Prospective Study of Placental Angiogenic Factors and Maternal Vascular Function Before and After Preeclampsia and Gestational Hypertension

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Background—Preeclampsia is a life-threatening pregnancy syndrome of uncertain origin. To elucidate the pathogenesis, we evaluated the temporal relationships between changes in vascular function and circulating biomarkers of angiogenic activity before and after the onset of preeclampsia and gestational hypertension.

Methods and Results—Maternal mean arterial pressure, uterine artery pulsatility index, brachial artery flow-mediated dilatation, and serum concentrations of placental growth factor (PIGF), soluble fms-like tyrosine kinase 1 (sFlt-1), and soluble endoglin were prospectively measured in 159 women from 10 weeks gestation until 12 weeks postpartum. At 10 to 17 weeks, women who developed preterm preeclampsia had lower serum PIGF (P=0.003), higher soluble endoglin (P=0.006), and higher sFlt-1:PIGF ratio (P=0.005) compared with women who later developed term preeclampsia, gestational hypertension, or normotensive pregnancy. At 10 to 17 weeks, mean arterial pressure inversely correlated with serum PIGF (r=−0.19, P=0.02); at 18 to 25 weeks, with soluble endoglin (r=0.18, P=0.02); and at 26 to 33 weeks, with sFlt-1 (r=0.28, P<0.001). At 23 to 25 weeks, uterine artery pulsatility index correlated with serum soluble endoglin (r=0.19, P=0.02) and sFlt-1 levels (r=0.17, P=0.03). Flow-mediated dilatation was higher during a pregnancy with gestational hypertension compared with preeclampsia (P=0.001). Twelve weeks postpartum, serum PIGF was higher in women who had a hypertensive pregnancy compared with a normotensive pregnancy (P<0.001).

Conclusions—These observations support a role for placenta-derived angiogenic biomarkers in the control of maternal vascular resistance of preeclampsia. Gestational hypertension develops differently, with a hyperdynamic circulation and angiogenic biomarker profile similar to normotensive pregnancy. Larger studies of unselected women are needed to ascertain whether measures of these angiogenic biomarkers assist with the prediction and prognosis of preeclampsia and whether postpartum measures of serum PIGF have a role in predicting future cardiovascular disease. (Circulation. 2010;122:478-487.)

Key Words: angiogenesis ● endothelium ● hypertension ● preeclampsia ● pregnancy

Preeclampsia is a multisystem disorder of pregnancy defined by the gestational onset of hypertension and proteinuria and poses a higher risk of future cardiovascular disease. Approximately 2% to 4% of first-time pregnancies in North America, Europe, and Australia are affected by preeclampsia, and a similar number of women develop high blood pressure without proteinuria during pregnancy, called gestational hypertension. In the United States, the incidence rates of both preeclampsia and gestational hypertension have increased over the last 20 years. These disorders of pregnancy account for between 15% and 25% of all maternal deaths, with the highest incidence in Latin America and the Caribbean. Both preeclampsia and gestational hypertension usually result in childbirth at term and offspring of normal birth weight. One in 200 pregnancies is complicated by preterm preeclampsia, which in the developed world is responsible for the majority of preeclampsia-related maternal and fetal morbidity and mortality.

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Prepregnancy risk factors for preeclampsia include nulliparity and risk factors for cardiovascular disease such as chronic hypertension, obesity, preexisting diabetes mellitus, hyperlipidemia, renal disease, and advanced maternal age, which may explain the strong association between the 2 disorders. However, most women with these risk factors do not develop preeclampsia, and many more women without these risk factors suffer preeclampsia, which has made it a
challenging condition to predict. Poor implantation of the placenta can precede the clinical onset of preeclampsia and is associated with an increase in uterine artery resistance that can be recognized by Doppler ultrasound. Furthermore, preeclampsia, like cardiovascular disease, is characterized by widespread maternal endothelial dysfunction. Despite these observations, the pathophysiological basis of preeclampsia remains uncertain, compromising the development of preventive interventions.

A pathogenic role for the angiogenic factors soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) has been demonstrated in pregnant animals. Adenovirus expressing sFlt-1 and given to pregnant rats causes a preeclampsia-like syndrome, exacerbated by the coadministration of sEng.

A rise in sFlt-1 and sEng and a reduction in placental growth factor (PlGF) have also been demonstrated in maternal serum 5 to 10 weeks before the onset of preeclampsia. It has been proposed that these changes may contribute to maternal endothelial dysfunction, but it remains unclear whether alterations in these factors contribute causally or are a consequence of preeclampsia. Furthermore, earlier observational studies have been retrospective, cross-sectional, or prospective but only from midpregnancy, making it uncertain whether these factors are altered earlier in pregnancy when the processes leading to preeclampsia are likely to be initiated. Moreover, no study has yet evaluated the relationship between angiogenic factors and maternal endothelial function.

We therefore studied prospectively from early pregnancy the sequence of changes in maternal circulating concentrations of PlGF, sEng, and sFlt-1 and their relationship to maternal blood pressure and endothelial function, as well as uterine artery blood flow. We reexamined these women 12 weeks postpartum to identify residual differences that might inform us about the mechanism linking preeclampsia to increased risk of future cardiovascular disease.

Methods

Recruitment of Subjects

The study was approved by the local Research Ethics Committee, and all participants gave written informed consent. During the first and early second trimester, 163 pregnant women were recruited from a single antenatal service (Chelsea and Westminster Hospital, London, UK). The majority of women were recruited when attending for a routine early pregnancy scan, but a proportion of women were actively recruited from an obstetric medicine clinic to enrich the cohort for women at risk of preeclampsia. Midwives gave all attending women an information sheet describing the study. Women who expressed an interest in participating were given further information (by M.N., who also obtained consent). Four subjects with twin pregnancies were excluded from the final analysis. Forty-five of the remaining 159 women had ≥1 of the following risk factors for preeclampsia: preexisting high blood pressure (n = 128), isolated gestational hypertension (n = 10), or preexisting diabetes mellitus (n = 4), thrombophila caused by factor V Leiden heterozygosity (n = 3) and homozgyosity (n = 1), previous fetal growth restriction (n = 1), and polycystic ovarian syndrome (n = 1).

At each visit, blood pressure was measured with an automated device (Omron MIT, Kyoto, Japan) validated for use in pregnancy, and body mass index (BMI) was calculated from measures of height and weight. Serum and plasma were prepared from venous whole blood, frozen, and stored anonymized at −70°C until analyzed. Large-artery endothelial function was serially assessed during pregnancy from 10 to 17 weeks gestation with ultrasound measurement of brachial artery flow-mediated dilatation (FMD) a median of 5.0 (interquartile range [IQR], 2 to 6) occasions and again at 12 weeks (IQR, 11 to 16 weeks) postpartum. Between 23 and 25 weeks gestation, uterine artery blood flow was measured by Doppler ultrasound. Blood samples were taken at 10 to 17 weeks (n = 150), 18 to 25 weeks (n = 158), 26 to 33 weeks (n = 157), 34 to 40 weeks (n = 137), and 11 to 16 weeks postpartum (n = 102). Gestational age was calculated from the 12-week dating scan, and gestational ranges were categorized according to completed weeks.

Preeclampsia was defined as new onset of a blood pressure >140/90 mm Hg on 2 separate occasions at least 4 hours apart, accompanied by proteinuria ≥300 mg/24 h or a ratio of urine protein to creatinine ≥30 mg/mmol, or ≥2 + on dipstick, in the absence of a urinary tract infection. Preeclampsia was defined as preterm if it developed up to and included 36 + 6 weeks. Gestational hypertension was defined as new-onset blood pressure >140/90 mm Hg without proteinuria after 20 weeks gestation.

Postpartum, two thirds of subjects (102 of 159) returned for further assessment. They were studied at a median of 12 weeks postpartum (IQR, 11 to 16 weeks). Of these women, 16 had developed preeclampsia (6 had preterm preeclampsia and 10 had term preeclampsia), 7 had gestational hypertension, and the remaining 79 were normotensive. All 102 subjects consented to a blood test, but 12 women declined further FMD assessment. There was no significant difference in recorded maternal phenotype at entry to the trial (10 to 17 weeks) between the 102 women who returned for follow-up and the 57 women who did not.

Assessment of Uterine Artery Blood Flow

Uterine artery Doppler velocimetry was assessed between 23 and 25 weeks gestation by a single operator (M.N.). An image of the uterine artery was obtained at the level at which it crosses the external iliac artery with a 3.5-MHz transducer with an angle correction of <60°. The pulsatility index (PI; a measure of diastolic flow) over 2 waves was calculated for each uterine artery. A mean PI was then calculated by averaging the PIs of each side.

Flow-Mediated Dilatation

Brachial artery FMD is a reliable measure of endothelial function. FMD was assessed serially an average of 5.0 (IQR, 2 to 6) times in 159 women during pregnancy and in 90 women 12 weeks postpartum. FMD was measured by a single operator (M.N.) in a quiet, temperature-controlled room in accordance with previously reported protocols.

Assays of Angiogenic Factors

PlGF, sFlt-1, and sEng were measured with commercially available ELISA kits (R&D Systems, Minneapolis, MN) in accordance with supplied protocols. The lowest limits of detection for PlGF, sFlt-1, and sEng for the kits used were 7, 3.5, and 7 pg/mL, respectively. When a sample level was undetectable, the lowest detectable value for that assay was assigned to that sample. All assays were performed by a single investigator (M.N.) and were analyzed blindly and in duplicate.

Statistical Analyses

Comparison at 10 to 17 weeks between characteristics of women considered at high or low risk for preeclampsia was made with an unpaired t test and χ² test. Subjects were also grouped according to pregnancy outcome into those who developed preterm preeclampsia (<37 weeks; n = 10), term preeclampsia (≥37 weeks; n = 11), or isolated gestational hypertension (n = 10) and those who remained normotensive (n = 128). Differences in maternal phenotype at 10 to 17 weeks gestation, according to pregnancy outcome group, were assessed with either 1-way ANOVA or the Fisher exact test.

For comparison of groups at a single time point (at 10 to 17, 23 to 25, or 12 weeks postpartum), cross-sectional analyses were per-
Table 1. Characteristics at 10 to 17 Weeks’ Gestation and Pregnancy Outcome for Women With and Without Risk Factors for Preeclampsia

<table>
<thead>
<tr>
<th></th>
<th>Low Risk (n=114)</th>
<th>High Risk (n=45)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.8±0.5</td>
<td>33.9±0.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Nulliparous, n (%)</td>
<td>95 (83)</td>
<td>19 (42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>80±1</td>
<td>90±2</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4±0.37</td>
<td>27.0±0.84</td>
<td>0.01</td>
</tr>
<tr>
<td>Normotensive pregnancy, n (%)</td>
<td>107 (93.8)</td>
<td>21 (46.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational hypertension, n (%)</td>
<td>1 (0.9)</td>
<td>9 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Term preeclampsia, n (%)</td>
<td>2 (1.8)</td>
<td>9 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Preterm preeclampsia, n (%)</td>
<td>4 (3.5)</td>
<td>6 (13.3)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are presented as a percentage of the cohort population or as mean±SEM. Comparisons used either an unpaired t test or χ² test.

formed with GraphPad Prism 5.0. Normally distributed data were compared by use of 1-way ANOVA, and data are presented as mean±SEM. Nonnormally distributed data were logarithmically transformed to normalize the data and analyzed by use of 1-way ANOVA. In all ANOVA analyses, the Bonferroni method was used to correct for multiple comparisons. Data were then back-transformed and expressed as geometric mean values and approximate SEMs, with a value of P<0.05 considered significant.

Longitudinal comparisons between the 4 pregnancy outcome groups were made across 3 or 4 time points from 10 to 17 weeks gestation until the end of pregnancy. Data were analyzed with STATA version 10 (Stata Corp, College Station, Tex). A growth curve model was fitted (using an “xtmixed” command). The 2 model components included fixed effects (time-dependent variables [polynomial functions of gestation]) and a clinical diagnosis variable, as well as random effects (random intercept and random slope terms). The polynomial order functions were determined by likelihood ratio test or χ² test.

The final analysis included 159 women. Fifteen of 45 women (33%) with preeclampsia risk factors (see Methods) developed preeclampsia (6 preterm preeclampsia and 9 term preeclampsia) compared with 6 of 114 women (5%) without risk factors (4 preterm preeclampsia and 2 term preeclampsia; Table 1). Of the remaining subjects with risk factors, 9 (20%) developed gestational hypertension, and 21 (47%) had a normotensive pregnancy. Of the remaining women without risk factors who did not develop preeclampsia, 1 developed gestational hypertension and 107 (94%) remained normotensive (Table 1).

At 10 to 17 weeks gestation, women with preexisting risk factors for preeclampsia had a higher MAP (P=0.01) and BMI (P=0.01) and were less likely to be nulliparous (P<0.001) compared with those without risk factors for preeclampsia (Table 1).

Maternal phenotype according to pregnancy outcome is recorded in Table 2. Longitudinal comparison of women between 10 and 40 weeks gestation showed that those who developed preterm preeclampsia or gestational hypertension had a greater BMI than those who remained normotensive (BMI, +4.8 kg/m² [95% CI, 2.0 to 7.6; P=0.001] and +3.5 kg/m² [95% CI, 0.8 to 6.1; P=0.009], respectively), whereas women who developed term preeclampsia had a BMI similar

Table 2. Subject Characteristics According to Pregnancy Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Normotensive Hypertension</th>
<th>Term Preeclampsia</th>
<th>Preterm Preeclampsia</th>
<th>P, ANOVA/Fisher Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>128</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>32.7 (4.9)</td>
<td>33.2 (7.1)</td>
<td>35.6 (1.0)</td>
<td>35.9 (3.0)</td>
</tr>
<tr>
<td>Nulliparous, n (%)</td>
<td>95 (74)</td>
<td>6 (60)</td>
<td>7 (64)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>MAP at 10–17 wk, mm Hg</td>
<td>81 (1)</td>
<td>92 (3)</td>
<td>94 (4)</td>
<td>92 (4)</td>
</tr>
<tr>
<td>Postpartum MAP, mm Hg</td>
<td>83 (1)</td>
<td>95 (4)</td>
<td>101 (5)</td>
<td>99 (4)</td>
</tr>
<tr>
<td>BMI at 10–17 wk, kg/m²</td>
<td>24.6 (0.4)</td>
<td>28.1 (1.5)</td>
<td>25.8 (2.1)</td>
<td>28.5 (1.9)</td>
</tr>
<tr>
<td>Postpartum BMI, kg/m²</td>
<td>24.6 (0.4)</td>
<td>27.4 (1.9)</td>
<td>25.6 (2.5)</td>
<td>26.8 (2.1)</td>
</tr>
<tr>
<td>Gestation at delivery, d</td>
<td>277 (1.6)</td>
<td>273 (3.8)</td>
<td>268 (2.9)</td>
<td>214 (11.2)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3346 (49)</td>
<td>3168 (185)</td>
<td>3144 (146)</td>
<td>1247 (275)</td>
</tr>
<tr>
<td>Birth centile</td>
<td>46.1 (2.6)</td>
<td>35.3 (10.8)</td>
<td>47.6 (11.0)</td>
<td>6 (3.3)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>8 (6)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Ethnicity, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>107</td>
<td>9</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Black/Caribbean</td>
<td>14</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Asian/Indian</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are given as mean (SEM). Comparisons were made with ANOVA or the Fisher exact test.
to that of women who remained normotensive (+0.88 kg/m²; 95% CI, −1.7 to 3.4; P = 0.5).

**Mean Arterial Pressure**

At 10 to 17 weeks gestation, MAP was elevated in women who later developed preterm preeclampsia, term preeclampsia, or gestational hypertension compared with women who remained normotensive (P < 0.001). The P value was derived from a linear mixed-effects model as described in Methods.

**Uterine Artery Blood Flow**

At 23 to 25 weeks gestation, women who developed preterm preeclampsia had a higher uterine artery PI (1.5 ± 0.2, mean ± SEM) compared with those who developed term preeclampsia (PI, 0.9 ± 0.1) or gestational hypertension (PI, 0.9 ± 0.1) or were normotensive (PI, 0.8 ± 0.3; P < 0.001; Figure 2).

**Brachial Artery FMD**

At 10 to 17 weeks, women who later developed gestational hypertension had a higher brachial artery FMD (12.9 ± 0.98%) compared with those who developed preterm preeclampsia (12.93 ± 0.98%) or term preeclampsia (8.48 ± 1.42%) and term preeclampsia (8.84 ± 1.52%; P = 0.04; Figure 3). Longitudinal comparison between 10 to 40 weeks gestation showed that the brachial artery FMD of women who developed gestational hypertension was 4.5% higher (95% CI, 1.64 to 6.46) during pregnancy compared with those who developed preeclampsia (preterm and term preeclampsia combined; P = 0.001; Figure 3).

Longitudinal analysis also showed that brachial artery FMD was lower in women who later developed preeclampsia (preterm and term preeclampsia combined) compared with those who did not (−1.7%; 95% CI, −3.3 to −0.13; P = 0.03; Figure 3). Women who remained normotensive had an intermediate level of FMD between those who later developed gestational hypertension and those who developed preeclampsia (Figure 3).

**Placental Growth Factor**

At 10 to 17 weeks gestation, women who went on to develop preterm preeclampsia had lower serum PlGF levels (18.9 ± 4.4 pg/mL, geometric mean ± SEM) compared with those who developed term preeclampsia (37.9 ± 4.5 pg/mL) or gestational hypertension (47.0 ± 10.4 pg/mL) and those who had a normotensive pregnancy (47.9 ± 2.6 pg/mL; ANOVA P < 0.001; Figure 4A). Longitudinal analysis showed that between 10 to 40 weeks gestation, maternal serum PlGF levels were 132% lower (95% CI, −165 to −99) in women who developed preeclampsia compared with those who did not develop gestational hypertension or gestational hypertension (P < 0.001; Figure 4A). Longitudinal analysis showed that maternal serum PlGF levels were 132% lower (95% CI, −165 to −99) in women who later developed preeclampsia compared with those who did not (P = 0.01).
who developed preterm preeclampsia compared with those who remained normotensive (P<0.001). Women who developed term preeclampsia and gestational hypertension also had lower serum levels of PlGF compared with those who remained normotensive, but these differences did not reach statistical significance (19% lower; 95% CI, 45 to 6; P=0.13; and 21% lower; 95% CI, 45 to 27; P=0.08, respectively; Figure 5A).

Soluble fms-Like Tyrosine Kinase 1
At 10 to 17 weeks, there was no significant difference in maternal serum sFlt-1 levels between all groups of women studied (preterm preeclampsia, 2414±622 pg/mL; geometric mean±SEM; term preeclampsia, 1688±210 pg/mL; gestational hypertension, 2219±349 pg/mL; normotensive pregnancy, 1729±85 pg/mL; ANOVA P=0.17; Figure 4B). Longitudinal analysis between 10 to 40 weeks gestation showed that women who developed preterm and term preeclampsia had higher circulating sFlt-1 levels compared with those who remained normotensive, 67% higher (95% CI, 35% to 99%; P<0.001) and 25% higher (95% CI, 2% to 49%; P=0.03), respectively. Women who developed gestational hypertension had levels of sFlt-1 similar to those who remained normotensive (20% higher; 95% CI, −6 to 46; P=0.13; Figure 5B).

sFlt-1:PlGF Ratio
At 10 to 17 weeks, the ratio of sFlt-1:PlGF was higher in women who later developed preterm preeclampsia (128.1±43.9, geometric mean±SEM) compared with all other groups (term preeclampsia, 9.1±0.7; gestational hypertension, 47.1±12.6; and normotensive pregnancy, 36.1±2.2; ANOVA P<0.001; Figure 4C). Longitudinal analysis between 10 to 40 weeks gestation showed that women who developed preterm preeclampsia had an sFlt-1:PlGF ratio that was 205% higher (95% CI, 158 to 251; P<0.001) compared with normotensive subjects. The sFlt-1:PlGF ratio was similar between women who developed term preeclampsia (11% lower; 95% CI, −48 to 27; P=0.58) or gestational hypertension (8.8% higher; 95% CI, −32 to 49; P=0.67) compared with those who remained normotensive (Figure 5C).

Soluble Endoglin
At 10 to 17 weeks gestation, maternal serum sEng levels were higher in women who later developed preterm preeclampsia (8553±1083 pg/mL, geometric mean±SEM) compared with all other groups (term preeclampsia, 5699±395 pg/mL; gestational hypertension, 5920±499 pg/mL; and normotensive pregnancies, 5177±142 pg/mL; ANOVA P<0.001; Figure 4D). Longitudinal analysis between 10 and 40 weeks gestation showed that women who developed preterm and
term preeclampsia had higher circulating maternal serum sEng levels compared with those who remained normoten-
sive, 79% higher (95% CI, 59 to 99; P = 0.001) and 25% higher (95% CI, 10 to 40; P = 0.001), respectively (Figure 5D). Women who developed gestational hypertension had serum sEng levels similar to those who remained normoten-
sive (11% higher; 95% CI, 3 to 25; P = 0.11; Figure 5D).

**Whole-Cohort Correlations Between Hemodynamic Measures and Angiogenic Factors**

At 10 to 17 weeks (n = 156), there was a weak inverse correlation between maternal MAP and serum PlGF levels (r = −0.19, P = 0.02) but no significant correlation between MAP and serum sFlt-1 or sEng levels. At 18 to 25 weeks (n = 159), the inverse correlation between maternal MAP and serum PlGF levels persisted (r = −0.17, P = 0.03), and a weak correlation between MAP and sEng became evident (r = 0.18, P = 0.02). At this gestation, there was no correlation between MAP and sFlt-1 or sFlt-1:PlGF ratio. At 26 to 33 weeks (n = 159), a correlation between MAP and maternal serum sFlt-1 and sFlt-1:PlGF ratio became evident (r = 0.28, P = 0.0003; and r = 0.29, P = 0.0003, respectively). The correlations between MAP and serum levels of PlGF and sEng persisted (r = −0.22, P = 0.006; and r = 0.17, P = 0.03, respectively). At 34 to 40 weeks (n = 139), correlations between maternal MAP and all 3 angiogenic biomarkers were strongest (MAP versus sFlt-1, r = 0.32, P = 0.0001; versus sEng, r = 0.29, P = 0.0005; versus PlGF, r = −0.22, P = 0.008; and versus sFlt-1:PlGF ratio, r = 0.33, P < 0.0001). There was no correlation between maternal serum levels of sFlt-1, sEng, or PlGF and brachial artery FMD at any time point during pregnancy. At 23 to 25 weeks gestation, uterine artery PI correlated with MAP (r = 0.27, P < 0.001) and weakly with maternal serum sEng levels (r = 0.19, P = 0.02) and sFlt-1 (r = 0.17, P = 0.03) but not with PlGF.

**Postpartum MAP, Endothelial Function, and Maternal PlGF, sFlt-1, and sEng Levels**

At 12 weeks postpartum (IQR, 6 to 32 weeks), cross-sectional analysis showed that women who had preterm preeclampsia (MAP, 99 ± 4 mm Hg, mean ± SEM), term preeclampsia (MAP, 100 ± 5 mm Hg), or gestational hypertension (MAP, 95 ± 4 mm Hg) continued to have a higher MAP than women who had completed a normotensive pregnancy (MAP, 84 ± 1 mm Hg; ANOVA P < 0.001). Women who had a normotensive pregnancy showed a fall in serum levels of
PIGF, sFlt-1, and sEng from their highest levels in the third trimester to their lowest levels 12 weeks postpartum. There was an ≈50-fold fall in PIGF concentration (424.9±24.0 to 8.1±0.17 pg/mL, geometric mean±SEM), a 25-fold fall in sFlt-1 levels (6721.1±461.8 to 264.2±11.6 pg/mL), and a 2.5-fold fall in sEng levels (10993±572 to 4256±72 pg/mL) from the third trimester to postpartum.

Contrary to observations during pregnancy, cross-sectional analysis showed that postpartum maternal serum PIGF levels were higher in women who had preterm preeclampsia (11.56±0.61 pg/mL), term preeclampsia (11.99±1.59 pg/mL), and gestational hypertension (10.77±0.06 pg/mL) compared with women who had a normotensive pregnancy (8.10±0.17 pg/mL; P=0.005; Table 3). There were no significant differences in postpartum serum levels of sEng, sFlt-1, or the sFlt-1:PIGF ratio between groups (Table 3).

Postpartum endothelial function, as measured by brachial artery FMD, appeared to be lower in women who had preterm preeclampsia (FMD, 7.3±2.6%) compared with those who had term preeclampsia (9.70±3.1%), gestational hypertension (9.99±3.1%), or normotensive pregnancies (9.64±0.6%), although these differences did not reach statistical significance.

**Additional Analyses**

Subgroup analysis of the 21 women who developed preeclampsia (preterm and term preeclampsia combined) showed an inverse correlation between MAP at 10 to 17 weeks and serum sFlt-1 levels at the time of preeclampsia onset (r=−0.563; 95% CI, −0.805 to −0.161; P=0.008; Figure 6). There was a weak inverse correlation between sEng and MAP at 10 to 17 weeks gestation (r=−0.443; 95% CI, −0.741 to −0.0004; P=0.044), but after removal of a single outlying data point, significance was lost (r=−0.366; 95% CI, −0.7029 to 0.1057; P=0.113). There was no correlation between 10- to 17-week MAP and serum PIGF levels or the sFlt-1:PIGF ratio at the time of preeclampsia diagnosis.

At 10 to 17 weeks gestation, women with preeclampsia risk factors (high-risk group; n=45) who developed either preeclampsia or gestational hypertension (n=24) had a higher MAP (97±2 mm Hg, mean±SEM) compared with those who went on to have a normotensive pregnancy (n=21; MAP, 84±2 mm Hg; P<0.001). At 10 to 17 weeks, women in the low-risk group who developed either preeclampsia or gestational hypertension (n=7) had an MAP of 84±4 mm Hg compared with 80±1 mm Hg for those who remained normotensive (n=107; P=0.29).

**Discussion**

This prospective study of pregnant women provides the first demonstration of the temporal sequence of changes in maternal vascular function and circulating levels of PIGF, sFlt-1, and sEng in women who later develop preeclampsia and gestational hypertension and the relationship between these measures. We support the previously described association between chronic hypertension and risk of preeclampsia and gestational hypertension. In our cohort, 14 of 31 women who developed either preeclampsia or gestational hypertension had a history of chronic hypertension. In early pregnancy, however, chronic hypertension can be masked by gestational vasodilatation. At 10 to 17 weeks, women who went on to develop preeclampsia or gestational hypertension had a blood pressure of 127/76±3/3 mm Hg (mean±SEM) compared with 112/65±10/9 mm Hg for those who had a normotensive pregnancy. A blood pressure of 127/76 mm Hg is often regarded as normal by midwives and obstetricians. Within the high-risk cohort, blood pressure at 10 to 17 weeks appeared to be the single most powerful clinical risk factor for preeclampsia or gestational hypertension, but alone it did not discriminate between the type of hypertensive pregnancy. Blood pressure outside of pregnancy has a continuous graded association

![Figure 6. Correlation between mean arterial blood pressure (MAP) at 10 to 17 weeks and maternal serum sFlt-1 concentrations at the time of preeclampsia onset. MAP at 10 to 17 weeks was inversely correlated with sFlt-1 (r=−0.563; 95% CI, −0.805 to −0.161; P=0.008).](image-url)
with future cardiovascular risk down to a blood pressure of as low as 115/75 mm Hg. The same graded association appears to be evident between blood pressure and preeclampsia risk in pregnancy.

Women with preexisting vascular diseases are predisposed to preeclampsia, and reduced endothelial function is a well-recognized feature of established preeclampsia. In our cohort of women, endothelial function, as measured by brachial artery FMD, was reduced in those who went on to develop term preeclampsia and more markedly reduced in those who developed preterm preeclampsia. However, we found that women who went on to develop gestational hypertension had higher values of FMD compared with women who had a normotensive or preeclamptic pregnancy. A high cardiac output has previously been observed in women with obesity and gestational hypertension and may be secondary to the hyperdynamic circulation associated with insulin resistance. Our finding of elevated FMD in women with gestational hypertension is compatible with these observations.

Microalbuminuria is also a marker of endothelial dysfunction, which is evident in women at risk of preeclampsia. Healthy renal glomeruli have a fenestrated endothelium that is maintained by vascular endothelial growth factor. During pregnancy, inhibition of vascular endothelial growth factor by sFlt-1 results in disruption of fenestrated endothelium and proteinuria. Compatible with this observation, we show that women with preeclampsia have a greater rise in sFlt-1 levels than women who have gestational hypertension or a normotensive pregnancy. However, toward the end of pregnancy, there is a rise in sFlt-1 levels in all groups, including normotensive pregnant women, which may contribute to the rise in albuminuria seen in healthy pregnancy.

Gestational proteinuria has also been associated with reduced serum levels of PIGF. In our cohort, however, women who developed term preeclampsia had higher serum levels of PIGF than those who developed gestational hypertension and who had no proteinuria. It is possible that the relative balance between vascular endothelial growth factor and sFlt-1 is more influential than serum PIGF levels alone in maintaining the integrity of glomerular endothelium.

Healthy placental development involves rapid angiogenesis, which appears to be partly driven by PIGF. Poor placental development is associated with a reduced concentration of PIGF in maternal serum. At 10 to 17 weeks, we found that maternal MAP inversely correlated with PIGF at a time when there was no such correlation with the circulating antiangiogenic factors sFlt-1 and sEng. It is therefore possible that prepregnancy maternal hypertension has a negative impact on placental development and PIGF expression. Low PIGF levels at 11 to 13 weeks have been shown in a case-control study to predict early- and late-onset preeclampsia. Our study supports a role for low PIGF levels in the early pathogenesis of preeclampsia.

sFlt-1 acts as a circulating antiangiogenic factor by binding PIGF and vascular endothelial growth factor. We showed that in early pregnancy women who later developed preeclampsia had a higher blood pressure and depressed FMD before a rise in circulating sFlt-1 levels. Not until 26 to 33 weeks did serum sFlt-1 levels correlate with MAP. It is possible therefore that impaired endothelial function represents a baseline risk factor for preeclampsia rather than developing as a consequence of elevated sFlt-1 levels, as has been proposed previously. In support of this suggestion, we show that women with higher MAPs in early pregnancy develop preeclampsia with lower serum concentrations of sFlt-1 and possibly sEng. Women with preexisting endothelial dysfunction may therefore be vulnerable to preeclampsia because they are more sensitive to a secondary rise in sFlt-1 and sEng. Administration of sFlt-1 and sEng to pregnant rats has also been shown to cause a preeclampsia-like syndrome with vasoconstriction of renal microvessels. Taken together, these observations support a role for sFlt-1 and possibly sEng in resistance vessel dysfunction during pregnancy.

In the whole cohort, the correlation between all 3 placental-derived angiogenic biomarkers and MAP became stronger as pregnancy progressed from 10 to 17 weeks through to 34 to 40 weeks of gestation. Such correlations support a progressively influential role for PIGF, sFlt-1, and sEng in the control of maternal vascular tone during pregnancy. This observation may explain the usually subclinical third-trimester rise in MAP.

In women who developed term preeclampsia, we showed a late rise in maternal sFlt-1 levels that in our prospectively followed cohort was balanced by a higher level of PIGF. This compensatory rise in PIGF and presumably placental development may explain why fetal growth is often preserved in women with term preeclampsia.

We observed that sEng, another soluble antiangiogenic factor, was elevated early in those pregnancies that resulted in preterm preeclampsia and preceded the rise in serum sFlt-1. Furthermore, a correlation between MAP and sEng preceded a similar correlation between sFlt-1 and MAP. sEng binds transforming growth factor-β in the circulation and prevents it from stimulating its diverse roles on the vascular system and immunity. We did not measure maternal serum transforming growth factor-β activity, but if bound to circulating sEng and prevented from stimulating membrane bound endoglin on placental tissues, it has been proposed that this could compromise placental growth and angiogenesis.

At 23 to 25 weeks, we found that maternal MAP correlated with uterine artery resistance and serum sFlt-1 and sEng levels, suggesting an interrelationship between these 4 factors. It is possible that these placenta-derived antiangiogenic factors antagonize the healthy gestational development of a low-resistance uteroplacental arterial system. Maintenance of high uterine artery resistance often precedes the clinical onset of preeclampsia, as in our cohort. Rising sFlt-1 and sEng concentrations in maternal serum may then aggravate preexisting maternal endothelial dysfunction, leading to hypertension and preeclampsia. The absence of a correlation between sFlt-1, sEng, and PIGF with maternal endothelial function, as measured by brachial artery FMD, may reflect different effects of these circulating factors on large as opposed to resistance arteries in pregnant women.

In our study, the calculated ratio of sFlt-1:PIGF in women destined to develop preterm preeclampsia was markedly elevated throughout pregnancy compared with all other groups. The present study was selectively enriched for women with a high
risk of preeclampsia and was neither designed nor powered to assess the performance of these measures in the prediction of preeclampsia. In our cohort, however, at 10 to 17 weeks, no women (of 142) who had a maternal serum sFlt-1:PlGF ratio <150 (arithmetic mean) developed preterm preeclampsia, whereas at 24 weeks gestation, no women (of 146) with an sFlt-1:PlGF ratio <50 (arithmetic mean) developed preterm preeclampsia. Larger prospective studies in unselected pregnancies are needed to critically evaluate the ability of these markers to discriminate women destined for a normotensive pregnancy from those at risk of preeclampsia.

Postpartum, maternal systolic blood pressure remained 10 to 15 mm Hg higher in all women who had a hypertensive pregnancy. In those who had preterm preeclampsia, we also demonstrated persistent postpartum endothelial dysfunction, confirming the observation of others and strengthening the association between preeclampsia and future cardiovascular disease. The similarity between early pregnancy and postpartum hemodynamic observations supports the notion that maternal cardiovascular differences predate gestational differences in placental angiogenic factors.

Contrary to observations during pregnancy, we observed that postpartum circulating levels of PlGF were higher in all groups of women who had a hypertensive pregnancy. PI not only promotes angiogenesis, an important function in pregnancy, but also stimulates atherosclerotic intimal thickening. In the Nurses’ Health Study, elevated PlGF levels were associated with an increased risk of coronary heart disease >10 years after a baseline test in asymptomatic women. The increased risk of cardiovascular disease after a pregnancy affected by preeclampsia or gestational hypertension may therefore be mediated in part through the capacity of elevated PlGF levels to increase atherosclerotic plaque formation. It is unclear why there should be a reciprocal relationship between low PI levels during hypertensive pregnancies and elevated PI levels in the same women postpartum.

Conclusions
Women who develop preeclampsia or gestational hypertension have an elevated MAP in early pregnancy, but those who develop preterm preeclampsia have an attenuated rise in PI, followed by an exaggerated rise in sEng and then sFlt-1. The correlation between sEng and sFlt-1 with elevated uterine artery PI at 23 to 25 weeks supports a role for these antiangiogenic factors in limiting uteroplacental angiogenesis, but this hypothesis requires direct testing. The strengthening relationship between all 3 angiogenic biomarkers and MAP during pregnancy supports a progressive role for the placenta on maternal vascular resistance. Women with higher MAPs at 10 to 17 weeks gestation develop preeclampsia with lower concentrations of sFlt-1, further supporting a pathogenic role for sFlt-1 through small-vessel dysfunction. Suppression of sFlt-1 and sEng or augmentation of PlGF forms novel therapeutic targets to prevent or ameliorate preeclampsia. Gestational hypertension develops differently from preeclampsia, with a hyperdynamic circulation and angiogenic biomarker profile similar to those in normotensive pregnancy, suggesting that a different therapeutic approach may optimize management. Women who develop preeclampsia also have large-vessel (brachial artery) endothelial dysfunction during pregnancy, which is independent of changes in serum PI, sEng, and sFlt-1 and persists postpartum. Postpartum, elevated serum PI levels in women who had preeclampsia and gestational hypertension may be partly responsible for promoting the increased risk of cardiovascular disease in these women.

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Disclosures
Dr Hingorani is a member of the editorial board of Drug and Therapeutics Bulletin and has acted as an adviser to GlaxoSmithKline and London Genetics. Dr Williams has acted as an adviser to Roche Diagnostics. Drs Hingorani and Williams have received honoraria for speaking at educational meetings sponsored by the pharmaceutical industry and have donated all or most of that honoraria to charity. The other authors report no conflicts.

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Angiogenic and Vascular Factors in Preeclampsia 487

Clinic Perspective

Preeclampsia, defined by the gestational onset of hypertension and proteinuria, affects 2% to 4% of first-time pregnancies and accounts for 15% to 25% of all maternal deaths in the United States and Europe. Delivery is the only cure but carries the risk of neonatal morbidity and mortality in cases of preterm preeclampsia. Understanding the pathogenesis of preeclampsia is necessary to improve outcomes. It has been proposed that rises in maternal serum levels of 2 antiangiogenic factors, soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin, and a reduction in placental growth factor contribute to maternal endothelial dysfunction and consequently preeclampsia. In this study, we prospectively evaluated the relationship between these factors and found that women who develop preterm preeclampsia have an attenuated rise in placental growth factor and excessive soluble endoglin, followed by an exaggerated rise in sFlt-1. The relationships between these biomarkers and maternal blood pressure strengthen as pregnancy progresses and at 23 to 25 weeks extend to an association with raised uterine artery resistance, a recognized precursor of preterm preeclampsia. Women who start pregnancy with an elevated blood pressure develop preeclampsia at lower circulating levels of sFlt-1, further supporting a pathogenic role for sFlt-1 through resistance vessel dysfunction. Gestational hypertension develops differently from preeclampsia, with a hyperdynamic circulation and angiogenic biomarker profile similar to normotensive pregnancy. sFlt-1, soluble endoglin, and placental growth factor may provide novel therapeutic targets to prevent or ameliorate preeclampsia.
Prospective Study of Placental Angiogenic Factors and Maternal Vascular Function Before and After Preeclampsia and Gestational Hypertension
Muna Noori, Ann E. Donald, Aspasia Angelakopoulou, Aroon D. Hingorani and David J. Williams

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The specific point refers to the sFlt-1:PlGF ratio at 10–17 weeks for women with term preeclampsia (TP). The correct ratio is 44.5 with SEM 3.6. The statistical comparison is not altered.

This affects Figures 4C and 5C, which are incorrect and should be replaced by the following figure panels:

Figure 4C:

![Figure 4C](image1)

Figure 5C:

![Figure 5C](image2)

The authors regret the error.

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This affects Figures 4C and 5C, which are incorrect and should be replaced by the following figure panels:

Figure 4C:
Maternal serum sFlt-1:PlGF Ratio

NP | GH | TP | PP

0  | 50 | 100 | 150 | 200
Figure 5C: The authors regret the error.