet activation, procoagulant effects, and lytic cell death. In addition, C5a stimulates monocytes to release soluble fms-like tyrosine kinase 1, which sequesters vascular endothelial growth factor and PlGF, contributing to hypertension and glomerular endotheliosis.

Our results introduce the novel finding that complement activation products C3a, C5a, and C5b-9 are excreted in urine in association with severe preeclampsia. Although urinary excretion of C3a, C5a, and C5b-9 was exaggerated in cases with severe preeclampsia compared with healthy controls, excretion of C5b-9 distinguished most clearly between severe preeclampsia and chronic hypertension. As a biomarker of disease, urinary C5b-9 was superior to plasma C5b-9, which could not distinguish between cases and hypertensive controls, supporting our hypothesis that complement markers in urine, rather than plasma, better reflect complement dysregulation.

Considering that plasma levels of C5a and C5b-9 were increased in subjects with preeclampsia, it is possible that urinary excretion of C5a and C5b-9 occurred because normal plasma clearance mechanisms were overwhelmed. However, we found that urinary complement levels (C3a, C5a, or C5b-9) did not correlate with plasma complement levels among women with severe preeclampsia, arguing against simple renal clearance of circulating complement proteins. Although urinary excretion of complement proteins correlates with blood pressure and proteinuria, among cases with preeclampsia, urinary complement markers correlated more strongly with each other than with markers of renal impairment. We hypothesize that exaggerated and coordinated urinary excretion of C3a, C5a, and C5b-9 in severe preeclampsia occurred because of complement-mediated inflammation and injury in the kidney, possibly at the level of the proximal tubule as seen in renal ischemia reperfusion injury.

Although urinary levels of C3a, C5a, and C5b-9 were all increased in cases with severe preeclampsia, downstream markers of C5 activation, particularly C5b-9, were more exaggerated in disease. Similarly, plasma levels of C5a and C5b-9, but not C3a, were increased in cases with severe preeclampsia and controls with chronic hypertension. Increased plasma C5 activation in controls with chronic hypertension may reflect heightened endothelial dysfunction or systemic inflammation and is consistent with the general finding that chronic hypertension in pregnancy predisposes to severe preeclampsia.

Taken together, our data suggest that complement dysregulation in ongoing disease occurs primarily at the level of C5. Animal models also support a dominant role for C5, relative to C3, in both complement-mediated kidney injury and adverse pregnancy outcomes. Levels of C5a and C5b-9 may rise out of proportion to C3a because of direct C5 activation (ie, enzymatic cleavage) by extrinsic serine proteases, bypassing proximal complement pathways. These extrinsic activators of C5 may be derived from leukocytes or the coagulation cascade (eg, thrombin). Alternatively, complement gene mutations (eg, CD46 gene mutations) may predispose to increased production of C5 convertases that generate C5a and C5b.

Presumably, C5a and C5b-9 should be generated in similar amounts from C5 activation. However, C5a has high affinity for its receptors and is rapidly cleared from circulation.
Furthermore, distinct C5a receptors in the kidney may be expressed in the proximal tubule or thick ascending limb of Henle loop (C5aR) or the distal convoluting tubule (C5L2),{40–42} which may limit the usefulness of urinary C5a as a biomarker of disease activity. C5b-9 seems to be a more reliable urinary marker for various renal diseases.{19,20} C5b-9 may be formed at the glomerular membrane and shed into the urine,{43} or complement proteins may pass through the glomerular membrane into the tubular lumen to stimulate complement activation and formation of C5b-9 in the proximal tubule.{44} Furthermore, our data also show that increased levels of urinary C5b-9, but not C3a or C5a, correlate well (ie, inversely) with urinary PIGF, a validated marker of the altered angiogenic state in preeclampsia.{45}

Our findings support the concept that severe preeclampsia is propagated by excess complement activation and eventual complement dysregulation, particularly at the level of C5. We propose that during gestation increasing amounts of aponectric fetoplacental debris{4,9,10} secondary to early placental aberrations, underlying comorbid conditions (eg, chronic hypertension), or genetic factors may trigger excess complement activation, which propagates systemic inflammation,{13,14,46,47}angiogenic imbalance,{15}endothelial dysfunction,{16,17}coagulation activation,{26,28,29,37}and oxidative stress,{20,51}with eventual kidney injury{17,18,33,34} and hypertension.{33,34} We suspect that complement-mediated inflammation and injury in the kidney are particularly detrimental in severe preeclampsia as suggested by marked urinary excretion of C5b-9 during active disease. Although heightened C5 activation occurred in pregnant grvidas with chronic hypertension, urinary excretion of C5b-9 was specific to preeclampsia. Our findings support urinary C5b-9 as a promising biomarker for severe preeclampsia, which may distinguish pathologic complement dysregulation from simply heightened complement activation. Furthermore, our finding that complement dysregulation is common in severe preeclampsia suggests that complement inhibition may be a viable treatment option.

**Perspectives**

Our conclusions are limited by sample size and heterogeneity of disease in severe preeclampsia. At this point, neither can we apply our findings to mild preeclampsia or atypical disease nor may we generalize them to grvidas with multiple gestation or baseline renal disease. We also cannot comment on the ability of urinary complement markers to predict disease onset. However, our findings support the hypothesis that active disease in severe preeclampsia is commonly associated with complement dysregulation, which is particularly manifest through exaggerated urinary excretion of C5b-9. In an ongoing pregnancy, if the nidus for complement activation is fetoplacental debris, the downward spiral of complement dysregulation will only abate with delivery and removal of the placenta. Delivery is a safe and preferred option at term or near-term, but delivery of an extremely premature neonate in the setting of early-onset severe preeclampsia is associated with high neonatal morbidity and mortality. Considering our data, we think that there is a compelling argument to attempt C5 blockade, in the setting of a clinical trial, as a putative treatment for early-onset severe preeclampsia. This rationale is supported by our recent report in which Eculizumab, a C5 inhibitor, was used to prolong pregnancy in the setting of severe preeclampsia/hemolysis, elevated liver enzymes, and low platelets syndrome.{52} Treatment with complement blockade offers the intriguing potential to stem ongoing disease in preeclampsia while safely prolonging pregnancy for both the mother and the child.

**Sources of Funding**

This study was supported by Eleanor and Miles Shore 50th Anniversary Fellowship Program for Scholars in Medicine, Harvard Medical School (Dr Feinberg, salary support).

**Disclosures**

None.

**References**


Novelty and Significance

What Is New?
- In association with severe preeclampsia, complement proteins are excreted in the urine.
- Urinary excretion of C5b-9 in pregnancy differentiates cases with severe preeclampsia from healthy controls and controls with chronic hypertension.

What Is Relevant?
- Complement dysregulation may propagate systemic inflammation, kidney injury, and hypertension in severe preeclampsia.
- Complement blockade, at the level of C5, is an intriguing therapeutic target for severe preeclampsia.

Summary
Severe preeclampsia is commonly associated with urinary excretion of downstream complement proteins. In particular, exaggerated urinary excretion of C5b-9 in pregnancy seems to distinguish cases with severe preeclampsia from healthy controls and controls with chronic hypertension. C5-mediated inflammation and injury in the kidney may be a central feature of disease, suggesting a therapeutic rationale for complement blockade.
Urinary Excretion of C5b-9 in Severe Preeclampsia: Tipping the Balance of Complement Activation in Pregnancy
Richard M. Burwick, Raina N. Fichorova, Hassan Y. Dawood, Hidemi S. Yamamoto and Bruce B. Feinberg

Hypertension. 2013;62:1040-1045; originally published online September 23, 2013; doi: 10.1161/HYPERTENSIONAHA.113.01420
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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