Low Placental Growth Factor Across Pregnancy Identifies a Subset of Women With Preterm Preeclampsia

Type 1 Versus Type 2 Preeclampsia?


Abstract—Preeclampsia is a heterogeneous syndrome affecting 3% to 5% of all pregnancies. An imbalance of the antiangiogenic and proangiogenic factors, soluble receptor fms-like tyrosine kinase 1 and placental growth factor (PGF), is thought to contribute to the pathophysiology of preeclampsia. Maternal plasma PGF and soluble receptor fms-like tyrosine kinase 1 were quantified by specific immunoassays in cross-sectional samples from 130 preeclamptic subjects and 342 normotensive controls at delivery and longitudinally in samples from 50 women who developed preeclampsia and 250 normotensive controls. Among women who developed preeclampsia, 46% (n=23) evidenced a pattern of consistently low maternal PGF across pregnancy below the lower 95% CI of controls from 15 weeks' gestation to term. In contrast, the remaining 54% (n=27) of women who developed preeclampsia had maternal PGF concentrations similar to or above (n=7) those of normotensive controls. Subjects with low PGF across pregnancy who developed preeclampsia evidenced significantly higher blood pressure in early pregnancy (P<0.05) and, after diagnosis, earlier gestational age at delivery (P<0.05) and more preterm birth (P<0.05) compared with preeclamptic patients with high PGF. A significant subset of women who develop preeclampsia show evidence of consistently low PGF across pregnancy. Low PGF with preeclampsia was associated with preterm delivery compared with preeclamptic patients with high PGF. Identifying women with consistently low plasma PGF during pregnancy may provide a greater understanding of preeclampsia pathophysiology and may provide more focused research and clinical activities. (Hypertension. 2012; 60:00-00.) ● Online Data Supplement

Key Words: pregnancy ■ preeclampsia ■ preterm birth ■ placental growth factor ■ soluble vascular endothelial growth factor receptor 1

Preeclampsia is a pregnancy-specific syndrome affecting 3% to 5% of all pregnancies and is a leading cause of maternal and fetal morbidity and mortality worldwide.1–3 Preeclampsia is diagnosed clinically by the presence of new-onset gestational hypertension and proteinuria after 20 weeks' gestation. Historically, these diagnostic criteria identify a subset of women at high risk for adverse maternal and fetal outcomes.4 However, preeclampsia is recognized as a complex and heterogeneous syndrome and far more than the diagnostic criteria of hypertension and proteinuria.5

The pathophysiology of preeclampsia remains incompletely elucidated; however, increased attention has been directed toward the role of angiogenic and antiangiogenic factors, including elevated soluble fms-like tyrosine kinase 1 receptor (sFLT1; also known as soluble vascular endothelial growth factor receptor 1) and lower placental growth factor (PGF).6–8 Concentrations of these factors are significantly different in women who develop preeclampsia several weeks before clinical manifestations of the disorder compared with women who have uncomplicated normotensive pregnancies.7–9 However, the biological variability in sFLT1 and PGF concentrations among subjects who later develop preeclampsia compared with normotensive controls appears to limit their clinical use as predictive markers.10–13 Conversely, the biological variability in sFLT1 and PGF concentrations observed in subjects who later develop preeclampsia may be useful in further differentiating the heterogeneous nature of preeclampsia and provide further insight into the pathophysiology of the syndrome.
The goal of this study was to investigate the heterogeneous nature of PGF and sFLT1 longitudinally among subjects who later develop preeclampsia compared with normotensive controls and to investigate whether longitudinal patterns of these factors may identify subsets of preeclamptic subjects with differences in demographics and pregnancy outcome.

Materials and Methods
A detailed Methods description is provided in the accompanying online-only Data Supplement.

Results
PGF and sFLT1 in Term and Preterm Preeclampsia
The demographic and clinical characteristics of the cross-sectional study subjects are shown in Table 1. Similar to previous reports, maternal plasma PGF was significantly lower and sFLT1 was significantly higher in women with preeclampsia compared with normotensive controls (Figure 1A and 1B). PGF was significantly lower and sFLT1 was significantly higher in women with term preeclampsia compared with controls (both *P*<0.0001). In addition, PGF was significantly lower and sFLT1 was significantly higher in women with preterm preeclampsia compared with women with term preeclampsia (*P*<0.0001 and *P*<0.02, respectively), as well as normotensive controls (*P*<0.0001 and *P*<0.0001, respectively).

Longitudinal Differences in PGF and sFLT1 Between Preeclampsia and Normotensive Controls
We next investigated differences in PGF and sFLT1 in maternal plasma samples collected longitudinally during pregnancy (Table 2). Maternal plasma PGF concentrations were not different between 4 and 15 weeks’ gestation in

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=342)</th>
<th>Term Preeclampsia (n=97)</th>
<th>Preterm Preeclampsia (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>23.9±5.7</td>
<td>26.0±6.1*</td>
<td>24.5±5.0</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>26.2±6.8</td>
<td>26.3±6.8</td>
<td>27.2±8.5</td>
</tr>
<tr>
<td>Average blood pressure before 20 wk gestation, mm Hg</td>
<td>113.6±8.1/60.1±5.6</td>
<td>115.6±10.6/70.1±6.9</td>
<td>115.5±10.4/70.6±7.5</td>
</tr>
<tr>
<td>Average blood pressure at delivery, mm Hg</td>
<td>121.5±11.4/73.3±7.6</td>
<td>147.6±12.4*/88.2±7.2*</td>
<td>159.1±18.9†/95.7±9.4†</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>40.0±1.0</td>
<td>39.4±1.1*</td>
<td>31.7±2.8†</td>
</tr>
<tr>
<td>Race, % black</td>
<td>98 (28.6%)</td>
<td>20 (20.6%)</td>
<td>6 (18.2%)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>185 (54.1)</td>
<td>34 (35.0)*</td>
<td>6 (18.2%)</td>
</tr>
<tr>
<td>Gestational age at sample collection, wk</td>
<td>38.3±3.6</td>
<td>39.3±1.1*</td>
<td>31.6±2.8†</td>
</tr>
<tr>
<td>Predelivery uric acid, mg/dL</td>
<td>5.0±1.2</td>
<td>5.8±1.3*</td>
<td>6.2±1.5*</td>
</tr>
<tr>
<td>Placenta weight, g (n)</td>
<td>461.5±91.6 (58)</td>
<td>471.6±94.4 (52)</td>
<td>267.9±97.9 (29)*†</td>
</tr>
<tr>
<td>Infant birth weight, g</td>
<td>3454.5±444.4</td>
<td>3198.4±494.6*</td>
<td>1586.5±664.6*†</td>
</tr>
<tr>
<td>Birth weight centile, %</td>
<td>54.0±26.2</td>
<td>41.4±28.3*</td>
<td>28.6±22.5*†</td>
</tr>
<tr>
<td>SGA infants, n (%)</td>
<td>9 (2.6)</td>
<td>14 (14.4)*</td>
<td>7 (21.2)*</td>
</tr>
</tbody>
</table>

Data are mean±SD. SGA, small for gestational age; BMI, body mass index.
*P<0.05 vs controls.
†P<0.01 vs term preeclampsia.

The box and whisker plots of maternal plasma placental growth factor (PGF; A) and maternal plasma soluble fms-like tyrosine kinase 1 receptor (sFLT1; B) at delivery. The ● are the median, the ○ are the 90th and 10th percentiles, and the top and bottom lines of the box are the 75th and 25th percentiles of the data for each group. Statistical significance is indicated by *P* values.
women who later developed preeclampsia compared with uncomplicated controls (Figure 2A). However, plasma PGF concentrations were significantly lower between 15.1 and 25.0 weeks’ gestation in women who later developed preeclampsia compared with normotensive controls (P<0.02; Figure 2A), and maternal plasma PGF remained significantly lower in preeclampsia cases compared with normotensive controls between 25.1 weeks’ gestation and term delivery (Figure 2A).

In contrast, maternal plasma sFLT1 concentrations were not different between 4.0 to 15.0 weeks’ or 15.1 to 25.0 weeks’ gestation between women who later developed preeclampsia compared with normotensive controls. However, plasma sFLT1 concentrations were significantly higher in preeclampsia cases compared with normotensive controls from 25.1 to 33.0 weeks’ and 33.1 weeks’ gestation to term (P<0.0001 for both; Figure 2B).

Receiver operating characteristic (ROC) curves were constructed using maternal concentrations of PGE, sFLT1, and the PGF/sFLT1 ratio as predictors of preeclampsia and preterm preeclampsia. ROC curves were constructed using samples obtained between 15 and 23 weeks’ gestation before the onset of preeclampsia. The area under the ROC curve for PGE predicting preeclampsia and preterm preeclampsia was 0.68 and 0.73, respectively. Similarly, the area under the ROC curve for maternal sFLT1 between 15 and 23 weeks was 0.51 for predicting preeclampsia and 0.56 for predicting preterm preeclampsia. The area under the ROC curve predicting preeclampsia using the PGF/sFLT1 ratio was 0.58 and 0.59 for predicting preterm preeclampsia. Using the best ROC curve and its optimal cutoff point the positive predictive value of PGE to predict preterm preeclampsia assuming a 1% occurrence rate would be 5%. These data are consistent with the heterogeneity of maternal plasma PGF and sFLT1 observed among women who later develop preeclampsia compared with normotensive controls, as shown in Figure 2A and 2B.

We next investigated when the maternal concentration of PGE and sFLT1 were different between women who developed preeclampsia compared with normotensive controls in relation to the clinical onset of the syndrome. Box and whisker plots of maternal plasma PGF concentrations according to the clinical onset of preeclampsia (time 0) show significantly lower PGF as early as 7.0 to 10.9 weeks before the onset of the syndrome in women who developed preeclampsia compared with controls matched for gestational age at sample collection (P<0.01) and remained significantly lower, including at the onset of the syndrome (P<0.0001; Figure 2C). Similarly, maternal plasma sFLT1 concentrations were significantly higher between 1.0 and 6.9 weeks before the onset of preeclampsia (P<0.0001) and remained significantly higher including at the onset of the syndrome (P<0.0001; Figure 2D). These data are consistent with previous studies and also further demonstrate the heterogeneous nature of these factors in pregnancy and preeclampsia.

**Low Maternal PGF Throughout Pregnancy Identifies a Subset of Preeclampsia Patients**

Although differences in maternal plasma PGF and sFLT1 during gestation between women who develop preeclampsia and normotensive controls were statistically significant, it was evident that there was considerable variability in the concentration of these factors among subjects who developed preeclampsia. Therefore, we asked whether this heterogeneity might resolve into identifiable longitudinal patterns suggestive of subsets of subjects. This investigation identified 2 distinct patterns of maternal plasma PGF during pregnancy among women who later developed preeclampsia. The first pattern of PGF among subjects who developed preeclampsia was similar to maternal plasma PGF concentrations observed among normotensive control subjects, in which plasma PGF concentrations rise sharply in early pregnancy and peak around 25 to 28 weeks’ gestation, after which PGF concentrations decrease sharply to term. The concentration of maternal plasma PGF in these preeclampsia patients, although lower at the time of the clinical presentation of the syndrome, was within (or greater than, n=7 subjects) the 95% CI of plasma PGF in normotensive control subjects (Figure 3A). Among the 50 preeclampsia subjects in this study, 27 (54%) exhibited this pattern of PGF across gestation. The second pattern of PGF was present in the remaining 23 women who developed preeclampsia (46%). In these subjects, PGF concentrations were consistently below the lower 95% CI of maternal PGF concentrations observed in normotensive control subjects from 15 weeks’ gestation to the end of pregnancy (Figure 3B). Interestingly, the pattern of plasma sFLT1 was also different across pregnancy between these 2 groups of preeclamptic subjects with differing patterns of PGF. The concentration of sFLT1 was not different between normotensive controls and preeclampsia subjects.
with the high PGF pattern until after 35 weeks’ gestation (P<0.05; Figure 3C). In contrast, as shown in Figure 3D, women who developed preeclampsia and exhibited the low PGF pattern exhibited a significant and earlier rise in plasma sFLT1 after 30 weeks’ gestation, as well as higher concentrations of sFLT1 in late pregnancy compared with normotensive controls (P<0.01). The PGF/sFLT1 ratios for the low and high PGF preeclampsia groups compared with controls follow the pattern of maternal PGF and are shown in Figure S1, available in the online-only Data Supplement.

We next investigated whether these markedly different patterns of PGF might identify subsets of preeclamptic women. To address this question we examined clinical data for differences between these 2 groups, as well as differences compared with normotensive control subjects. Compared with preeclampsia subjects with the high PGF pattern, preeclampsia subjects with the low PGF pattern delivered significantly earlier (37.2±3.8 versus 38.6±1.9 weeks’ gestation; P<0.05) and had more preterm births (39% versus 11%; P<0.05), higher average blood pressures before 20 weeks’ gestation (P<0.001), more small-for-gestational-age (SGA) infants (22% versus 11%; P=0.30), and lower average infant birth weight centiles (34.9±24.8% versus 41.6±32.8%; P=0.42) but were not statistically different. We also examined outcomes in normotensive control subjects with the lowest 5% of PGF concentrations across pregnancy (n=12) similar to the low PGF preeclampsia group, and these subjects did not deliver earlier or have smaller infants compared with controls without low PGF (Table 3). Interestingly, normotensive controls with low PGF evidenced higher average predelivery systolic blood pressures compared with normotensive controls without low PGF (P<0.03) and a trend toward significantly higher predelivery diastolic blood pressure (P=0.08). In addition, there were significantly fewer smokers among the preeclamptic and normotensive control subjects with low PGF compared with controls without low PGF; however, the pattern of low PGF persisted after controlling for smoking among the preeclampsia subjects. Overall, there are 2 distinct patterns of maternal circulating PGF concentrations during pregnancy among women who later...
develop preeclampsia in the absence of differences in maternal adiposity or race, and these PGF patterns are associated with differences in maternal and infant outcomes among preeclamptic subjects.

Discussion

In this study we observed 2 distinct patterns of maternal plasma PGF across pregnancy. One PGF pattern resembled that observed among uncomplicated normotensive pregnant women, whereas the other maternal plasma PGF pattern was consistently below the lower 95% CI of plasma PGF observed among uncomplicated pregnant women after 15 weeks’ gestation to delivery. This low pattern of maternal PGF was present in nearly half (46%; 23 of 50) of the women who developed preeclampsia in this study. It is obvious that subjects with this low PGF pattern before clinically evident preeclampsia account for the statistically significant differences in PGF observed among the entire longitudinal cohort of women who develop preeclampsia. Finally, we observed that preeclamptic subjects with the consistent low PGF pattern across pregnancy exhibited differences in clinical outcome data compared with preeclamptic subjects with the high PGF pattern, including significantly elevated blood pressure before 20 weeks’ gestation, significantly earlier gestational age at delivery, more preterm births, and more SGA infants. Low PGF alone was not sufficient to account for these findings in the absence of other components of preeclampsia pathophysiology because these clinical differences were not present in the 5% of normotensive control women with similarly low PGF concentrations across pregnancy (although blood pressure was slightly higher at delivery in this group). However, these data support the heterogeneous nature of preeclampsia and suggest that these strikingly different patterns of maternal PGF across pregnancy among women who later develop preeclampsia may identify important subsets of preeclamptic patients.

In addition, we have confirmed that women with preeclampsia have significantly lower plasma PGF and higher sflt1 concentrations compared with women with uncomplicated pregnancies and that these differences are greatest among women with preterm preeclampsia. We also observed that overall maternal plasma PGF concentrations were significantly lower by midpregnancy (15–25 weeks’ gestation) and remained lower among women who later developed preeclampsia in the absence of differences in maternal adiposity or race, and these PGF patterns are associated with differences in maternal and infant outcomes among preeclamptic subjects.

Figure 3. Median±95% CI of plasma placental growth factor (PGF) concentrations (A and B) and soluble fms-like tyrosine kinase 1 receptor (sFLT1) concentrations (C and D) from 10 to 40 weeks’ gestation. Women with uncomplicated pregnancies (solid black line), women who developed preeclampsia and had plasma PGF concentrations within the 95% CI of control uncomplicated pregnant women (dashed blue line, A and C), and women who developed preeclampsia and had plasma PGF concentrations below the 95% CI of control uncomplicated pregnant women from 15 weeks’ gestation to delivery (solid red line, B and D).
and that maternal sFLT1 concentrations were significantly elevated later in pregnancy (25–33 weeks’ gestation) and remained elevated among women who developed preeclampsia compared with women with uncomplicated pregnancies. These differences in PGF and sFLT1 preceded the clinical onset of preeclampsia by 7 to 11 weeks and 1 to 7 weeks, respectively. These data are consistent with other published studies that have suggested these analytes, alone or in combination, as potential predictive markers of preeclampsia. However, the heterogeneity of PGF and sFLT1 concentrations among women who develop preeclampsia limits their practical use as predictive markers of the syndrome, as evidenced by the poor ROC curves obtained using PGF, sFLT1, and the PGF/sFLT1 ratio values from samples obtained between 15 and 23 weeks’ gestation before the clinical onset of the syndrome.

The variability of PGF and sFLT1 among pregnant women and women who develop preeclampsia is evident in many studies. PGF is a member of the vascular endothelial growth factor subfamily, it is expressed by trophoblast cells, and it has both vasculogenetic and angiogenetic functions. PGF’s angiogenic abilities have been speculated to play a role in normal pregnancy, and changes in the concentrations of PGF and sFLT1 have been proposed in the pathophysiology of preeclampsia. However, given the heterogeneous nature of PGF and sFLT1 among women who develop preeclampsia, it seems unlikely that a single pattern or mechanism can explain all cases of preeclampsia. In addition, these data indicate that these angiogenic markers are not necessarily causative for the incomplete or failed uterine spiral artery remodeling reported in preeclampsia, because their concentrations are similar to those observed among uncomplicated pregnant women in early pregnancy when these placental abnormalities begin to develop.

Several studies have reported that the circulating concentration of maternal PGF is significantly lower in early pregnancy among women who later develop preeclampsia compared with women with uncomplicated pregnancies and that low maternal PGF concentrations are more pronounced among women who develop early onset preeclampsia and/or deliver SGA infants. As shown in this study, the observation of low PGF in early pregnancy among all women who later develop preeclampsia was driven by an identifiable subset of women who exhibited consistently low PGF across pregnancy, below the 95% CI of uncomplicated pregnant women. Importantly, the subset of preeclamptic women who exhibited consistently low PGF across pregnancy accounted for almost half of the preeclampsia cases in this longitudinal investigation. In addition, these subjects were more likely to deliver preterm and to have SGA infants compared with preeclamptic women with PGF concentrations similar to uncomplicated controls.

Table 3. Characteristics of Control and Preeclampsia Subjects and Their Infants According to Maternal PGF Profile During Pregnancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=238)</th>
<th>Controls, Low PGF (n=12)</th>
<th>Preeclampsia, High PGF (n=27)</th>
<th>Preeclampsia, Low PGF (n=23)</th>
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</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>22.3±4.4</td>
<td>26.7±6.5*</td>
<td>23.4±5.4</td>
<td>23.1±6.0</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>25.2±6.4</td>
<td>28.9±7.4</td>
<td>25.2±5.6</td>
<td>27.4±6.4</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>40.1±1.0</td>
<td>40.6±1.1</td>
<td>38.6±1.9*</td>
<td>37.2±3.8*</td>
</tr>
<tr>
<td>Preterm birth, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (11)</td>
<td>10 (43)†</td>
</tr>
<tr>
<td>Predelivery uric acid, mg/dL</td>
<td>4.9±1.1</td>
<td>4.6±0.8</td>
<td>5.8±1.3*</td>
<td>5.5±1.4*</td>
</tr>
<tr>
<td>Average blood pressure before 20 wk gestation, mm Hg</td>
<td>112.8±7.5/68.4±5.5</td>
<td>113.6±9.1/70.2±5.1</td>
<td>113.3±6.5/67±6.8</td>
<td>118.7±9.1*/71.4±4.7†</td>
</tr>
<tr>
<td>Average blood pressure predelivery, mm Hg</td>
<td>119.9±10.9/72.7±7.9</td>
<td>127.5±16.5*/76.7±5.3</td>
<td>143.6±13.0*/87.6±7.3*</td>
<td>148.3±13.1*/91.2±8.7*</td>
</tr>
<tr>
<td>Race, % black</td>
<td>78 (33%)</td>
<td>3 (25%)</td>
<td>9 (33%)</td>
<td>9 (39%)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>145 (61)</td>
<td>2 (17)*</td>
<td>13 (48)</td>
<td>8 (35)*</td>
</tr>
<tr>
<td>Placental weight, g (n)</td>
<td>466.2±88.9 (45)</td>
<td>400.0±70.7 (3)</td>
<td>436.7±98.4 (15)</td>
<td>396.1±140.8 (15)*</td>
</tr>
<tr>
<td>Infant birth weight, g</td>
<td>3431.9±429.4</td>
<td>3585.7±349.6</td>
<td>3066.2±666.6*</td>
<td>2601.9±825.2†</td>
</tr>
<tr>
<td>Infant length, cm</td>
<td>50.6±3.8</td>
<td>51.1±2.1</td>
<td>49.7±3.6</td>
<td>47.9±3.5*</td>
</tr>
<tr>
<td>Infant head circumference, cm</td>
<td>34.2±1.6</td>
<td>34.9±1.3</td>
<td>32.7±2.2*</td>
<td>32.8±2.1*</td>
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<tr>
<td>Birth weight centile, %</td>
<td>51.9±26.0</td>
<td>60.2±25.1</td>
<td>41.6±32.8</td>
<td>34.9±24.8*</td>
</tr>
<tr>
<td>SGA infants, n (%)</td>
<td>7 (3)</td>
<td>0 (0)</td>
<td>3 (11)*</td>
<td>5 (22)*</td>
</tr>
</tbody>
</table>

Data are mean±SD. SGA indicates small for gestational age; BMI, body mass index.
*P<0.05 vs controls.
†P<0.05 vs preeclampsia.
angiogenic signaling (low PGF) and half of the cases of preeclampsia are the result of insensitivity to angiogenic signaling (high PGF) or are determined by a different pathogenic factor. Based on these 2 different patterns of maternal PGF we speculate that this may represent a relevant division of preeclampsia, low versus high PGF or type 1 versus type 2 preeclampsia. This finding may be similar to that of diabetes mellitus with its well-defined and understood type 1 (low insulin) versus type 2 (high insulin) classification. In addition, the presence of low and high patterns of maternal PGF in preeclampsia explains previous published data regarding the poor predictive nature of PGF and sFLT1 for preeclampsia. If low PGF in preeclampsia is present in only half of all cases, then it is obvious that many cases of preeclampsia would be missed if only using this marker. Conversely, these longitudinal data suggest that women with persistent PGF concentrations below the lowest 5% of concentrations observed in uncomplicated pregnant women after 15 weeks’ gestation have a 30% chance of developing preeclampsia, and this group of women appears to represent half of the cases of preeclampsia. It is important for this observation to be duplicated in another independent longitudinal study.

Despite the large number of normotensive control subjects investigated in the longitudinal portion of this study, this group is limited in that none of these subjects had preterm birth and relatively few had SGA infants (3%). Therefore, differences in preterm birth and SGA infants between controls and the preeclampsia subjects are more pronounced and are likely elevated compared with more heterogeneous control populations. However, because of this limitation, this study focused its attention primarily on differences between the low versus high PGF preeclampsia groups, as well as comparisons between the low PGF normotensive control group and the low PGF preeclampsia group, indicating that the low maternal PGF pattern is not just a consequence of SGA.

Perspectives

It is well accepted that preeclampsia is a heterogeneous syndrome.\(^5,26\) Most studies address the heterogeneity of preeclampsia by dividing subjects according to severity of the syndrome and clinically relevant outcomes, such as early onset/preterm delivery and SGA infants. In many cases these studies also investigate differences in biological markers, such as PGF and sFLT1, according to the previously defined clinical outcomes.\(^5,26\) Identifying such subsets might aid in the understanding of pathophysiology and perhaps allow individual preventive therapy.\(^27\) In this study we have identified 2 different patterns of PGF among women who develop preeclampsia and investigated how women who develop preeclampsia based on significant differences in the patterns of PGF are similar and different in their clinical outcome. Using this approach we have found that a significant proportion of women (46%) who develop preeclampsia show evidence of a consistent pattern of low PGF at or below the 95% CI of normotensive controls from 15 weeks’ gestation onward and that this group of preeclamptic women have elevated blood pressures in early pregnancy, more preterm births, and SGA infants compared with preeclamptic women with PGF concentrations similar to normotensive controls. Based on these findings, we propose that dividing preeclampsia cases by differences in patterns of pathophysiologically relevant analytes may be useful for future research studies, as well as provide insights into pathophysiologic and clinically relevant subtypes and whether analyte patterns can be predicted by demographic or preexisting clinical factors.\(^28\)

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Disclosures

None.

References


Novelty and Significance

What Is New?
- In a longitudinal analysis, 46% of patients who later developed preeclampsia evidenced maternal PGF consistently below the lower 95% CI of uncomplicated pregnant women across pregnancy.
- Preeclampsia patients with consistently low PGF have significantly higher blood pressures, deliver earlier, and have more preterm births compared with preeclampsia patients without low PGF.

What Is Relevant?
- Preeclampsia is a significant hypertensive complication of pregnancy affecting 3% to 5% of all pregnancies and is a leading cause of maternal and fetal morbidity and mortality worldwide.

Summary
A significant proportion of women (46%) who develop preeclampsia show evidence of a pattern of consistently low PGF at or below the 95% CI of normotensive pregnant patients from 15 weeks’ gestation onward. Investigating differences in patterns of pathophysiologically relevant analytes in preeclampsia cases may be useful for future research studies, as well as provide insights into pathophysiologic and clinically relevant subtypes.
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LOW PGF ACROSS PREGNANCY IDENTIFIES A SUBSET OF WOMEN WITH PRETERM PREECLAMPSIA; TYPE 1 vs. TYPE 2 PREECLAMPSIA?

Online supplement

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Clinical and Translational Research 5
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MATERIAL AND METHODS

Cross-sectional Study Population

We conducted a retrospective case-control study using banked maternal plasma samples collected between 1997 and 2005 from an ongoing investigation of preeclampsia at the University of Pittsburgh, Magee-Womens Hospital and Magee-Womens Research Institute, Pittsburgh, PA USA. The study was approved by the institutional review board and informed consent was obtained from all subjects. Samples collected at the time of admittance to labor and delivery were available from 342 women with uncomplicated normotensive pregnancies and 130 women with preeclampsia. All subjects were nulliparous.

Preeclampsia was defined by the research criteria recommended by the National High Blood Pressure Education Program\(^1\): gestational hypertension, proteinuria and return of all abnormalities to normal by 12 weeks postpartum. Gestational hypertension was defined as blood pressure persistently $\geq 140$ mmHg systolic and/or $\geq 90$ mmHg diastolic for the first time after 20 weeks of gestation. Proteinuria was the excretion of $\geq 300$ mg of protein in 24 hours, or a dipstick of 2 plus, or a catheterized sample of 1 plus, or a protein creatinine ratio of $\geq 0.3$. The diagnosis of preeclampsia was confirmed by chart review by a jury of research and clinical investigators.

Preterm birth was defined as < 37 and 0/7 completed weeks of gestation. Gestational age-specific birth weight centiles were based upon data from Magee-Womens Hospital adjusted for sex and race. Small-for-gestational age (SGA) was defined as birth weight $\leq$ 10th centile. SGA infants with clinical or pathological evidence of chronic intrauterine infection or chromosomal abnormalities were excluded from the study. Maternal race and smoking status were by self-report at enrollment. Pre-pregnancy body mass index $[\text{weight (kg)/height (m)}^2]$ was based on measured height and maternal self-report of pre-pregnancy weight at the initial visit. Control subjects were women with uncomplicated, normotensive pregnancies who delivered healthy babies. Patients with multiple gestations, chronic hypertension, diabetes, evidence of infection, renal disease or other significant metabolic disorder, or a history of illicit drug use were excluded.

The demographic and clinical characteristics of the cross-sectional study subjects are shown in Table 1 separating preeclamptic women who delivered at term or preterm. Preeclampsia patients had higher uric acid concentrations, infants with lower birth weight centiles, a higher percentage of SGA infants and an earlier gestational age at delivery. Preterm preeclampsia cases had significantly higher blood pressure at admission to labor and delivery, smaller babies, smaller birth weight centiles, and smaller placentas compared to term preeclampsia cases (p<0.01 all). Consistent with previous epidemiologic data $^2,^3$, there were significantly fewer smokers in preeclampsia subjects compared to controls (p<0.05).

Longitudinal Study Population

The longitudinal study subjects were recruited at the time of presentation to Magee-Womens Hospital for prenatal care and all subjects provided informed consent. Blood samples were collected across pregnancy at the time of clinically indicated sampling. Similar to the cross-sectional study subjects, all subjects were nulliparous healthy women without known medical complications and consisted of 250 women with uncomplicated normotensive control
pregnancies and 50 women who developed preeclampsia. Definitions for preeclampsia and uncomplicated normotensive control pregnancies were the same as that described for the cross-sectional study population.

The demographic and clinical characteristics of the longitudinal study subjects are shown in Table 2. Among the longitudinal subjects, maternal blood pressure was measured on average three times prior to 20 weeks of gestation, with a range from 1 to 6 measurements. Specifically, prior to 20 weeks maternal blood pressure was measured one time in 13 subjects, two times in 34 subjects, three times in 114 subjects, four times in 105 subjects, five times in 30 subjects, and six times in 4 subjects. Similar to the cross-sectional study subjects, longitudinal subjects who developed preeclampsia delivered significantly earlier \((p<0.001)\), had babies with lower birth weight centiles \((p<0.01)\), and more SGA infants \((p<0.05)\) compared to controls. In addition, fewer women were smokers among the subjects who developed preeclampsia compared to normotensive controls \((p<0.05)\), and preeclampsia subjects had higher average systolic blood pressures before 20 weeks gestation \((p<0.05)\), and by definition significantly higher blood pressure at admission to labor and delivery \((p<0.001)\).

**Patient samples**

Maternal venous EDTA plasma samples were collected, aliquoted and stored at –70°C until assayed. One thousand and ninety nine (1099) total maternal plasma samples were available for the longitudinal analysis. As many as five plasma samples were collected from a single subject across pregnancy. Samples were collected as early as 4 weeks gestation and thereafter up to delivery. A sample was collected at the time of clinical diagnosis for all subjects with preeclampsia. 39%, 48%, and 9% of women contributed three, four, or five total samples, respectively. The gestational age of samples at the time of collection was determined from the best obstetrical estimate of gestational age (early ultrasound when available or firm LMP consistent with second trimester ultrasound).

**Measurement of plasma PGF and sFLT1**

The maternal plasma concentration of free PGF and sFLT1 were quantified using prototype reagents on the Abbott Architect i2000SR (Abbott Park, IL). The inter-assay variability for sFLT1 was 3.1-4.5% and for free PGF was between 1.4-5.5%. Since the reagents for PGF and sFLT1 quantification have not been published before, we compared these reagents to those used by most other published studies. Data obtained using the Abbott reagents were found to be comparable to analyses obtained from a separate collection of 969 maternal plasma samples using commercially available ELISA reagents for PGF and sFLT1 (R&D Systems, Minneapolis, MN; PGF \(r^2=0.92\) and sFLT1 \(r^2=0.90\)). Bland-Altman plots (data not shown) indicate the Abbott reagents give proportionally higher values for PGF and sFLT1 (mean difference between assays 6.42±0.14ng/ml for sFLT1 and 114.52±38.44pg/ml for PGF).

**Statistical analysis**

Maternal and newborn demographic data are presented as the mean±SD. Longitudinal samples were analyzed as continuous data for individual subjects as well as divided into four separate gestational age distributions according to collection times during pregnancy: between 4 and 15 weeks, 15.1 to 25 weeks, 25.1 to 33 weeks, and 33.1 to 42.3 weeks, one sample per subject within each distribution of gestational age. Data were also analyzed cross-sectionally, using all available samples within intervals of gestational age and according to the time before
the onset of preeclampsia. In the intervals when there was more than one sample per subject, the earliest sample was used. Investigation of differences in maternal PGF and sFLT1 in relation to the clinical onset of preeclampsia was performed using all available samples from subjects who developed preeclampsia distributing them into time blocks according to when the sample was collected in relation to the clinical onset of preeclampsia for each subject (onset of clinically recognized preeclampsia is time 0), and two separate samples from two control subjects were matched within one week of the same gestational age in weeks to the sample from the preeclampsia subject. Receiver operator characteristic (ROC) curves were constructed to evaluate the predictive potential of PGF, sFLT1, and the PGF/sFlt ratio using the values obtained from samples collected between 15 and 23 weeks gestation before the clinical onset of preeclampsia. The distribution of plasma concentrations of PGF and sFLT1 was found to be non-normally distributed and the distribution was normalized by taking the natural logarithm of the data before statistical analysis. JMP 5.0.1a (Cary, NC) and Sigma Plot 12 software (San Jose, CA) were used to analyze the data. Data were analyzed by ANOVA or Student’s unpaired t-test where appropriate, and categorical variables using the chi-square test. Statistical significance was accepted at p<0.05.

References

Figure S1. Box and whisker plots of maternal plasma PGF/sFLT1 ratio (A) in low PGF control subjects, n=12 (B) in high PGF preeclampsia subjects, n=27 and (C) in low PGF preeclampsia subjects, n=23. Open boxes are controls and shaded boxes are comparison subjects: (A) low PGF control subjects, comparison group are controls minus the 12 low PGF controls, (B) high PGF preeclampsia subjects and (C) low PGF preeclampsia subjects. The filled black circles are the median, the open circles are the 90th and 10th percentiles, and the top and bottom lines of the box are the 75th and 25th percentiles of the data for each group. Statistical significance is indicated by p values.