Angiogenic Factors in Superimposed Preeclampsia
A Longitudinal Study of Women With Chronic Hypertension During Pregnancy

Uma Perni, Cristina Sison, Vijay Sharma, Geri Helseth, Amret Hawfield, Manikkam Suthanthiran, Phyllis August

Abstract—Imbalances in circulating angiogenic factors contribute to the pathogenesis of preeclampsia. To characterize levels of angiogenic factors in pregnant women with chronic hypertension, we prospectively followed 109 women and measured soluble fms-like tyrosine kinase 1 (sFlt1), soluble endoglin, and placental growth factor at 12, 20, 28, and 36 weeks’ gestation and postpartum. Superimposed preeclampsia developed in 37 (34%) and was early onset (≤34 weeks) in 9 and later onset (>34 weeks) in 28. Circulating levels of sFlt1 and the ratio of sFlt1 to placental growth factor were higher before clinical diagnosis at 20 weeks’ gestation in women who subsequently developed early onset preeclampsia between 28 and 34 weeks compared with levels in women who never developed preeclampsia (P<0.001) or who developed late-onset preeclampsia (P=0.001). Circulating levels of sFlt1, soluble endoglin, and the ratio of sFlt1:placental growth factor were also significantly higher, and placental growth factor levels were significantly lower at the time of clinical diagnosis of superimposed preeclampsia in women with either early or late-onset superimposed preeclampsia compared with levels at similar gestational ages in those with uncomplicated chronic hypertension. We conclude that alterations in angiogenic factors are detectable before clinical diagnosis of superimposed preeclampsia, whereas alterations were observed only at the time of diagnosis in women with late-onset superimposed preeclampsia. Longitudinal measurements of angiogenic factors may help anticipate early onset superimposed preeclampsia and facilitate diagnosis of superimposed preeclampsia in women with chronic hypertension. (Hypertension. 2012;59:740-746.)

Key Words: superimposed preeclampsia ■ angiogenic factors ■ chronic hypertension in pregnancy
The current investigation was designed to characterize the levels of circulating sFlt1, sEng, and PlGF in women with preexisting hypertension and to investigate whether longitudinal profiling can help distinguish chronically hypertensive pregnant women who develop SPE from those who do not develop SPE. In this study, we have leveraged and performed a secondary analysis of the peripheral blood specimens originally collected longitudinally in the context of a placebo-controlled, double-blinded randomized trial of calcium supplementation in chronically hypertensive pregnant women and demonstrate significant alterations in sFlt1, sEng, and PlGF before the clinical diagnosis of early onset (<34 weeks) SPE and at the time of diagnosis in women with both early and late-onset SPE. Our findings supporting the hypothesis that measurement of angiogenic factors may be helpful in the diagnosis of SPE in women with chronic hypertension form the basis of this report.

Methods

Subjects

Clinical data and venous peripheral blood were collected as part of the Chronic Hypertension in Pregnancy Study, a placebo-controlled, double-blinded randomized trial of calcium for the prevention of PE in women with preexisting chronic hypertension performed at New York Presbyterian-Weill Cornell, which concluded in 1999.20 The study protocol was approved by the institutional review board at Weill-Cornell Medical College, and informed consent was obtained at the initial visit. Eligible women were 18 to 45 years of age who were 12 to 15 weeks pregnant and had blood pressure ≥140/90 mm Hg either before pregnancy or in the first trimester or who were on active treatment for chronic hypertension. Women with a serum creatinine ≥1.2 mg/dL or creatinine clearance <75 mL/min were excluded from the Chronic Hypertension in Pregnancy Study. Data analysis was carried out on a cohort of 109 women whose pregnancies progressed beyond 20 weeks.

Study Protocol

Pregnant women with chronic hypertension were randomly assigned to treatment with either calcium carbonate (2000 mg daily) or placebo. Blood pressure, weight, and brief history and physical were performed at baseline (12–15 weeks’ gestation) and every 4 weeks until the third trimester and then every 1 to 2 weeks until delivery. At baseline; 20, 28, and 36 weeks; and 6 weeks post partum, maternal venous blood was collected in standard serum separator tubes and stored at −80°C until analysis for angiogenic factors 8 years later. Previous studies have documented the stability of angiogenic factors in specimens stored for >12 years.16 Additional institutional review board approval was obtained to analyze stored specimens for angiogenic factors. Soluble Flt1, sEng, and PlGF were measured using solid-phase, quantitative sandwich ELISA using ELISA kits (R&D Systems Inc, Minneapolis, MN) and following the manufacturer’s instructions. All of the samples were run in duplicate by a single investigator (U.P.), who was blinded to clinical outcomes. A standard curve was constructed using recombinant human sFlt1, sEng, or PlGF, and a curve-fitting software program was used to quantify the concentration of sFlt1, human sEng, or recombinant PlGF. According to the kit manufacturer, the mean minimum detectable dose of sFlt1 is 3.5 pg/mL, and the intra-assay and interassay median coefficients of variation (CVs) were 3.2% and 7.0%, respectively, and in our laboratory the intra-assay and interassay CVs were 2.4% and 10.7%, respectively. The mean minimum detectable dose of sEng is reported as 7.0 pg/mL, and the intra-assay and interassay CVs were 3.0% and 6.5%, respectively; in our laboratory the intra-assay and interassay median CVs were 1.9% and 12.7%, respectively. The mean minimum detectable dose of PlGF is reported as 7.0 pg/mL; the intra-assay and interassay CVs were 5.6% and 11.0%, respectively; and in our laboratory the intra-assay and interassay CVs were 1.6% and 9.7%, respectively.

Pregnancy outcomes were ascertained by detailed chart review of each case shortly after delivery by the principal investigator of the clinical trial, and each patient was given either a diagnosis of SPE or no SPE. We diagnosed SPE if there was a significant increase in blood pressure compared with baseline (≥30 mm Hg systolic, 15 mm Hg diastolic) in association with new-onset proteinuria (either 300 mg per 24 hours or ≥2+ by dipstick on ≥2 occasions). If proteinuria was present at baseline, SPE was diagnosed if there was doubling of urinary protein excretion after 20 weeks’ gestation in association with a significant increase in BP. SPE was also diagnosed if blood pressure was elevated and there were elevated liver enzymes (2 times baseline) and a low platelet count (<100,000). Cases of SPE were classified as early onset (<34 weeks’ gestation) or late onset (≥34 weeks’ gestation).

Statistical Analysis

All of the statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Continuous variables were summarized using mean and SD, and comparisons between groups were made using Student t test or Mann-Whitney test as appropriate for 2-group comparisons and either ANOVA or Kruskal-Wallis test as appropriate for multiple group comparisons. Post hoc testing using a Bonferroni-type adjustment (P<0.01) was used to compare early and late SPE with women with no PE. Categorical variables were summarized using proportions, and comparisons between groups were made using either the χ² test or Fisher exact test, as appropriate. Multivariable modeling using logistic regression along with a backward elimination algorithm was performed to determine whether levels of sFlt1, sEng, or sFlt1/PlGF were independent predictors of developing either early onset or late-onset SPE. All of the P values are 2 tailed, and a P value of <0.05 was considered significant.

Results

We enrolled 110 pregnant women with chronic hypertension in our clinical trial. Stored aliquots of sera were available from all but 1 study participant. The mean ±SD age of the 109 subjects was 33±5.6 years, most were overweight or obese, and ≈40% were black. Forty-one percent were nulliparous, and of the 64 multiparous women, 30% had a history of previous PE. Thirty seven (34%) of the 109 women developed SPE, 9 (8%) of 37 developed early onset (<34 weeks) SPE, and the remaining 28 (25%) developed later onset SPE (after 34 weeks; Table 1). Women who developed early onset SPE were more likely to have secondary hypertension compared with women with later onset SPE or no SPE; 1 had type 1 diabetes mellitus, 1 had lupus with previous nephritis, and 1 had a well-functioning renal allograft. As expected, women with both early onset and late-onset SPE were more likely to have PE in a previous pregnancy compared with women with no SPE (P=0.04). There were no statistically significant differences in age, race, parity, body mass index, blood pressure, or renal function assessed at 12 weeks’ gestation in women who developed early onset, late-onset, or no SPE (Table 1). Women with early onset SPE delivered earlier and had smaller and more growth-restricted infants compared with women with either late or no SPE (P=0.0001; Table 2).

Circulating Levels of Angiogenic Factors in Sequential Serum Samples

Circulating levels of both antiangiogenic (sFlt1 and sEng) and proangiogenic (PlGF) factors were increased during pregnancy in all of the women compared with postpartum
levels (Table 3 and Figures 1 and 2). Calcium therapy had no influence on any of the angiogenic factor levels at any time during pregnancy (data not shown). Baseline levels (obtained between 12 and 14 weeks' gestation) of all of the factors were similar among women with early, late, or no SPE. sFlt1 levels were significantly higher at 20 and 28 weeks in women with early onset SPE compared with women with later onset or no SPE. sFlt1 was significantly higher in women with early onset SPE compared with women with later onset or no SPE. sFlt1 was significantly higher in women with late-onset compared with no SPE only at 36 weeks (Figure 1A and Table 3). sEng levels were higher at 28 weeks in women with early onset SPE compared with those with late or no SPE and were higher at 36 weeks in women with late-onset SPE compared with women without SPE (Figure 1B and Table 3). In contrast to the circulating levels of antiangiogenic sFlt1 or sEng, the levels of proangiogenic PlGF were significantly lower at 28 weeks in women with early onset SPE compared with those without SPE and were also lower at 28 and 36 weeks in women with late-onset SPE compared with no SPE (Figure 2A and Table 3). Angiogenic factor levels measured 6 to 10 weeks postpartum were similar in all of the groups.

We calculated the ratio of sFlt1:PlGF because it captures the reciprocal changes between sFlt1 and PlGF and has been shown to be strongly associated with PE. Values of this ratio were significantly higher at 20 and 28 weeks in women with early onset SPE compared with the late-onset or no SPE groups (Figure 2B and Table 3); the sFlt1/PlGF ratio was also significantly higher at 36 weeks in women with late-onset SPE compared with those with no SPE.

Prediction of SPE

There has been considerable interest in determining whether alterations in angiogenic factors can identify women at increased risk for PE. We reported previously that 3 easily ascertained clinical and laboratory variables (systolic BP, serum uric acid, and plasma renin activity) measured at 20 weeks predict SPE in women with chronic hypertension. We reassessed the ability to predict SPE in this population using our previous prediction model cut points (serum uric acid: >3.6 mg/dL; plasma renin activity: <4 ng/mL per hour; systolic blood pressure: >140 mm Hg) and analysis involving receiver operating characteristic curve showed that the area under the curve was 0.764. Inclusion of angiogenic factor levels in this prediction model and application of a backward elimination algorithm showed that only sFlt1/PlGF improved the predictive accuracy of the model, with the area under the curve increasing from 0.764 to 0.852.

Discussion

We conducted a longitudinal study of pregnant women with chronic hypertension and report that circulating levels of the antiangiogenic factor sFlt1 and the ratio of sFlt1/PlGF were higher at midpregnancy (20 weeks) in women who were subsequently diagnosed with early onset (<34 weeks' gestation) SPE compared with women who did not develop SPE and also compared with those who were diagnosed with late-onset SPE (≥34 weeks' gestation). sFlt1 levels were also higher at 28 weeks in women with early onset SPE and at 36

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early PE (N=9)</th>
<th>Late PE (N=28)</th>
<th>No PE (N=72)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>28.7±4.97†</td>
<td>36.4±3.3†‡</td>
<td>37.5±3.9</td>
<td></td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>1341±715*</td>
<td>2759.2±638‡‡</td>
<td>3031.4±739</td>
<td></td>
</tr>
<tr>
<td>SGA (%)</td>
<td>3/8 (38)§</td>
<td>3/27 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarian section (%)</td>
<td>7/8 (88)</td>
<td></td>
<td></td>
<td>17/27 (63)</td>
</tr>
</tbody>
</table>

PE indicates preeclampsia; early PE, superimposed preeclampsia diagnosed before 34 wk gestation; late PE, superimposed preeclampsia diagnosed at or after 34 wk gestation; no PE, no superimposed preeclampsia; gestational age at delivery; SGA, small for gestational age. Continuous variables are shown as mean±SD. *P<0.0001 vs late PE and no PE. †P<0.002 vs no PE. ‡P<0.017 vs no PE. §P<0.001 vs no PE. ¶P<0.02 vs no PE. **P<0.02 vs no PE.
Table 3. Levels of Angiogenic Factors During Pregnancy by Diagnosis Group and Gestational Age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Early PE (N=9)</th>
<th>Late PE (N=28)</th>
<th>No PE (N=73)</th>
<th>P*</th>
<th>Early PE vs Late PE†</th>
<th>Early PE vs No PE†</th>
<th>Late PE vs No PE†</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 wk (baseline)</td>
<td>n=9</td>
<td>n=23</td>
<td>n=65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sFlt1, pg/mL</td>
<td>2205.3±1169</td>
<td>1471.8±881.4</td>
<td>1834.5±1039.8</td>
<td>0.054</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Endoglin, ng/mL</td>
<td>7.7±2.0</td>
<td>6.7±2.7</td>
<td>7.2±3.3</td>
<td>0.216</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>PlGF, pg/mL</td>
<td>100.8±73.6</td>
<td>74.1±61.3</td>
<td>77.0±56.5</td>
<td>0.796</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>sFlt1/PlGF</td>
<td>35.8±26.5</td>
<td>27.2±18.9</td>
<td>38.4±53.7</td>
<td>0.445</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>PI GF/endoglin</td>
<td>14.4±13.6</td>
<td>11.5±7.5</td>
<td>14.6±21.9</td>
<td>0.996</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>20 wk</td>
<td>n=8</td>
<td>n=24</td>
<td>n=64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sFlt1, pg/mL</td>
<td>5813.6±4899</td>
<td>1426.6±1132.2</td>
<td>2076.0±2452.1</td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Endoglin, ng/mL</td>
<td>25.2±37.5</td>
<td>6.6±4.3</td>
<td>6.3±2.8</td>
<td>0.057</td>
<td>0.039</td>
<td>0.036</td>
<td>0.307</td>
</tr>
<tr>
<td>PlGF, pg/mL</td>
<td>136.3±109.5</td>
<td>228.8±150.2</td>
<td>272.6±169.8</td>
<td>0.045</td>
<td>0.112</td>
<td>0.021</td>
<td>0.236</td>
</tr>
<tr>
<td>sFlt1/PlGF</td>
<td>131.8±192.4</td>
<td>7.4±5.3</td>
<td>10.3±16.5</td>
<td>0.001</td>
<td>0.0007</td>
<td>0.001</td>
<td>0.240</td>
</tr>
<tr>
<td>PI GF/endoglin</td>
<td>26.0±28.5</td>
<td>39.3±21.8</td>
<td>45.4±25.1</td>
<td>0.101</td>
<td>0.122</td>
<td>0.052</td>
<td>0.323</td>
</tr>
<tr>
<td>28 wk</td>
<td>n=8</td>
<td>n=25</td>
<td>n=65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sFlt1, pg/mL</td>
<td>13139±11848</td>
<td>2702.1±3546.2</td>
<td>2176.2±1998.3</td>
<td>0.002</td>
<td>0.002</td>
<td>0.001</td>
<td>0.466</td>
</tr>
<tr>
<td>Endoglin, ng/mL</td>
<td>36.1±35.7</td>
<td>9.3±6.9</td>
<td>7.4±4.0</td>
<td>0.004</td>
<td>0.012</td>
<td>0.001</td>
<td>0.340</td>
</tr>
<tr>
<td>PlGF, pg/mL</td>
<td>247.6±368.9</td>
<td>422.3±272.5</td>
<td>525.2±243.4</td>
<td>0.003</td>
<td>0.038</td>
<td>0.006</td>
<td>0.024</td>
</tr>
<tr>
<td>sFlt1/PlGF</td>
<td>496.7±670.2</td>
<td>9.3±13.1</td>
<td>5.5±6.2</td>
<td>0.007</td>
<td>0.011</td>
<td>0.003</td>
<td>0.368</td>
</tr>
<tr>
<td>PI GF/endoglin</td>
<td>35.5±63.5</td>
<td>62.2±47.0</td>
<td>166.8±667.3</td>
<td>0.001</td>
<td>0.027</td>
<td>0.003</td>
<td>0.018</td>
</tr>
<tr>
<td>36 wk</td>
<td>n=8</td>
<td>n=24</td>
<td>n=58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sFlt1, pg/mL</td>
<td>NC§</td>
<td>8341±6979</td>
<td>4423±2995</td>
<td>0.014</td>
<td>...</td>
<td>...</td>
<td>0.014</td>
</tr>
<tr>
<td>Endoglin, ng/mL</td>
<td>NC</td>
<td>21.90±17.11</td>
<td>12.99±7.79</td>
<td>0.016</td>
<td>...</td>
<td>...</td>
<td>0.016</td>
</tr>
<tr>
<td>PlGF, pg/mL</td>
<td>NC</td>
<td>258.9±142.9</td>
<td>424.1±208.5</td>
<td>0.0003</td>
<td>...</td>
<td>...</td>
<td>0.0003</td>
</tr>
<tr>
<td>sFlt1/PlGF</td>
<td>NC</td>
<td>52.1±79.20</td>
<td>13.96±13.28</td>
<td>0.0001</td>
<td>...</td>
<td>...</td>
<td>0.0001</td>
</tr>
<tr>
<td>PI GF/endoglin</td>
<td>NC</td>
<td>19.65±18.37</td>
<td>74.28±238.0</td>
<td>0.0001</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Postpartum§</td>
<td>n=8</td>
<td>n=21</td>
<td>n=53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sFlt1, pg/mL</td>
<td>223.0±45.96</td>
<td>180.0±52.94</td>
<td>216.9±189.3</td>
<td>0.057</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Endoglin, ng/mL</td>
<td>6.35±1.65</td>
<td>5.71±1.95</td>
<td>5.50±1.33</td>
<td>0.395</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>PlGF, pg/mL</td>
<td>11.15±3.56</td>
<td>11.12±2.70</td>
<td>10.26±2.15</td>
<td>0.553</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>sFlt1/PlGF</td>
<td>21.19±6.05</td>
<td>17.17±6.80</td>
<td>21.84±19.76</td>
<td>0.122</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>PI GF/endoglin</td>
<td>1.81±0.51</td>
<td>2.15±0.89</td>
<td>1.93±0.50</td>
<td>0.556</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Levels of angiogenic factors are expressed as mean±SD. PE indicates preeclampsia; sFlt1, soluble fms-like tyrosine kinase 1; PI GF, placental growth factor; early PE, superimposed preeclampsia diagnosed before 34 wk gestation; late PE, superimposed preeclampsia diagnosed at or after 34 wk gestation; no PE, no superimposed preeclampsia.

*P value was calculated using Kruskal-Wallis test, P<0.05 considered significant.
†P value was calculated using Mann-Whitney test, P<0.01 considered significant.
‡P<0.05.
§NC indicates that specimens were not collected because patients with early PE had already delivered by 36 wk.
¶Postpartum specimens were collected 6 wk after delivery.

weeks in those with late-onset SPE compared with levels at similar gestational ages in those without SPE.

Endoglin is a homodimeric receptor for transforming growth factor-β family members and plays an important role in angiogenesis, hematopoiesis, and cardiovascular development; PI GF is an angiogenic factor that belongs to the vascular endothelial growth factor family. Circulating levels of sEng were higher and PI GF lower when SPE was already diagnosed by clinical criteria in women with early onset and late-onset SPE compared with women without SPE. The ratio of sFlt1/PI GF was higher at the time of clinical diagnosis in both early onset and late-onset SPE, compared with women who never developed SPE. The ratio of PI GF/endoglin was lower at the time of clinical diagnosis in early and late PE compared with women who did not develop SPE.

These alterations in angiogenic factors in women who developed SPE mimic those reported in women who develop PE who were previously normotensive and suggest similarities in the pathogenesis of PE and SPE.14–16,21 Although the alterations in angiogenic factors were more striking in women with early onset compared with late-onset SPE, angiogenic factor alterations were clearly detectable in those with late-onset SPE close to the time of diagnosis. It has been suggested that severe placental dysfunction is a distinguishing feature of early onset PE, whereas women who develop PE close to term are more likely to have maternal predispos-
Figure 1. Circulating levels of soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) in women with early onset superimposed preeclampsia (SPE), late-onset SPE, or no PE. A and B show mean ± SD levels of sFlt1 and sEng during pregnancy and 6 weeks postpartum in women with chronic hypertension with no SPE (light blue broken lines); those with early onset SPE (solid red lines); and those with late-onset SPE (broken green lines). Specimens were collected within a window of 2 weeks before or after the time points on the x axis. Women with early onset SPE did not contribute any specimens at 36 weeks because they had all delivered. A, Levels of sFlt1 were higher in women with early onset SPE at 20 weeks’ (P = 0.001) and 28 weeks’ (P < 0.002) gestation compared with the other groups. sFlt1 levels were lower in women with late-onset SPE (solid red lines); and those with late-onset SPE (broken green lines). Specimens were collected within a window of 2 weeks before or after the time points on the x axis. Women with early onset SPE did not contribute any specimens at 36 weeks because they had all delivered. B, sEng levels were higher in women with early onset SPE at 20 and 28 weeks’ gestation compared with those with no SPE (P = 0.014). B, sEng levels were higher in women with early onset SPE at 20 and 28 weeks’ gestation compared with those with no SPE (P = 0.014).

Figure 2. Levels of placental growth factor (PIGF) and the ratio of soluble fms-like tyrosine kinase 1 (sFlt1) to PIGF in women with early onset superimposed preeclampsia (SPE), late-onset SPE, or no PE. A and B show mean ± SD levels of PIGF and the ratio of sFlt1/PIGF during pregnancy and 6 weeks postpartum in women with chronic hypertension with no SPE (light blue broken lines); those with early onset SPE (solid red lines); and those with late-onset SPE (broken green lines). Specimens were collected within a window of 2 weeks before or after the time points on the x axis. A, Levels of PIGF were lower in women with early onset SPE at 20 and 28 weeks’ gestation compared with those with no SPE (P = 0.021 and P = 0.006, respectively) and were lower at 28 weeks compared with those with late-onset SPE (P = 0.038). PIGF levels were lower in women with late-onset SPE at 36 weeks compared with those with no SPE (P = 0.0003). B, sFlt1/PIGF ratio was higher in women with early onset SPE at 20 and 28 weeks’ gestation compared with those with either late-onset SPE (P = 0.0007 and P = 0.011, respectively) or no SPE (P = 0.001 and P = 0.003). sFlt1/PIGF ratio was higher in women with late-onset SPE at 36 weeks compared with those with no SPE (P = 0.0001).
ing risk factors, such as hypertension, obesity, and diabetes mellitus, and less likely to have clinically apparent placental dysfunction.\textsuperscript{22–26} It has also been suggested that women with chronic hypertension or diabetes mellitus develop PE in the context of smaller increments of sFlt and sEng, because there is already preexisting maternal endothelial cell dysfunction.\textsuperscript{27} Our finding of more significant alterations in angiogenic factors in early versus late-onset SPE are consistent with reports that more severe placental dysfunction distinguishes early onset from late-onset SPE in women without preexisting hypertension. It should be noted that angiogenic factors were clearly altered even in late-onset SPE close to the time of diagnosis, suggesting that placental dysfunction plays a role in both early onset, as well as late-onset, SPE, albeit to a different degree. We did not collect data on angiogenic factors in normotensive pregnant controls, thus we cannot determine whether the levels in chronic hypertensives who did not develop PE were similar to normotensive pregnant women, although results from other published studies suggest that they are.\textsuperscript{17,18} The levels that we report in women who developed SPE are also similar to previous reports in women who developed SPE and who were sampled only twice during pregnancy.\textsuperscript{17} Our study, in which angiogenic factors were measured every 8 weeks and postpartum in the same patients, both confirms and adds to these previous reports.

The 9 women with early onset SPE had different baseline characteristics compared with the other groups. They had slightly lower body mass index, were more likely to have secondary causes of hypertension, and some had baseline proteinuria. Diagnosing PE in women with preexisting hypertension and proteinuria is challenging, thus the elevations in sFlt1, sEng, and sFlt/PlGF that we observed suggest that measurement of these factors might be useful for diagnosing SPE in these women.

There has been considerable interest in identifying biomarkers for the prediction of PE. Although serum levels of angiogenic factors are clearly different in PE compared with normotensive pregnancy at the time of diagnosis, as well as weeks before, whether they have sufficient sensitivity or specificity to be useful in clinical prediction has not been resolved. We performed multivariable modeling to determine predictors of SPE, because we have reported previously that easily ascertained clinical and laboratory measurements (systolic blood pressure, uric acid, and plasma renin activity) obtained at 20 weeks may be useful for prediction.\textsuperscript{20} Although the addition of the ratio of sFlt1/PlGF to the 3-variable model that we reported previously resulted in an increased area under the curve for prediction of early onset PE, our sample size was not large enough to accurately evaluate prediction.

Our study has limitations. More than one third of the women were multigravidas, and some had baseline proteinuria, characteristics known to confound the diagnosis of PE.\textsuperscript{28} However, the accuracy of the diagnosis of SPE is supported by the significant differences in relevant clinical outcomes between women with early SPE and no SPE, as well as differences between women with late SPE and no SPE. Women with both early and late SPE delivered earlier, had smaller infants, had a higher proportion of infants with growth restriction, and more cesarean sections compared with women without SPE (Table 2). Additional studies evaluating the relationship between angiogenic factors and these adverse outcomes would be of interest and might strengthen our finding that angiogenic factor measurements identify women at risk.

Our aim was to gain insight into the pathogenesis of SPE, and we believe our results show that early onset SPE compared with late-onset SPE is associated with more striking alterations in angiogenic factors both before clinically apparent disease and at the time of diagnosis, similar to observations in early onset PE in women who were normotensive before pregnancy.\textsuperscript{18–25,29} Although small sample size is another limitation of our study, management and follow-up were standardized, PE was diagnosed in all of the cases by 1 investigator (P.A.), and data collection was complete, with almost all of the subjects contributing both clinical and laboratory information at all of the time points. Our findings provide insight into pathogenesis of SPE, and we suggest that the utility of measuring angiogenic factors to predict and/or diagnose SPE requires further validation and is of potential clinical significance.

**Perspectives**

Women with chronic hypertension are at increased risk for developing SPE, a condition with significant maternal and fetal morbidity and mortality. Altered levels of circulating angiogenic factors have been reported in women with PE who were previously normotensive and also in cross-sectional studies of women with chronic hypertension in pregnancy. In a longitudinal prospective study of women with chronic hypertension, we observed similar alterations in angiogenic factors before and at the time of diagnosis in women with SPE. Our findings suggest similarities in the pathogenesis of PE and SPE. Measurement of sFlt1 and the ratio of sFlt1/PlGF may be useful diagnostic tests in women with chronic hypertension, particularly those with baseline proteinuria or secondary hypertension in whom recognizing PE is often more challenging.

**Sources of Funding**

This work was supported by National Institutes of Health grant R01 HL 488-46.

**Disclosures**

None.

**References**


Angiogenic Factors in Superimposed Preeclampsia: A Longitudinal Study of Women With Chronic Hypertension During Pregnancy
Uma Perni, Cristina Sison, Vijay Sharma, Geri Helseth, Amret Hawfield, Manikkam Suthanthiran and Phyllis August

*Hypertension*. 2012;59:740-746; originally published online February 6, 2012; doi: 10.1161/HYPERTENSIONAHA.111.181735

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/59/3/740

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/