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Direct evidence of complement activation in HELLP syndrome: a link to atypical hemolytic uremic syndrome

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Abstract

HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) is a severe variant of preeclampsia whose pathogenesis remains unclear. Recent evidence and clinical similarities suggest a link to atypical hemolytic uremic syndrome (aHUS), a disease of excessive activation of the alternative complement pathway effectively treated with a complement inhibitor, eculizumab. Therefore, we utilized a functional complement assay, the modified Ham test, to test sera of women with classic or atypical HELLP syndrome, preeclampsia with severe features, normal pregnancies and healthy non-pregnant women. Sera were also evaluated using levels of the terminal product of complement activation (C5b-9). We tested the *in vitro* ability of eculizumab to inhibit complement activation in HELLP serum. Increased complement activation was found in participants with classic or atypical HELLP compared to normal pregnancy and non-pregnant controls. Mixing HELLP serum with eculizumab containing serum resulted in a significant decrease in cell killing compared to HELLP serum alone. In conclusion, HELLP syndrome is associated with increased complement activation demonstrated by the modified Ham test. This assay may aid in the diagnosis of HELLP syndrome and confirm its pathophysiology relates to aHUS.

Introduction

Preeclampsia is a multisystem disorder of pregnancy which manifests as hypertension, proteinuria, and/or other end organ damage as a result of endothelial dysfunction, and occurs in 3–5% of all pregnancies [1]. Aside from its high prevalence, preeclampsia accounts for

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10–30% of all preterm deliveries in developed countries [2, 3]. HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) is a severe variant of preeclampsia. Hematologist are often consulted because of thrombocytopenia and microangiopathy. First defined by Weinstein in 1982, HELLP syndrome has a reported incidence of up to 0.8% of all pregnancies [4]. For classic HELLP syndrome the Tennessee and Mississippi classifications propose clinical criteria using platelet count, lactate dehydrogenase (LDH) levels, bilirubin and aspartate aminotransferase (AST) with or without alanine aminotransferase (ALT) levels to establish the diagnosis (Table 1) [5, 6]. It is further acknowledged that many women with preeclampsia may have some but not all of these laboratory findings such as isolated thrombocytopenia or elevated liver enzymes without the classic disease. These women are categorized as “impending,” “partial”, “incomplete” or “atypical” HELLP and the criterion for this disease process varies [7, 8]

HELLP syndrome may result in severe morbidity and mortality to both the mother and fetus. Disseminated intravascular coagulopathy is the most frequent severe maternal complication followed by hepatic rupture and bleeding [7]. Delivery is the treatment of choice, but preterm delivery may have severe consequences to the neonate. Neonatal mortality and morbidity are significantly higher if the fetus is delivered before 34 weeks [9]. Treatment of HELLP prior to delivery is largely supportive and consists of fetal monitoring, steroids to increase fetal lung maturity, magnesium sulfate for maternal seizure prophylaxis, and pharmacologic management of hypertension [10–12].

Although the pathogenesis of HELLP is believed to be on the spectrum of preeclampsia via abnormal placentation, endothelial dysfunction, and release of vasoactive substances in the first trimester [13]), the cellular biology and natural history of disease that results in its distinct phenotype is not well defined. However, recent evidence implicates complement activation in its pathogenesis [14]. Studies have postulated an up-regulation of the alternative pathway of complement (APC) using markers in serum and urine (C5b-9 or membrane attack complex/MAC) [15, 16]. Recently, mutations in the APC have been found in up to 20% of HELLP patients [17]. Similar mutations are also found in atypical hemolytic uremic syndrome (aHUS), a disease of excess APC activation caused by inherited or acquired defects in the regulation of the APC [18]. Indeed, mothers with HELLP syndrome have manifestations very similar to aHUS which mainly involve microangiopathic hemolytic anemia (MAHA), thrombocytopenia, thrombotic microangiopathy, renal dysfunction, hypertension, seizures and altered mental status. Unlike HELLP, where the mainstay of therapy is limited to supportive care, expectant management and ultimately delivery, aHUS is treated with a C5 monoclonal antibody, eculizumab [19]. Eculizumab is approved by the Food and Drug Administration for the treatment of: a) paroxysmal nocturnal hemoglobinuria (PNH), a complement-mediated hemolytic anemia due to deficiency of the complement regulatory proteins CD55 and CD59 from the red cell membrane[20, 21] and b) aHUS, a complement-driven thrombotic microangiopathy due to excessive complement activation in the serum [22]. Eculizumab is now considered the treatment of choice for both aHUS and PNH [23]. Eculizumab treatment has been also recently described in a case report of a HELLP patient with favorable effects [24].

We recently developed a sensitive and specific serum-based assay to detect heightened activity of the alternative pathway of complement ("modified Ham test") that readily distinguishes aHUS from other thrombotic microangiopathies such as thrombotic thrombocytopenic purpura [25] The principle of the modified Ham test is similar to the acidified serum (Ham) test that was developed by Dr. Thomas Ham in the 1930s to diagnose PNH [26, 27]. In PNH there is a genetic mutation, *PIGA* that causes deficiency or absence of glycosylphosphatidylinositol (GPI). Therefore, proteins anchored by GPI are missing. Two of those missing proteins are complement regulatory proteins (CD55 and CD59) on red cells [28]. Acidifying normal human serum activates the alternative pathway of complement and leads to specific lysis of PNH erythrocytes secondary to increased vulnerability to the activated complement in acidified serum, as shown in Figure 1A. In aHUS, there are usually genetic mutations or antibodies that lead to constitutive activation of the alternative pathway of complement in the patient's serum. Thus, when PNH-like reagent cells (GPI-deficient or *PIGA*-null TF-1 cells) are incubated with aHUS serum they rapidly accumulate C5b-9 and undergo cell death within hours [25]. Complement-induced death in the modified Ham test prevents conversion of a cell proliferation reagent (WST-1) into a red dye, formazan (Figure 1B). This colorimetric reaction allows one to detect complement-mediated killing in hours.

We hypothesized that a subset of women with atypical HELLP and classic HELLP syndrome may have increased APC activation similar to aHUS. To test this hypothesis, we used the modified Ham test to assess complement activation in patients with severe preeclampsia/HELLP syndrome.

Methods and Materials

Study population

We performed an observational, case-controlled study of women with classic HELLP (Group 1), atypical HELLP (Group 2), preeclampsia with severe features (Group 3), women with normal pregnancies (Group 4), and non-pregnant healthy women (Group 5). All participants, other than group 5, were greater than 23 weeks pregnant with no specified matching of gestational ages among groups. Preeclampsia with severe features was defined by the ACOG (American Congress of Obstetricians and Gynecologists) executive summary on hypertension in pregnancy [10]. Classic HELLP syndrome was defined as satisfying either Mississippi or Tennessee criteria. Atypical HELLP syndrome was defined as having at least one laboratory abnormality found in the Mississippi or Tennessee criteria for HELLP syndrome [5–7].

Groups 1, 2, and 3 were recruited antenatally from the Johns Hopkins Hospital and Johns Hopkins Bayview Medical Center from September 1, 2014 to August 31, 2015, and Group 4 and Group 5 were recruited from the institution's outpatient centers. Women with known sickle cell disease, systemic lupus erythematous, antiphospholipid antibody syndrome, or previously diagnosed microangiopathic and hemolytic diseases were excluded from all groups. Women with any hypertensive diseases of pregnancy were excluded from Group 4. All participants gave written informed consent, and the study was approved by the Johns Hopkins University Institutional Review Board.

Blood from groups 1, 2, and 3 were collected at the time of admission and diagnosis, and blood from Group 4 and 5 was collected in the outpatient setting at the time of consent. The blood from all five groups was collected in serum separation tubes and was centrifuged at 4 °C. Serum was separated and stored at −80 °C. Serum samples were processed and stored within 4 hours after the blood was drawn to prevent *ex vivo* complement activation. Coded samples were sent de-identified in the laboratory for further testing.

Given the expense of eculizumab treatment and its limited availability, the *in vitro* effects of eculizumab were tested utilizing serum from a patient treated with eculizumab for paroxysmal nocturnal hemoglobinuria (PNH), as previously described [29]. Eculizumab containing serum was drawn following informed consent from a treated patient within 60 minutes of eculizumab infusion, as a source of optimal eculizumab concentration

Serum C5b-9 levels

Serum C5b-9 levels were determined using a commercially available ELISA (enzyme-linked immunosorbent assay) kit (Quidel, San Diego, CA) according to the manufacturer's instructions.

Modified Ham test

The modified Ham test was performed as previously described[16]. Briefly, PNH-like reagent cells (*PIGA*-null TF-1 cell line) previously established in our laboratory were used as targets of complement-mediated killing. [17]. *PIGA*-null TF-1 cells were plated in U-shaped 96-well plates at a density of 4,000 cells / well and cultured until confluent. Then, cells were washed with PBS and incubated with 20% of serum in Gelatin Veronal Buffer (GVB, Sigma Aldrich, St Louis, MO) in triplicates for 30 minutes at 37 °C. Cells were washed again with PBS and incubated with the cell proliferation reagent 4-[3-(4-lodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1.3-benzene disulfonate / WST-1 (Roche, Switzerland) for 2 hours at 37 °C. The proliferation reagent was diluted in the cell culture medium at a concentration of 1:10 and 100 μl of its solution was added per well. Absorbance was measured in an iMark Microplate Absorbance Reader (Bio-rad, Hercules, CA) at 490 nm with a reference wavelength at 595 nm.

Heat-inactivated serum was used as a negative control. Heat inactivation was performed the same day of the experiment, incubating the serum at 56 °C for 30 minutes. Normal human AB serum (H4522, Sigma-Aldrich, St Louis, MO) was used as an internal control of the assay. Absorbance values of each sample were normalized after subtraction of the absorbance value of a blank well. Percentage of viable cells was expressed as a ratio of the absorbance of each sample multiplied by 100, to the absorbance of the same sample's heat-inactivated control. Thus, percentage of non-viable cells (cell killing) was calculated using the following formula: 100 − (sample absorbance * 100 / heat-inactivated sample's absorbance). Assay validation studies have indicated that sample storage at room temperature for more than 4 hours or overnight in a refrigerator results in higher than 50% reduction of cell killing in the modified Ham test. Results are not affected by one cycle of sample freeze/thaw at −80°C if all preparations are made on ice.

In vitro evaluation of complement inhibition

Complement inhibition *in vitro* was evaluated using the modified Ham test. Eculizumab (ECU) containing serum from a PNH patient was mixed at different ratios with HELLP sera (50–50%, 25–75% and 12.5–87.5% of HELLP and ECU sera, respectively). Total amount of serum in the assay remained unchanged (20%). Complement inhibition was also evaluated by adding 25 μg/mL of an anti-C5 monoclonal antibody solution (Alexion Pharmaceuticals). The total amount of serum in this assay remained unchanged at 20% as well.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 20.0 for Windows (SPSS, Chicago, IL). The independent samples Student t-test was used to compare differences between the mean values of two groups. One-way ANOVA with Bonferroni's correction or nonparametric tests were used to compare means between more than two groups. Receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off value, sensitivity and specificity of HELLP diagnosis by the modified Ham test. A p-value ≤0.05 was considered statistically significant. Statistical power analysis was performed retrospectively calculating the observed power in a univariate analysis model.

Results

Study population

We studied sera from 14 women with classic HELLP syndrome (Group 1) and atypical (Group 2) HELLP syndrome, 7 women with preeclampsia with severe features (Group 3), 11 women with normal pregnancies (Group 4), and 8 controls (Group 5). Age was similar among participants with preeclampsia with severe features with or without HELLP (27.4±3.8 versus 26.3±5.4 and 28.6±5.0 for Groups 1, 2 and 3, p=0.596). Laboratory and clinical characteristics of participants in Groups 1, 2, and 3 are shown in Table 2. Two participants studied antepartum with preeclampsia with severe features but not HELLP, were also evaluated postpartum when they were diagnosed with classic HELLP and atypical HELLP.

Serum C5b-9 levels

We first studied C5b-9 (membrane attack complex) levels as a biomarker of terminal complement activation. C5b-9 is the terminal product of the complement cascade that lands on the cell membrane inducing complement-mediated cell killing. Although C5b-9 has been found increased in diseases with excess complement activation such as aHUS, the levels significantly overlap with diseases not characterized by systemic complement activation [30]. In the present study we, too, found no significant difference in serum C5b-9 levels among participants with preeclampsia with severe features with and without HELLP and normal controls (p=0.808), as shown in Figure 2.

Modified Ham test

We next sought to investigate complement activation in an assay that distinguished systemic complement activation observed in aHUS, the modified Ham test. The modified Ham test

reflects complement activation as a percentage of complement-mediated cell killing and is thereby a functional assay. Greater than 21.5% killing of reagent cells was indicative of aHUS in our previous study of the modified Ham test[16]. As expected, healthy pregnant (Group 4) and non-pregnant (Group 5) controls presented similarly low cell killing (4.9±7.0% versus 3.3±3.36%, p=0.100). Participants in Groups 1 and 2 showed significantly higher percentage of cell killing than participants with normal pregnancy (34.3±24.6%and 26.0±14.8% versus 4.9±7.0%, p=0.001 and p=0.002 respectively). The observed power in the univariate analysis model was 86.7%. Also, there was a trend towards significantly increased killing in Group 1 (classic HELLP) compared to Group 3 (preeclampsia with severe features) (34.3±24.6% versus 13.0±11.7% p=0.074). Interestingly, cell killing was above the cut-off value determined for aHUS diagnosis (21.5%) in three out of four participants diagnosed with classic HELLP and seven out of ten participants diagnosed with atypical HELLP; cell killing due to complement was in the range seen for aHUS. One of the participants (case 3 in Table 2), had an increase in killing antenatally, and immediately after delivery was diagnosed with classic HELLP syndrome, suggesting she may have a genetic predisposition to HELLP as is often observed in aHUS. The other participant (case 5 in Table 2) had minimal cell killing antepartum but returned on postpartum day 4 with atypical HELLP syndrome and cell killing minimally above threshold value. Results are summarized in Figure 3A.

To determine a cut-off value above which the modified Ham test would be considered positive for HELLP diagnosis, we performed a ROC curve analysis that showed a significant area under the curve (area under the curve=0.848, p-value <0.001). We were able to determine a percentage of non-viable cells higher than 20.5% as the cut-off value for the diagnosis of HELLP with 78.6% sensitivity and 92.9% specificity. ROC curve is shown in Figure 3B.

In vitro evaluation of complement inhibition

Eculizumab is highly effective in treating diseases of terminal complement activation such as PNH and aHUS. Therefore, we hypothesized that eculizumab would abrogate cell killing caused by HELLP serum *in vitro*. As expected, eculizumab-containing serum alone caused no increase in cell killing. However, mixing HELLP serum with increased cell killing with eculizumab-containing serum resulted in a significant decrease of cell killing compared to atypical HELLP sera alone suggesting that the cell killing induced from HELLP patient serum is complement-mediated and can be abrogated by terminal complement inhibition (p=0.007), Figure 4. In order to show that eculizumab was directly responsible for inhibiting cell killing in the mixing studies, we added an anti-C5 monoclonal antibody (Alexion pharmaceuticals) directly to HELLP patient serum and performed the modified Ham test. As shown, in Figure 5, the anti-C5 monoclonal antibody markedly reduced cell killing at a dose of 25μg/mL.

Discussion

The present study provides evidence that there is increased complement activation through the alternative pathway of complement in a subset of women with preeclampsia with severe

features and classic or atypical HELLP syndrome. This provides evidence of a link among preeclampsia, HELLP syndrome, and diseases of excess complement activation, namely aHUS. Furthermore, the modified Ham test appears to be a useful biomarker that may assist in the diagnosis of HELLP syndrome and if validated may help guide future clinical trials. HELLP syndrome is categorized as a severe form of preeclampsia. Limitations in the diagnosis of HELLP syndrome are the different criteria for classic HELLP syndrome and atypical HELLP syndrome, and a lack of reliable biomarkers. Moreover, severe preeclampsia, atypical and classical HELLP likely represent a clinical continuum and some patients are delivered before meeting clinical criteria for classical HELLP. In addition, LDH, AST and bilirubin are not specific for liver dysfunction and their elevation may be caused by intravascular hemolysis alone.

aHUS involves kidney injury and thrombotic microangiopathy similar to many cases of severe preeclampsia and HELLP. aHUS results from both inherited and acquired defects in the regulation of the alternative pathway of complement [18]. Mutations in genes regulating or activating the alternative pathway of complement involve serum (such as complement factor H and I, complement component C3) and cell membrane factors (such as CD46 or membrane cofactor protein and thrombomodulin). Genetic mutations in these complement regulatory genes are found in 50–60% of patients diagnosed with aHUS [31, 32] and triggers (e.g, pregnancy, surgery, inflammation etc.) are considered crucial for the manifestation of the disease two-hit model[33]. Pregnancy is a common trigger for aHUS, accounting for 7–20% of cases, and 80% of pregnancy-associated aHUS is diagnosed in the postpartum period [34].

Genetic testing for aHUS is expensive, takes weeks to get the results and fails to diagnose almost 50% of cases; thus, there has been extensive research into finding more reliable biomarkers such as serum or urine levels of C5b-9, a marker of terminal complement activation. Although elevated in plasma of aHUS patients [30], C5b-9 levels cannot reliably distinguish aHUS from other thrombotic microagiopathies, such as thrombotic thrombocytopenic purpura (TTP) [25, 30]. Serum C5b-9 levels did not prove a reliable marker of complement activation in pregnant women with preeclampsia with severe features with or without HELLP. However, utilizing the modified Ham test we were able to show that classic and atypical HELLP sera cause increased complement-mediated cell killing to the same degree as aHUS serum. Indeed, the cut-off value defined for HELLP diagnosis was 20.5% whereas the cut-off value previously defined for aHUS was 21.5% [35]. Interestingly, one participant studied both antepartum and postpartum exhibited increased cell killing both times (case 3). The other participant who was studied both antepartum and postpartum showed no significant killing in the antepartum state, but was later diagnosed with atypical HELLP and showed significantly increased killing upon postpartum readmission, albeit marginally above threshold (case 4). If these findings are validated in larger cohorts, the modified Ham test may serve as a diagnostic test and help to identify patients who might benefit from complement inhibition treatment. Further studies are needed to determine whether or not the assay has predictive value for HELLP syndrome.

We have also shown that complement inhibition by eculizumab can effectively block activation of the alternative pathway of complement demonstrated as increased cell killing

using the modified Ham test in HELLP serum. Through terminal complement inhibition by the monoclonal antibody, eculizumab has been established as a treatment of choice for aHUS. We decided to test whether complement inhibition is effective in HELLP serum utilizing this *in vitro* experiment. This experiment provides a mechanistic basis behind an already published case report of successful treatment with eculizumab ([24].

Demonstration of excess activation of the alternative pathway of complement in HELLP suggest that terminal complement inhibition may be effective in treating a subset of patients with HELLP. There is now increasing experience using eculizumab use in pregnant women with PNH [36–39] or pregnancy-related aHUS [40, 41]. No adverse maternal or fetal outcomes and no complications after breast feeding have been reported and eculizumab is not detected in fetal plasma [38, 42, 43]; the drug to not cross the placenta well and does not seem to get into breast milk.

Our study has limitations. First, the modified Ham test was negative in three out of fourteen participants with classic or atypical HELLP syndrome. Several possible explanations exist. The modified Ham test is serum-based and theoretically may not detect increased complement activation caused by mutations in cell membrane factors such as CD46 and thrombomodulin. Mutations in a membrane complement regulatory factor (CD46 or membrane cofactor protein) have been documented in case reports of patients with HELLP [24, 44] and in 4 out of 59 patients (6.8%) with preeclampsia with severe features and/or HELLP syndrome studied [17]. Since mutations in other membrane complement regulatory factors in HELLP have not been studied yet, membrane abnormalities may account the negative results of the modified Ham test. Alternatively, other mechanisms and pathways beyond complement activation may also be involved in the spectrum of preeclampsia and HELLP syndrome. Another limitation is our relatively small sample size. Certainly, further studies are needed in a larger cohort of pregnant women.

In conclusion, we have shown that preeclampsia with severe features along with classic and atypical HELLP syndrome may be considered, at least in part, a disease of excessive complement activation. The modified Ham test may be a promising tool to identify patients with increased complement activation who might benefit from therapies that block complement activation. If confirmed in a larger cohort, the modified Ham test may be a valuable assay to select patients for such a clinical trial.

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Highlights

- Serum from HELLP patients show activation of the alternative pathway of complement, similar to patients with atypical hemolytic uremic syndrome.
- Terminal complement blockade is effective in modulating excess complement in HELLP patients in vitro.

Math Formulae

$$\text{Percentage of non- viable cells} = 100 - (\text{sample absorbance} * 100 / \text{heat- inactivated sampl sample's absorbance})$$

Figure 1.
Model of the principle of the Ham and the modified Ham test.

Figure 2.

Serum CSb-9 levels patients with preeclampsia with severe features with and without HELLP and normal - pregnant controls. No significant difference was found among the three groups (p=0.808).

Figure 3. Modified Ham test in the HELLP syndrome

A. Participants with classic HELLP (cHELLP) and atypical HELLP (aHELLP) showed significantly higher percentage of cell killing than participants with normal pregnancy (34.3±24.6%and 26.0±14.8% versus 4.9±7.0%, p=0.001 and p=0.002 respectively)

B. The ROC curve analysis showed a significant area under the curve (area under the curve=0.848, p-value <0.001). We were able to determine a percentage of non-viable cells higher than 20.5% as the cut-off value for the diagnosis of HELLP with 78.6% sensitivity and 92.9% specificity.

Figure 4.

Complement inhibition by anti-C5 monoclonal antibody (Eculizumab) abrogates cell killing in the modified Ham test

Figure 5. Anti-C5 monoclonal antibody inhibits complement in HELLP serum

The modified Ham test was performed using 20% serum from a healthy control, a patient with atypical HUS (positive control), a patient with classic HELLP, and a patient with severe preeclampsia with increased killing in the modified Ham test. Each participant is represented as (*) with serum alone, and with (†) the addition of anti-C5 antibody 25 μg/mL. The y-axis represents percent cell kill after 2 hours in the modified Ham test. Cell kill of greater than 20.5% is considered positive in this assay. The addition of anti-C5 antibody (25mg/mL) to serum of all patient samples reduced cell kill in the modified Ham test to less than 20%.

Table 1

Tennessee and Mississippi Criteria for HELLP Syndrome

Lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT)

Tennessee Criteria	Mississippi Criteria	Atypical HELLP Criteria
Microangiopathic hemolytic anemia, LDH > 600	Hemolysis (increased LDH and anemia)	Defined as having at least one, but not every lab abnormality from either the Mississippi or Tennessee Criteria
Platelet Count < 100,000 cells/microL	Platelet Count < 150,000 cells/microL	
Total Bilirubin > 1.2 mg/dL or LDH > 600 IU/L	LDH> 600 IU/L, AST >40 IU/L and/or ALT>40 IU/L	
AST > 70 IU/L		

Table 2

Laboratory and clinical characteristics of participants with classic HELLP, atypical HELLP, and preeclampsia with severe features at the time of sample collection

ID	Diagnosis	Gestational age (weeks)	Race	Age (years)	BMI (kg/m^2)	SBP* (mmHg)	DBP* (mmHg)	AST (IU/L)	ALT (IU/L)	Proteinuria	PLT (× 10^8/ μl)	LDH (mg/dl)	Creatinine (mg/dl)	Neonatal Weight	% Weight	SGA Neonate	% killing
1	Classic HELLP (Group 1)	34.2	Caucasian	23	24.3	162	96	84	68	Yes	132	325	0.7	1790	5	Yes	50
2		30.6	Caucasian	32	24	155	90	109	79	NA	73	260	0.7	1490	15	No	0
3app		PPD 1	African American	25	31.1	200	100	1485	952	Yes	52	1623	0.6	1190	1	Yes	33
4		31.6	Caucasian	25	34.4	180	120	99	56	No	88	689	1	1680	1	Yes	54
5app	Atypical HELLP (Group 2)	PPD 4	African American	27	32.1	174	98	95	80	Yes	214	303	1	1880	25	No	21
6		30.2	African American	27	41.86	215	105	43	52	Yes	180	276	1.6	1040	1	Yes	48
7		28.2	African American	25	44.1	200	100	106	57	Yes	272	449	1.1	1125	50	No	41
8		30.5	Asian	28	22.5	193	77	17	18	Yes	135	231	0.9	1420	1	Yes	0
9		36.1	African American	35	25.8	184	100	10	7	Yes	117	316	0.7	2410	25	No	33
10		24.6	African American	25	32.5	172	106	89	147	Yes	204	440	1	380	1	Yes	6
11		31.6	African American	30	28.7	172	97	89	72	Yes	197	288	0.9	1410	2	Yes	34
12		33.1	Caucasian	35	27.6	188	109	36	47	Yes	132	356	1.3	1490	1	Yes	30
13		33.6	Caucasian	29	28.1	157	105	297	126	Yes	234	245	0.8	2510	69	No	21
14		30.6	African American	39	36.3	174	107	366	351	No	199	446	0.7	1610	29	No	26
15	Preeclampsia with severe features (Group 3)	36.5	Caucasian	34	40.9	175	93	37	22	Yes	163	202	0.5	2310	8	Yes	0
16		33.2	African American	19	22.1	170	110	22	13	Yes	238	240	0.8	1730	3	Yes	20
17		31.6	African American	30	22.4	188	109	17	13	Yes	160	271	1	1760	40	No	25
18		40.2	Caucasian	29	48.4	204	96	17	14	No	272	NA	0.6	4390	99	No	19
19		32.1	Caucasian	20	51.7	180	103	20	13	Yes	241	223	0.8	1810	9	Yes	0
3ap		31.1	African American	25	31.1	200	100	31	28	Yes	291	222	0.6	1190	1	Yes	25
4ap		32.4	African American	27	32.1	174	98	21	18	Yes	168	274	0.9	1880	40	No	2

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; aspartate aminotransferase (AST); alanine aminotransferase (ALT); PLT: platelet count; lactate dehydrogenase (LDH); SGA: small gestational age; NA: Not available; antepartum (ap); postpartum (pp), postpartum day (PPD);

*defines highest antepartum value; defines lower antepartum value, all lab values correlate with the day of blood draw for cell killing