Complement activation and regulation in preeclampsia and hemolysis, elevated liver enzymes, and low platelet count syndrome

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The Complement System

The complement system, which comprised more than 30 soluble and membrane-bound complement proteins, is critical to human health owing to its central role in host defense and innate immunity.\(^1,2\) Complement proteins provide immediate defense against foreign pathogens, such as viruses and bacteria, through opsonization, inflammation, and cell membrane attack. Complement proteins are activated via enzymatic cleavage through at least 3 distinct pathways as follows: (1) the classical pathway, in which complement is activated when substances such as DNA, apoptotic cells, C-reactive protein, or immune complexes bind to complement component 1q (C1q)\(^3\); (2) the lectin complement pathway, where complement is activated when mannose-binding lectins or ficolins bind carbohydrate patterns on foreign pathogens;\(^4\) or (3) the alternative complement pathway, where complement is continuously activated at a low level to probe foreign, modified self-, and unaltered self-structures (Figure 1).\(^5,6\)

The complement system is critical to human health owing to its central role in host defense and innate immunity. During pregnancy, the complement system must be appropriately regulated to allow for immunologic tolerance to the developing fetus and placenta. Although some degree of complement activation can be seen in normal pregnancy, the fetus seems to be protected in part through the placental expression of complement regulatory proteins, which inhibit complement activation at different steps along the complement activation cascade. In women who develop preeclampsia and hemolysis, elevated liver enzymes, and low platelet count syndrome, there is a shift toward increased complement activation and decreased complement regulation. There is an increase in placental deposition of C5b-9, which is the terminal effector of classical, lectin, and alternative complement pathways. C5b-9 deposition stimulates trophoblasts to secrete soluble fms-like tyrosine kinase-1, which sequesters vascular endothelial growth factor and placental growth factor. Pathogenic mutations or deletions in complement regulatory genes, which predispose to increased complement activation, have been detected in women with preeclampsia and hemolysis, elevated liver enzymes, and low platelet count syndrome. Before the disease, biomarkers of alternative complement pathway activation are increased; during active disease, biomarkers of terminal complement pathway activation are increased. Urinary excretion of C5b-9 is associated with preeclampsia with severe features and distinguishes it from other hypertensive disorders of pregnancy. Taken together, existing data link preeclampsia and hemolysis, elevated liver enzymes, and low platelet count syndrome with increased activation of the terminal complement pathway that, in some cases, may be influenced by genetic alterations in complement regulators. These findings suggest that the inhibition of the terminal complement pathway, possibly through C5 blockade, may be an effective strategy to treat preeclampsia and hemolysis, elevated liver enzymes, and low platelet count syndrome, but this strategy warrants further evaluation in clinical trials.

Key words: alternative pathway, anaphylatoxins, atypical hemolytic uremic syndrome, classical pathway, complement C4, complement C5, complement inactivating agents, complement membrane attack complex, complement system proteins, HELLP syndrome, immune system, placenta, preeclampsia, pregnancy

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C8 (C8), and complement protein C9 (C9) to form the terminal complement complex (C5b-9), also known as the membrane attack complex (MAC) because of its ability to form transmembrane pores and propagate cell lysis. Numerous complement proteins regulate both amplification and degradation of C3b and formation of C5b-9 on the cell surface, thereby limiting harm to self. These complement regulators may be soluble (eg, complement factor H [CFH]−, factor I−, and factor H−related proteins) or membrane bound (eg, cluster of differentiation 55 [CD55], cluster of differentiation 46 [CD46], cluster of differentiation 55 [CD55], and cluster of differentiation 59 [CD59]).

**Complement activation in pregnancy**

During pregnancy, the host complement system must be appropriately regulated to allow for immunologic tolerance to the developing fetus and placenta, which are themselves semiallogenic or, in the setting of donor egg and surrogate pregnancy, fully allogenic. Although some degree of complement activation can be seen in normal pregnancy owing to multiple factors including placental apoptotic debris, cell-free fetal DNA and RNA, and immune complex formation, the fetus seems to be protected in part through the placent al expression of complement regulatory proteins, which inhibit complement activation at different steps along the complement activation cascade (Figure 1).

Various complement activation products have been measured in normal pregnancy to show that systemic complement activation is present. Richani et al showed that C3a and C5a are increased in healthy pregnant women at 20 to 42 weeks’ gestation compared with nonpregnant women (median, C3a, 2365 vs 1340 ng/mL; C5a, 12.4 vs 4.1 ng/mL; both, P<.001). Derzsy et al showed that soluble C5b-9 levels are increased in the plasma of women with normal pregnancy at 36 to 37 weeks’ gestation compared with healthy nonpregnant women (median, C5b-9, 60 vs 33 ng/mL; P<.001). Systemic complement activation in pregnancy is balanced by a concomitant increase in soluble complement regulators, such as CFH, membrane-bound complement regulators, including CD46, CD55, and CD59, which are widely expressed on placent al trophoblast cells from at least 6 weeks’ gestation to term.

**Preeclampsia and Hemolysis, Elevated Liver Enzymes, and Low Platelet Count Syndrome**

Preeclampsia is defined by hypertension and proteinuria that develops in the second half of pregnancy. When there is severe hypertension (≥160/110 mm Hg) or end-organ injury (eg, elevated serum creatinine, pulmonary edema, severe headache), with or without proteinuria, it is termed preeclampsia with severe features. Those with microangiopathic hemolysis, elevated liver enzymes, and low platelet count (HELLP) are diagnosed with HELLP syndrome. Regardless of the terminology, pregnant women with preeclampsia without severe features, preeclampsia with severe features, or HELLP syndrome, all improve soon after the placenta is removed at delivery.

Preeclampsia has long been considered a placentally mediated disease. Early impairments soluble fms-like tyrosine kinase-1 (sFlt-1) in placent al blood flow and oxygenation, such as with poor spiral artery remodeling, may increase placental inflammation and apoptosis over time, with systemic activation of leukocytes and endothelial cells. In women who develop preeclampsia, there is necrotic shedding of placent al trophoblast into the maternal circulation and increased trophoblast release of sFlt-1 an antiangiogenic factor that binds and sequesters placent al growth factor (PIGF) and vascular endothelial growth factor (VEGF). Increased levels of sFlt-1 and reduced levels of PIGF and VEGF are associated with hypertension and proteinuria and the development of preeclampsia.

There is an increasing recognition that complement proteins are also involved in the pathophysiology of preeclampsia and HELLP syndrome, through excess complement activation, decreased complement regulation, or both.

**Placental Complement Deposition**

The terminal effector of the complement pathway is C5b-9, which has both lytic and sublytic inflammatory effects. Therefore, placent al deposition of C5b-9 may contribute to placent al injury in women who develop preeclampsia.

In 2008, Rampersad et al showed that trophoblast deposition of C5b-9 was increased in cell culture under hypoxic conditions and C5b-9 deposition enhanced apoptotic cell death. Moreover, the surface density of C5b-9 staining and colocalization of fibrin-C5b-9 complexes were increased 2-fold in placent al sections from preeclampsic pregnancies compared with those from uncomplicated pregnancies. Subsequent studies by Banadakoppa et al and Collier et al also found increased trophoblast deposition of C5b-9 in placentas from women with preeclampsia and HELLP syndrome compared with placentas from a healthy pregnancy. In the study by Collier et al, the degree of placent al C5b-9 staining was similar in preeclampsia and HELLP syndrome.

Although C5b-9 is the shared terminal effector of all complement pathways, investigators have sought to determine whether placent al C5b-9 deposition is driven by a specific activation pathway. In 2012, Buurma et al showed that placent al trophoblast staining for complement component 4d (C4d) was more often detected in preeclampsic placentas than control placentas (C4d positive, 50% vs 3%; P=.0001). The authors noted that C4d is a component of both the classical and lectin pathways, and therefore, they investigated the presence of...
of C1q and mannose-binding lectin to determine which pathway was responsible. Mannose-binding lectin was never observed, making it unlikely that placental C4d deposits were a result of lectin pathway activation. When C4d was present in a diffuse staining pattern at the syncytiotrophoblast surface, it colocalized with C1q, indicating that C4d deposits were most likely the result of classical pathway activation. Collier et al.44 also found increased C4d deposition in preeclamptic and HELLP placentas vs controls (Figure 2), and like their findings with C5b-9, the level of placental C4d staining was no different between preeclampsia and HELLP syndrome.44

Although placental trophoblast deposition of C5b-9 seems to be increased in preeclamptic placentas, it remains unclear whether this is caused by increased complement activation or impaired regulation. Buurma et al.15 found that placental mRNA expressions of complement regulatory proteins CD55 and CD59 were increased 2-fold and 4-fold, respectively, in the placentas of women who delivered with pre eclampsia compared with the placentas from uncomplicated deliveries. The investigators hypothesized that up-regulation of CD55 and CD59 in the face of increased complement activation suggests the presence of a feedback mechanism to maintain trophoblast integrity.

Complement and Antiangiogenic Factors

Because placental release of antiangiogenic factor sFlt-1 has been consistently implicated in the development of preeclampsia,40,41 there has been interest in the relationship between complement proteins and angiogenic factors. Placental ischemia and hypoxia have been shown to increase the trophoblast release of sFlt-1,46,47 but increasing data suggest that terminal complement proteins C5a and C5b-9 may also be implicated in this process.

In 2006, using antibody independent mouse models of complement dysregulation, Girardi et al.48 showed that complement activation products, particularly C5a, induced the dysregulation of angiogenic factors. Specifically, C5a stimulated monocytes to produce soluble VEGF receptor 1 (VEGFR1) (also known as sFlt-1), which sequesters VEGF and contributed to abnormal placental development, fetal growth restriction, and death. When the effects of C5a were blocked, by inhibiting the interaction of C5a with its receptor complement component 5a receptor (C5aR), the release of sFlt-1 was prevented and fetal death was averted. Langer et al.19 subsequently showed that C5a was capable of polarizing macrophages toward an antiangiogenic phenotype, through increased expression and secretion of soluble VEGFR1

FIGURE 1
Schematic of complement activation and regulation at the placental interface

The complement cascade may be activated (complement activation) through distinct pathways. The classical pathway is activated by substances such as apoptotic cells, DNA, and immune complexes; the lectin pathway is activated by carbohydrate patterns on foreign surfaces; and the alternative pathway is constitutively active through spontaneous hydrolysis. These pathways converge to generate C3 convertases, which cleave C3 to generate activation products C3a (anaphylatoxin) and C3b (opsonin). The accumulation of C3b leads to the generation of C5 convertases, which cleave C5 of the terminal pathway to generate C5a (anaphylatoxin) and C5b, which combines with complement proteins C6 to C9 to form C5b-9 (MAC). C5b-9 may incorporate into cell membranes, pictured above (red cylinder on placental surface) with sublytic or lytic effects. C5b-9 may also be released in an active soluble form (soluble C5b-9), pictured above (red cylinder released from placental surface). The complement cascade may be inhibited by (1) soluble complement regulatory proteins, including CFH and CFI, which function together to inactivate C3b and down-regulate complement activation, and CFHR1, which blocks C5 convertase activity and interferes with membranous C5b-9 deposition, and (2) membrane-bound complement regulatory proteins, which may be expressed on the trophoblast membrane as pictured above (green rectangles on placental surface: CD46, CD55, CD59); CD46 blocks the activation of C3 into C3a and C3b; CD55 inhibits the actions of C3b and reduces C5 activation; and CD59 blocks the actions of C5b-9, the MAC.

C3, complement protein C3; C5, complement protein C5; C6, complement protein C6; C9, complement protein C9; CFB, complement factor B; CFH, complement factor H; CFHR, complement factor H related; CFI, complement factor I; MAC, membrane attack complex.

(sFlt-1). The supernatant from C5a-stimulated human monocytes, enriched with sFlt-1, inhibited the VEGF-induced capillary-like tube formation, and this effect was mitigated by C5aR inhibition. Banadakoppa et al.\textsuperscript{a1} took these experiments a step further by correlating trophoblast release of sFlt-1 with the degree of complement activation. Investigators observed increased trophoblast levels of sFlt-1 with increased complement activation, but as the degree of complement activation increased, cellular levels of sFlt-1 decreased. They hypothesized that complement activation induced secretion of sFlt-1 protein from trophoblast cells. To confirm this, they measured sFlt-1 protein levels in cell culture and they found that levels increased in conjunction with greater complement activation. Moreover, secretion of sFlt-1 correlated most closely with trophoblast release of C5b-9. In 2019, Collier et al.\textsuperscript{44} also showed that placental C5b-9 and sFlt-1 levels were strongly associated (R=0.59, P=0.01) and placental sFlt-1 signaling was spatially correlated in proximity to syncytiotrophoblast membrane C4d and C5b-9 immunofluorescence signal.\textsuperscript{14}

**Complement Gene Mutations**

Knowledge of complement gene mutations has increased significantly over the past decade, in large part owing to a better understanding of atypical hemolytic uremic syndrome (aHUS), which is a complement-mediated disorder. Complement gene mutations are detected in approximately 60% of aHUS cases,\textsuperscript{31,52} including pregnancy-associated cases.\textsuperscript{53–55} Most genetic aHUS cases are heterozygous and are attributed to the genes CFH (25% to 30% of cases), CD46 (8% to 10%), and C3 and complement factor I (CFI) (4% to 8%, respectively).\textsuperscript{56,57} Mutations with a minor allele frequency of 1% to 5% have generally been referred to as uncommon variants, whereas those with a minor allele frequency of <1% are considered rare variants.\textsuperscript{58} Determining whether a complement gene mutation is benign or pathogenic (clinically meaningful) is challenging, and such determinations have relied on functional testing or on detailed statistical analysis of allele frequencies in patient cohorts compared with reference genomic datasets.

Complement gene mutations have also been identified in pregnant women with preeclampsia and HELLP syndrome without apparent aHUS, and these studies are presented in Table 1.\textsuperscript{61–65} The clinical phenotype of aHUS and HELLP syndrome overlap,\textsuperscript{55,66} and the 2 conditions can be difficult to distinguish in the peripartum period, although HELLP syndrome usually resolves after delivery, whereas aHUS typically does not. Nonetheless, the overlapping clinical phenotype of these conditions suggests that there may be shared biologic underpinnings. An underlying complement gene mutation could help to explain why some pregnant women develop severe preeclampsia and HELLP syndrome in the setting of complement activation whereas others do not.

**Complement Mutations in Preeclampsia and HELLP Syndrome**

In 2007, Fang et al.\textsuperscript{57} reported a patient with HELLP syndrome who carried a membrane cofactor protein (MCP) gene variant (A304V) known to be associated with aHUS. The A304V variant was found in the transmembrane domain of MCP, and compared with wild type, the variant allowed more C3b deposition in response to increased antibody challenge. The authors concluded that the A304V variant in MCP had a reduced functional ability to regulate complement activation in situ. Subsequently, numerous investigators have evaluated the MCP gene in preeclampsia and HELLP syndrome. From a total of 264 women with preeclampsia or HELLP syndrome, MCP variants were detected in 8% of cases (21/264) (Table 1).\textsuperscript{61–65} However, the prevalence ranged widely (0%–12%), possibly caused by heterogeneous clinical phenotypes and ethnic variation.
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Case population</th>
<th>N</th>
<th>Complement genes tested</th>
<th>Any variant</th>
<th>C3</th>
<th>CFB</th>
<th>CFH</th>
<th>CFHR1, CFHR3, CFHR5</th>
<th>CFI</th>
<th>MCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fakhouri et al, 2008</td>
<td>HELLP and renal involvement</td>
<td>11</td>
<td>CFH, CFI, MCP</td>
<td>4/11 (36)</td>
<td>N/A</td>
<td>N/A</td>
<td>1/11 (9.1)</td>
<td>N/A</td>
<td>2/11 (18)</td>
<td>1/11 (9.1)</td>
</tr>
<tr>
<td>Salmon et al, 2011</td>
<td>SLE or APL Ab with preeclampsia or HELLP</td>
<td>40</td>
<td>CFH, CFI, MCP</td>
<td>7/40 (18)</td>
<td>N/A</td>
<td>N/A</td>
<td>1/40 (2.5)</td>
<td>N/A</td>
<td>2/40 (5.0)</td>
<td>4/40 (10)</td>
</tr>
<tr>
<td>Salmon et al, 2011</td>
<td>Nonautoimmune severe preeclampsia or HELLP</td>
<td>59</td>
<td>CFH, CFI, MCP</td>
<td>5/59 (8.5)</td>
<td>N/A</td>
<td>N/A</td>
<td>0/59 (0)</td>
<td>N/A</td>
<td>1/59 (1.7)</td>
<td>4/59 (6.8)</td>
</tr>
<tr>
<td>Crovetto et al, 2012</td>
<td>HELLP</td>
<td>33</td>
<td>C3, CFB, CFH, CFI, MCP</td>
<td>2/33 (6.1)</td>
<td>0/33 (0)</td>
<td>0/33 (0)</td>
<td>0/33 (0)</td>
<td>N/A</td>
<td>1/33 (3.0)</td>
<td>1/33 (3.0)</td>
</tr>
<tr>
<td>Lokki et al, 2015</td>
<td>Severe preeclampsia</td>
<td>95</td>
<td>MCP</td>
<td>11/95 (12)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>11/95 (12)</td>
</tr>
<tr>
<td>Vaught et al, 2018</td>
<td>Partial HELLP</td>
<td>14</td>
<td>C3, CFB, CFH, CFHR1, CFHR3, CFHR5, CFI, MCP</td>
<td>3/14 (21)</td>
<td>2/14 (14)</td>
<td>0/14 (0)</td>
<td>0/14 (0)</td>
<td>3/14 (21)</td>
<td>1/14 (7.1)</td>
<td>0/14 (0)</td>
</tr>
<tr>
<td>Vaught et al, 2018</td>
<td>HELLP</td>
<td>11</td>
<td>C3, CFB, CFH, CFHR1, CFHR3, CFHR5, CFI, MCP</td>
<td>5/11 (45)</td>
<td>1/11 (9.1)</td>
<td>0/11 (0)</td>
<td>0/11 (0)</td>
<td>4/11 (36)</td>
<td>0/11 (0)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>Preeclampsia or HELLP</td>
<td>263</td>
<td>C3, CFB, CFH, CFHR1, CFHR3, CFHR5, CFI, MCP</td>
<td>37/263 (14)</td>
<td>3/58 (5.2)</td>
<td>0/58 (0)</td>
<td>2/168 (1.2)</td>
<td>7/25 (28)</td>
<td>7/168 (4.2)</td>
<td>21/263 (8.0)</td>
</tr>
</tbody>
</table>

Data are presented as number/total number (percentage).

APL Ab, antiphospholipid antibody positive; C3, complement protein C3; CFB, complement factor B; CFH, complement factor H; CFHR, complement factor H related; CFI, complement factor I; HELLP, hemolysis, elevated liver enzymes, and low platelet count syndrome; MCP, membrane cofactor protein (CD46); N/A, not assessed; SLE, systemic lupus erythematosus.

*a* Descriptions per investigators; severe preeclampsia and partial HELLP now termed preeclampsia with severe features; *b* Any variant of the ones listed in column 4, complement genes tested.

In a cohort of 40 women with pre eclampsia and autoimmune disease (lupus or antiphospholipid antibodies), Salmon et al found MCP variants in 4 women (10%), 3 of whom had the A304V variant. There were no MCP variants found in 34 matched controls. Investigators then evaluated the A304V MCP variant in women with preeclampsia or HELLP syndrome, but without autoimmune disease, and the variant was found in 6.8% of cases (4/59) compared with 2.1% of matched controls with healthy pregnancy (3/143). However, other studies of MCP (CD46) gene variants in preeclampsia and HELLP syndrome have been less compelling. Lokki et al evaluated the A304V MCP variant in 95 primiparous women with severe preeclampsia in a Finnish cohort. Heterozygosity for the A304V variant was observed in 12% of preeclampsia cases (11/95) compared with 11% of healthy controls (21/188) (odds ratio [OR], 0.94; 95% confidence interval [CI], 0.45–2.0). Notably, 3 other studies, with a combined 55 women with HELLP syndrome, detected MCP variants in only 3.6% of cases (2/55).

Of the studies listed in Table 1, mutations in CFH and CFI were detected in 1.2% (2 of 168) and 4.2% (7/168) of women with preeclampsia or HELLP syndrome, respectively. Both CFH and CFI are soluble complement regulators that function to inactivate C3b and thereby down-regulate complement activation. Pathogenic mutations in CFH and CFI are usually loss-of-function, leading to increased C3b deposition and increased complement activation. For example, Fahouri et al described a patient with HELLP syndrome who had a CFH mutation (p.Arg303Gln) located in the SCR-1/5 domain important for C3b binding and decay-accelerating activity. Another patient with HELLP syndrome had a CFI mutation (p.Arg345Gln, c.1034G>A), with functional analysis demonstrating a marked defect in both C3b and C4b cofactor activities.

In 2018, Vaught et al analyzed an expanded number of complement genes to identify rare variants and deletions in CFH-related genes (CFH-related protein 1 [CFHR1], CFH-related protein 3 [CFHR3], and CFH-related protein 5 [CFHR5]). Homozygous CFHR1-CFHR3 deletion has been reported in approximately 2% of the population and has a known association with aHUS. Complement gene variants or CFHR deletions were detected in 21% of women with partial HELLP (now called preeclampsia with severe features) (3 of 14) and 45% of women with HELLP syndrome (5 of 11). Notably, 5 of these women had homozygous deletions in CFHR genes, including 4 with homozygous CFHR1-CFHR3 deletion (3 cases of preeclampsia with severe features, 1 case of HELLP) and 1 with homozygous CFHR1 deletion (HELLP). CFHR1 blocks C5 convertase activity and interferes with membranous C5b-9 deposition, and therefore, deletion of CFHR1 may result in increased C5b-9 formation.

It should be noted that most complement genetic studies have assessed maternal but not fetal genetic status. The fetus has 50% chance of inheriting a heterozygous maternal variant (discordant maternal-fetal status) but 50% chance of not inheriting it (discordant maternal-fetal status). In addition, the fetus may inherit complement gene mutations from the paternal side. Thus, fetal complement genetic testing is urgently needed in future investigations.

Complement Biomarkers Before the Disease

The presence of complement genetic variants may predispose some women to develop preeclampsia and HELLP syndrome. However, even when there is a known complement gene mutation, penetrance is limited. Thus, it would be helpful to determine whether abnormal complement activation is present before the onset of disease.

In 1989, Haeger et al showed that women who developed preeclampsia had higher plasma C3a levels, but not C5a levels, 1 month before delivery compared with unmatched healthy controls. Nearly 20 years later, Lynch et al evaluated alternative complement pathway fragment Bb in a prospective study of 701 women. Investigators found that women with Bb levels in the upper 90th percentile before 20 weeks’ gestation had 3.3 times greater relative risk of preeclampsia (adjusted OR [aOR], 3.8; 95% CI, 1.6–9; P<.002) after adjustment for confounders. In contrast, soluble C5b-9 levels were not increased in early gestation (10–15 weeks) among women who developed preeclampsia. Taken together, these results show that the activation of the alternative complement pathway, but not the terminal complement pathway, is increased in early pregnancy among women who develop preeclampsia.

In a larger cohort of women (n=1002) with plasma samples collected before 20 weeks’ gestation, Lynch et al showed that C3a levels were higher in women who developed any hypertensive disorder of pregnancy (848 vs 757 ng/L; P<.001), but not preeclampsia specifically. Investigators noted that the development of preeclampsia was most likely in those with underlying obesity (body mass index, >30 kg/m²) and elevated Bb levels (aOR, 10.0; 95% CI, 3.3–30; P<.001) or C3a levels (aOR, 8.8; 95% CI, 3–24; P<.001) before 20 weeks’ gestation. These results demonstrated a combined impact of obesity and elevated complement on the development of preeclampsia.

In 2015, Banadakoppa et al evaluated complement split products in amniotic fluid collected from 731 women at the time of genetic amniocentesis. Compared with women with uncomplicated term pregnancy, those who developed early-onset preeclampsia before 34 weeks’ gestation had higher amniotic fluid C3a levels (318.7 vs 254.5 ng/mL; P=0.04) and Bb levels (1127 vs 749 ng/mL; P=0.03). Median levels of C4a and C5a were not significantly different between the groups. These results show that complement activation is not only increased in the plasma in early pregnancy but also in the amniotic fluid before the onset of preeclampsia.

Previous studies evaluated complement markers in samples collected mostly between 10 and 20 weeks’ gestation. In 2020, He et al evaluated 500 women, using plasma samples collected
Complement biomarkers in preeclampsia and HELLP syndrome

<table>
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<tr>
<th>Author, year</th>
<th>Casesa (n)</th>
<th>Controlsb (n)</th>
<th>Specimen</th>
<th>C3a</th>
<th>C5a</th>
<th>C5b-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haeger et al,70 1989</td>
<td>Preeclampsia (14)</td>
<td>Healthy, unmatched (16)</td>
<td>Plasma</td>
<td>4.0×</td>
<td>2.6×</td>
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<td>Plasma</td>
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<td>3.2×</td>
<td>n.d.</td>
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<td>Healthy, unmatched (60)</td>
<td>Plasma</td>
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<td>1.3×</td>
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<tr>
<td>Soto et al,78 2013</td>
<td>Preeclampsia without SGA (54)</td>
<td>Healthy, unmatched (134)</td>
<td>Plasma</td>
<td>n.d.</td>
<td>1.6×</td>
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<tr>
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<td>Preeclampsia with SGA (52)</td>
<td>Healthy, unmatched (134)</td>
<td>Plasma</td>
<td>n.d.</td>
<td>1.6×</td>
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<td>He et al,81 2016</td>
<td>Early-onset severe preeclampsia (30)</td>
<td>Healthy, GA matched (30)</td>
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<td>18×</td>
<td>2.7×</td>
<td>2.8×</td>
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<td>Late-onset severe preeclampsia (30)</td>
<td>Healthy, GA matched (30)</td>
<td>Plasma</td>
<td>115×</td>
<td>6.1×</td>
<td>2.2×</td>
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<td>Burwick et al,82 2018</td>
<td>Preeclampsia with severe features (104)</td>
<td>Healthy, GA matched (54)</td>
<td>Plasma</td>
<td>N/A</td>
<td>N/A</td>
<td>2.0×</td>
</tr>
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<td>Burwick et al,83 2019</td>
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<td>Healthy, unmatched (16)</td>
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<td>n.d.</td>
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<td>Burwick et al,83 2019</td>
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<td>Healthy, GA matched (25)</td>
<td>Urine</td>
<td>3.7×</td>
<td>5.4×</td>
<td>&gt;4.3×</td>
</tr>
<tr>
<td>Burwick et al,82 2018</td>
<td>Preeclampsia with severe features (104)</td>
<td>Healthy, GA matched (54)</td>
<td>Urine</td>
<td>N/A</td>
<td>N/A</td>
<td>4.5×</td>
</tr>
<tr>
<td>Burwick et al,83 2019</td>
<td>Preeclampsia (16)</td>
<td>Healthy, unmatched (16)</td>
<td>Urine</td>
<td>N/A</td>
<td>n.d.</td>
<td>14×</td>
</tr>
</tbody>
</table>

Data are fold change in cases vs controls, with P<.05 unless stated otherwise.

GA, gestational age; HELLP, hemolysis, elevated liver enzymes, and low platelet count; N/A, not applicable; n.d., no difference; SGA, small for gestational age.

a Descriptions per investigator; severe preeclampsia is now termed preeclampsia with severe features; b Controls labeled as unmatched if the matching criteria were not described in the study design.


Complement Biomarkers in Active Disease

Biomarker studies suggest that before the onset of preeclampsia and HELLP syndrome, there is an early strain on the alternative complement pathway, but not the terminal pathway. However, data have shown significant activation of the terminal complement pathway in the setting of active disease. Summary findings from complement biomarker studies among women with active preeclampsia and HELLP syndrome are presented in Table 2.78–83

In 1989, Haeger et al70,77 first reported an increase in both C3a and C5a in women with preeclampsia and HELLP syndrome. Subsequent studies in the past decade have been mixed, in part owing to varying study design and assay systems. Plasma C3a levels were increased compared with healthy controls in 4 studies7,76,77,81 whereas 2 others found no difference.78,79 Plasma C5a levels were increased in preeclampsia or HELLP compared with healthy controls in 5 studies76,77,81 whereas 2 others found no difference.80,83 Notably, 5 studies found that plasma C5b-9 levels were increased in preeclampsia7,78,79,82,83 whereas 2 studies found no difference.77,80

All complement biomarker studies among women with severe preeclampsia (now preeclampsia with severe features) showed a significant increase in plasma C5a and C5b-9 levels compared with healthy controls, suggesting that terminal complement activation may be more consistently elevated in severe disease. However, Burwick et al79,82 showed that plasma C5a and C5b-9 levels did not differentiate preeclampsia with severe features from other hypertensive disorders of pregnancy, including gestational hypertension, chronic hypertension, and preeclampsia without severe features. These findings suggest that plasma markers of terminal complement activation (C5a and C5b-9) may be broadly increased in hypertensive disorders of pregnancy.

In urine studies, Burwick et al79 showed that C5a levels were increased 5-fold in women with severe preeclampsia compared with matched healthy controls. Urine C5b-9 levels were also increased more than 4-fold among women with severe preeclampsia, and
this finding was seen across 3 different studies. In contrast to plasma C5b-9 levels, increased urinary C5b-9 levels were specific to women with severe preeclampsia (median [interquartile range] urine C5b-9: severe preeclampsia, 4.3 [1.2–15.1] ng/mL; chronic hypertension, 0 [0–0] ng/mL; healthy controls, 0 [0–0] ng/mL; P.<.0001). Urinary excretion of C5b-9 was detected in 96% of cases with severe preeclampsia, 12% of controls with chronic hypertension, and 8% of healthy controls.

In a multicenter observational study, Burwick et al showed that urine C5b-9 concentrations differentiated pre-eclampsia with severe features from other hypertensive disorders of pregnancy (chronic hypertension, gestational hypertension, or preeclampsia without severe features), with area under the receiver operating characteristic curve of 0.74 (95% CI, 0.68–0.80). Among women with preeclampsia with severe features, 40% (42 of 104) had urine C5b-9 concentration of ≥22 ng/mL compared with 0% of women with chronic or gestational hypertension (0 of 137) (P.<.001) and 11% of those with preeclampsia without severe features (6 of 57) (P.<.001). After multivariable logistic regression, preeclampsia with severe features was more likely in women with urine C5b-9 of ≥22 ng/mL (aOR, 10.0; 95% CI, 3.5–29; P.<.001).

Under normal circumstances, urinary excretion of C5b-9 is not expected because of its large size (>1,000,000 Daltons). However, C5b-9 may form at the glomerular membrane with shedding into the urine, or C5b-9 may result from complement-mediated inflammation and cellular injury at the proximal tubule. Burwick et al showed that urinary excretion of C5b-9 increased in association with other urinary biomarkers indicative of glomerular and proximal tubule injury. Guseh et al showed that in comparison with women without C5b-9 in urine, those with detectable urinary C5b-9 levels had significantly increased plasma concentrations of sFlt-1 (32.029 vs 4556 pg/mL; P.<.0001) and significantly decreased concentrations of PI GF (15.6 vs 226 pg/mL; P.<.0001) and VEGF (119 vs 153 pg/mL; P.=.001). These findings demonstrated that urinary excretion of C5b-9 correlated strongly with the antiangiogenic state in women with pre-eclampsia. Although these findings are intriguing, urinary C5b-9 measurements are not yet validated for clinical use, limiting immediate applicability.

Terminal Complement Blockade
Taken together, existing data show that complement regulation is impaired, and terminal complement activation is increased, in many women who develop preeclampsia and HELLP syndrome. These findings suggest that inhibition of the terminal complement pathway (eg, C5 blockade) may be an effective strategy to mitigate disease.

The only C5 blocker currently used in pregnant and lactating women is eculizumab, a humanized monoclonal antibody against C5, which inhibits cleavage of C5 into C5a and C5b and prevents formation of the terminal complement complex C5b-9. It was approved by the Food and Drug Administration to treat paroxysmal nocturnal hemoglobinuria (PNH) in 2007 and aHUS in 2011, owing to its therapeutic efficacy in both conditions. PNH is an acquired hemolytic disorder that occurs when hematopoietic stem cells in the bone marrow lose their ability to anchor complement regulatory proteins CD55 and CD59 to the cell surface, thereby predisposing to complement-mediated red cell hemolysis. In contrast, aHUS is a complement-mediated thrombotic microangiopathy disorder, often attributed to inherited complement mutations. However, the penetrance of complement gene mutations is limited, approximately 50% by the age of 45 years, and aHUS many not manifest clinically until there is a complement amplifying event, such as pregnancy.

Pregnancy safety data for eculizumab stem from its use in pregnant women with PNH and aHUS. Among pregnant women with PNH treated with eculizumab, Kelly et al found that eculizumab was detectable in 7 of 20 cord blood samples (range, 11.8–21.1 ug/mL), suggesting that it crosses the placenta at low levels. Although immunoglobulin G monoclonal antibodies are transported across the placenta through the neonatal crystallizable fragment receptor (FcRn), eculizumab was engineered with human immunoglobulin G2 and immunoglobulin G4 heavy-chain sequences to form a hybrid constant region with reduced affinity for FcRn. Hallstensen et al showed that treatment with eculizumab during pregnancy does not seem to alter the complement system activity of the newborn. Kelly et al also examined 10 breast milk samples among women receiving eculizumab, and the drug was not detected in any of the samples, leading the authors to conclude that breastfeeding is safe. However, continued vigilance is warranted given the small sample size.

Complement Blockade in Preeclampsia and Hemolysis, Elevated Liver Enzymes, and Low Platelet Count Syndrome
Data from PNH and aHUS have been used to strengthen the argument for C5 blockade in preeclampsia and HELLP syndrome. In ex vivo experiments, Vaught et al showed that complement-mediated hemolysis was increased when the serum of women with aHUS, severe preeclampsia, or HELLP syndrome was mixed with PNH red cells lacking CD55 and CD59 (modified Ham test). Likewise, Palomo et al showed that C5b-9 deposition on endothelial cells was increased after exposure to activated plasma from women with aHUS, severe preeclampsia, or HELLP syndrome. The magnitude of complement-mediated hemolysis and endothelial C5b-9 deposition in these 2 studies, respectively, was similar in subjects with preeclampsia, HELLP, or aHUS, suggesting that aberrant complement activation was a common feature of these disorders. Importantly, both studies showed that complement-mediated effects were mitigated by C5 blockade with eculizumab.

Although terminal complement blockade with eculizumab is effective for the treatment of PNH and aHUS, there is only a single case report describing the successful off-label use of eculizumab to...
treat preeclampsia and HELLP syndrome in pregnancy. However, others have shown that on-label use of eculizumab is effective in treating pregnant women with aHUS who develop preeclampsia or those who develop postpartum aHUS after a delivery complicated by preeclampsia and HELLP syndrome. In 2019, Lu et al described a pregnant woman who was diagnosed with aHUS at 22 weeks’ gestation and initiated on eculizumab treatment. At 24 weeks’ gestation, she was also diagnosed with superimposed preeclampsia and eculizumab treatment was continued, with prolongation of pregnancy an additional 25 days. In retrospective laboratory analyses, the authors found that the sFlt-1 to PlGF ratio decreased after the initiation of eculizumab, and they hypothesized that eculizumab may have moderated the antiangiogenic activity in preeclampsia. In 2020, Lokki et al described a patient who developed postpartum aHUS after delivery for preeclampsia and HELLP syndrome at 34 weeks’ gestation. The patient developed severe renal failure requiring dialysis, but she quickly recovered after the diagnosis of aHUS was made and treatment with eculizumab was initiated.

In 2013, Burwick and Feinberg were the first to show that C5 blockade with eculizumab could also be used effectively to treat preeclampsia and HELLP syndrome, in the absence of aHUS. They described a previously healthy pregnant woman who developed severe preeclampsia and HELLP syndrome at 26 weeks’ gestation. After the off-label use of eculizumab, there was resolution of hemolysis and thrombocytopenia and normalization of liver enzymes (Figure 3). Maternal and fetal status improved sufficiently to prolong pregnancy an additional 17 days, allowing more time for fetal maturation and decreasing the likelihood of neonatal morbidity. In a subsequent report, the authors reported that the concentration of C5b-9 in the blood and urine decreased in conjunction with C5 blockade and disease remission. Although there was a good clinical outcome, the authors noted a persistent elevation of C5a after C5 blockade, and they cautioned that extrinsic factors such as thrombin may also generate C5a independent of the C5 convertase. Finally, they also cautioned that eculizumab or eculizumab-C5 complexes may cross the placenta or deposit in the maternal kidney, and long-term effects are unknown. Therefore, the benefits of eculizumab or other C5 monoclonal antibodies for the treatment of preeclampsia and HELLP syndrome require further evaluation in the setting of a clinical trial.

Discussion

The current literature demonstrates that complement activation and regulation are altered in women who develop preeclampsia and HELLP syndrome. The initial stages of disease may begin in early pregnancy, with complement proteins altering trophoblast development and mediating placental inflammation and apoptosis. Early activation of the alternative complement pathway and underlying medical conditions such as obesity magnify the likelihood of developing preeclampsia. Pathogenic complement gene mutations or deletions also confer an increased risk, owing to an impaired ability to regulate complement activation in pregnancy. Terminal complement pathway activation leads to the formation of C5a and C5b-9, which can polarize monocytes, macrophages, and trophoblast cells to the antiangiogenic phenotype, with the release of sFlt-1 into
the maternal circulation. In active disease, there is an increased formation of C5b-9 in the blood and urine, and urinary detection of C5b-9 differentiates preeclampsia with severe features from other hypertensive disorders of pregnancy.

Terminal complement pathway activation seems to exceed the complement regulation in women who develop preeclampsia and HELLP syndrome. Research to date suggests that the degree of complement activation is largely similar in preeclampsia with severe features and HELLP syndrome, consistent with our understanding of the disease spectrum. We found no evidence that HELLP syndrome is a distinct entity from preeclampsia, but rather the 2 conditions share pathophysiologic underpinnings, with excess complement activation as a common feature. Therefore, research in this area should evaluate women with both preeclampsia and HELLP syndrome to capture more pregnancies with complement-mediated disease.

The role of complement proteins in preeclampsia and HELLP syndrome is now evident but more studies are needed to understand how complement protein biomarkers and complement genetic variants may be used clinically to guide prediction, diagnosis, and treatment of disease. Some data may be extrapolated from existing studies on aHUS and other thrombotic microangiopathy disorders, but pregnancy is a unique time with distinct considerations for the mother and the developing fetus. Future clinical trials aimed at preventing or treating preeclampsia and HELLP syndrome should consider complement blocking therapies, including C5 blockade, but there remains an urgent and unmet need to evaluate the safety and efficacy of novel complement therapeutic agents in pregnant and lactating women.

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GLOSSARY

aHUS: Atypical hemolytic uremic syndrome.
Anaphylatoxin: A complement component which causes smooth muscle contraction, vasodilatation, histamine release from mast cells, and enhanced vascular permeability.
CD46: Cluster of differentiation 46, also known as membrane cofactor protein.
CD55: Cluster of differentiation 55, also known as decay-accelerating factor.
CD59: Cluster of differentiation 59, also known as protecin.
CFB: Complement factor B.
CFH: Complement factor H.
CFHR: Complement factor H related.
CFI: Complement factor I.
C1q: Complement component 1q.
C3: Complement component 3.
C3a: Complement component 3a.
C3b: Complement component 3b.
C4: Complement component 4.
C4d: Complement component 4d.
C5: Complement component 5.
C5a: Complement component 5a.
C5AR1: Complement component 5a receptor.
C5b: Complement component 5b.
C5b-9: Terminal complement complex, also known as membrane attack complex.
HELLP: Hemolysis, elevated liver enzymes, low platelet count.
MAC: Membrane attack complex (or C5b-9).
MCP: Membrane cofactor protein (or CD46).
Opsonin: A complement component which binds to foreign pathogens or cells making them more susceptible to phagocytosis.
PIGF: Placental growth factor.
PNH: Paroxysmal nocturnal hemoglobinuria.
sFlt-1: Soluble fms-like tyrosine kinase-1.
VEGF: Vascular endothelial growth factor.
VEGFR1: Vascular endothelial growth factor receptor 1.

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