Circulating Angiogenic Factors and Risk of Adverse Maternal and Perinatal Outcomes in Twin Pregnancies With Suspected Preeclampsia

Sarosh Rana, Michele R. Hacker, Anna Merport Modest, Saira Salahuddin, Kee-Hak Lim, Stefan Verlohren, Frank H. Perschel, S. Ananth Karumanchi

Abstract—To evaluate whether angiogenic factor levels correlate with preeclampsia-related adverse maternal and perinatal outcomes in women with twin pregnancy, we studied 79 women with suspected preeclampsia in the 3rd trimester. Antiangiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) and proangiogenic placental growth factor (PlGF) were measured at presentation on an automated platform. An adverse outcome was defined as hemolysis, elevated liver enzymes, and low platelets syndrome; disseminated intravascular coagulation; abortion; pulmonary edema; cerebral hemorrhage; maternal, fetal, and neonatal death; eclampsia; acute renal failure; small for gestational age; and indicated delivery. All outcomes were ascertained 2 weeks after initial evaluation. Comparing the 52 women (65.8%) who experienced an adverse outcome with the 27 women (34.2%) without an adverse outcome, the median sFlt-1 was elevated (11461.5 pg/mL [8794.0–14847.5] versus 7495.0 pg/mL [3498.0–10482.0; \(P=0.0004\)]), PI GF was reduced (162.5 pg/mL [98.0–226.5] versus 224.0 pg/mL [156.0–449.0]; \(P=0.005\)), and sFlt-1/PlGF ratio was elevated (74.2 [43.5–110.5] versus 36.2 [7.1–71.3]; \(P=0.0005\)). Among those presenting <34 weeks (n=40), the difference in sFlt-1/PI GF ratio was more striking (97.7 [76.6–178.1] versus 31.7 [6.5–48.7]; \(P=0.001\)). Addition of sFlt-1/PI GF to the highest systolic blood pressure and proteinuria improved prediction of adverse outcomes. We conclude that in women with twin pregnancy and suspected preeclampsia, the sFlt-1/PI GF ratio at the time of initial evaluation is associated with subsequent adverse maternal and perinatal outcomes. These findings are similar to those in singleton pregnancies and may implicate common pathogenic pathways. (Hypertension. 2012;60:00-00.)

Key Words: angiogenic factors □ twin pregnancy □ preeclampsia □ adverse outcomes

Preeclampsia is a pregnancy-specific hypertensive disorder with an overall incidence of 5% to 8% among all pregnancies.1 Preeclampsia/eclampsia (characterized by seizures related to preeclampsia) accounts for more than 50,000 maternal deaths worldwide each year,2 and it is the second most common obstetric cause of stillbirths and early neonatal deaths in developing countries.3 It is a major cause of iatrogenic preterm delivery in developed countries.4 Women with twin gestation, which account for 5% of pregnancies in the United States, are at increased risk for the development of hypertensive disorders of pregnancy, with a reported incidence of 12.9% to 37%. The incidence of preeclampsia is 2 to 3 times higher for women with twin pregnancies than for women with singleton pregnancies.5,6

Preeclampsia is thought to be a placental disease characterized by inadequate trophoblast invasion of maternal spiral arteries leading to release of systemic factors that are responsible for the maternal syndrome.7,8 However, uterine artery Doppler abnormalities reflecting placental vascular disorder are typically not seen in twin pregnancies when associated with normal fetal growth.9,10 Moreover, there is no increase in hypoxia in placentas from the twin gestation compared with singletons when there is no concurrent fetal growth restriction.11 Recently, angiogenic imbalance has been shown to be present in women with preeclampsia. Specifically, levels of the antiangiogenic protein soluble fms-like tyrosine kinase-1 (sFlt-1) are elevated, and levels of proangiogenic proteins, such as placental growth factor (PI GF), are reduced in women with preeclampsia.12–15 Women with twin pregnancies have levels of sFlt-1 that are twice those of singletons11,16; however, the elevated levels of sFlt-1 are not associated with primary placental pathology, but rather are caused by increased placental mass seen in twins; this possibly leads to the increased risk of preeclampsia seen in these patients.11

Received March 28, 2012; accepted June 3, 2012.
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© 2012 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.112.195065
Prediction of adverse outcomes among women with pre-eclampsia remains a challenge. In a recent, large, international study evaluating multiple clinical parameters to develop a model to predict preeclampsia-related adverse outcomes, Von Dadelszen et al showed that the model predicted adverse maternal outcomes occurring within the first 48 hours after eligibility, with modest predictive values up to 7 days. The number of patients with twin gestation was small and their data were not presented separately. In another study, Klien et al looked at whether the uterine artery pulsatility index at 20 to 22 weeks gestation could predict adverse outcomes in twin gestation. They found that an increased mean lowest and highest pulsatility index is associated with a higher risk of preeclampsia and adverse pregnancy outcomes. However, the obvious limitation of any ultrasound test is availability of the equipment and inter- and intraobserver variability, depending on the experience of the sonographer.

Angiogenic factors have been shown to have prognostic value in patients presenting for evaluation of preeclampsia. In a retrospective study, Romero et al showed that levels of proangiogenic and antiangiogenic factors in triage supported the diagnosis of preeclampsia; however, twin pregnancies were excluded. We have recently shown that among women with a singleton pregnancy presenting to obstetric triage for evaluation of preeclampsia, levels of sFlt-1 and PlGF were not only related to the diagnosis of preeclampsia, but also predicted preeclampsia-related adverse outcomes within 2 weeks. The ratio of sFlt-1/PlGF out-performed all clinical and laboratory data currently used to predict adverse outcomes. In addition, these proteins were measured on an automated platform requiring small amounts of sample volume, and results are available in minutes, making use of angiogenic factors in this clinical scenario feasible.

The purpose of this study was to evaluate the role of angiogenic factors for prediction of adverse outcomes in women with twin gestation who present for evaluation of preeclampsia. We hypothesized that, similar to singleton pregnancies, angiogenic proteins can be used for risk stratification and prediction of adverse outcomes in women with twin pregnancies.

### Methods

#### Study Design

This cohort came from an ongoing prospective cohort study evaluating the role of angiogenic factors in women with suspicion of preeclampsia, which is described in detail elsewhere. In the present study, we included all women with twin pregnancies who presented to Beth Israel Deaconess Medical Center in Boston, MA, and who were evaluated for preeclampsia from July 2009 through August 2011. These women were either referred by their obstetric provider because of signs of preeclampsia or they self-presented with symptoms of preeclampsia. The need for an evaluation for preeclampsia was determined by a primary care provider (registered nurse practitioner, resident or attending physician). Indications for evaluation included: elevated blood pressure; proteinuria; or any symptoms

<table>
<thead>
<tr>
<th>Table 1: Participant Characteristics</th>
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<tbody>
<tr>
<td><strong>Characteristic</strong></td>
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<tr>
<td>--------------------</td>
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<tr>
<td><strong>n</strong></td>
</tr>
<tr>
<td><strong>Age (y), median (IQR)</strong></td>
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<tr>
<td><strong>Body mass index (kg/m²), median (IQR)</strong></td>
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<tr>
<td><strong>Race, n (%)</strong></td>
</tr>
<tr>
<td><strong>White</strong></td>
</tr>
<tr>
<td><strong>Black</strong></td>
</tr>
<tr>
<td><strong>Asian</strong></td>
</tr>
<tr>
<td><strong>Other/unknown</strong></td>
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<tr>
<td><strong>Ethnicity, n (%)</strong></td>
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<td><strong>Hispanic</strong></td>
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<tr>
<td><strong>Non-Hispanic</strong></td>
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<tr>
<td><strong>Unknown</strong></td>
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<tr>
<td><strong>Smoking, n (%)</strong></td>
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<td><strong>Never</strong></td>
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<tr>
<td><strong>Past/quit before pregnancy</strong></td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
</tr>
<tr>
<td><strong>Medical history, n (%)</strong></td>
</tr>
<tr>
<td><strong>Chronic hypertension</strong></td>
</tr>
<tr>
<td><strong>Preeclampsia</strong></td>
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<tr>
<td><strong>Renal disease</strong></td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
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<tr>
<td><strong>Nulliparous</strong></td>
</tr>
<tr>
<td><strong>Yes</strong></td>
</tr>
<tr>
<td><strong>No</strong></td>
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</tbody>
</table>

IQR indicates interquartile range.
associated with preeclampsia, such as headache, visual symptoms, right upper quadrant pain, or edema. The plasma sample obtained at the time of initial presentation was collected and stored at −80°C. The study was approved by the Beth Israel Deaconess Medical Center Institutional Review Board, and all patients provided informed consent.

### sFlt-1 and PlGF Assays
Automated assays for sFlt-1 and PlGF were performed at the clinical laboratory of Charite Hospital (Berlin, Germany) using the commercially available assays on Elecsys platform (Roche Diagnostics) as previously described. The interassay coefficient of variation for sFlt-1 and PlGF immunoassays ranged from 2.6% to 3.0% and 2.0% to 2.4%, respectively. The assay operators were blinded to the clinical information of the participants. All assays were performed after the diagnosis and outcomes were ascertained for each participant.

### Diagnoses and Outcomes
Determination of clinical diagnoses and adverse outcomes was based on information collected from the time of presentation through the subsequent 2 weeks. The diagnoses of preeclampsia, gestational hypertension, and chronic hypertension were based on standard criteria endorsed by the American College of Obstetricians and Gynecologists. An adverse maternal outcome was defined as the presence of hypertension (blood pressure ≥140/90 mm Hg on 2 occasions, 2 hours to 2 weeks apart) plus at least 1 of the following: elevated aspartate aminotransferase (≥80 U/L) or alanine aminotransferase (≥80 U/L), platelet count ≤100×10⁹/L, disseminated intravascular coagulation, abruption (clinical and/or pathological), pulmonary edema, cerebral hemorrhage, eclampsia, abnormal renal function (creatinine ≥132.6 μmol/L), or maternal death. The adverse fetal/neonatal outcomes included iatrogenic delivery for hypertensive complications of pregnancy as reported by the primary obstetrician, small-for-gestational-age birth weight (≤10th percentile for gestational age), abnormal umbilical artery Doppler (absent or reverse flow), fetal death, and neonatal death (in either twin). Diagnoses and adverse outcomes were adjudicated by 2 study staff before availability of assay results.

### Statistical Analysis
All analyses were conducted using Statistical Analysis System (SAS 9.3, SAS Institute) and STATA (version 12). Data are reported as mean±SD, median (interquartile range), or proportion depending on data type and distribution. Parametric and nonparametric tests were used as appropriate. All tests were 2-sided and probability values <0.05 were considered statistically significant.

The sFlt-1/PlGF ratio (value of sFlt-1 measured in pg/mL, divided by the value of PlGF measured in pg/mL) was used as a measure of circulating angiogenic imbalance, based on previous studies showing that this marker was most accurate in discriminating women with and without preeclampsia. To determine the clinical utility of the sFlt-1/PlGF ratio in prediction of adverse outcomes, we used receiver operating characteristic analysis, which employed logistic regression to calculate the area under the curve and the associated 95% CIs. We performed this analysis for individual predictors, such as blood pressure, proteinuria, and sFlt1/PlGF ratio, as well as for combinations of predictors. Confidence intervals that include 0.50 suggest that the predictor or set of predictors are no better than is random guessing which women will develop an adverse outcome within 2 weeks. The Spearman correlation coefficient was used to assess the association between sFlt-1/PlGF ratio and pregnancy duration from time of evaluation for preeclampsia to delivery.

### Results

#### Demographics and Clinical Characteristics
From July 2009 through August 2011, 95 women with twin gestation were evaluated for preeclampsia. Six women were excluded (3 women had no sample available, 2 women had no preeclampsia evaluation performed, and 1 sample was postpartum). Ten women declined consent. A total of 79 women were analyzed with a median maternal age of 33.0 years.
Table 3. Angiogenic Protein Levels Stratified by Hypertensive Disorder Diagnosed and Presence of Adverse Outcome Within 2 Weeks in All Subjects

<table>
<thead>
<tr>
<th>Angiogenic Factors</th>
<th>No Hypertensive Disorder</th>
<th>Gestational Hypertension</th>
<th>P Value*</th>
<th>Preeclampsia</th>
<th>P Value†</th>
<th>No Adverse Outcome</th>
<th>Adverse Outcome</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td>46</td>
<td>27</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sFlt-1 (pg/ml)</td>
<td>5976.0 (3408.5–11 103.0)</td>
<td>7479.0 (4355.5–11 169.5)</td>
<td>0.75</td>
<td>10 790.0 (8975.0–14 714.0)</td>
<td>0.01</td>
<td>7495.0 (3498.0–10 482.0)</td>
<td>11 461.5 (6794.0–14 847.5)</td>
<td>0.0004</td>
</tr>
<tr>
<td>PlGF (pg/ml)</td>
<td>212.0 (109.5–483.0)</td>
<td>183.5 (112.5–334.0)</td>
<td>0.78</td>
<td>175.5 (104.0–229.0)</td>
<td>0.23</td>
<td>224.0 (156.0–449.0)</td>
<td>162.5 (98.0–226.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>sFlt-1/PlGF ratio</td>
<td>47.4 (5.5–82.2)</td>
<td>53.7 (20.2–72.7)</td>
<td>0.83</td>
<td>72.2 (42.5–111.9)</td>
<td>0.052</td>
<td>36.2 (7.1–71.3)</td>
<td>74.2 (43.5–110.5)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range).
*Comparison between women without a hypertensive disorder and women with gestational hypertension.
†Comparison between women without a hypertensive disorder and women with preeclampsia.
‡Comparison between women with and without an adverse outcome.

PlGF indicates placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1.

Factors (31.0–38.0) and a median body mass index of 30.7 (28.2–34.7). Within the 2 weeks following presentation and enrollment, 52 women (65.8%) experienced at least 1 adverse maternal or fetal outcome and 27 women (34.2%) did not.

Women who did and did not experience an adverse outcome in the 2 weeks after presentation were similar with respect to age, body mass index, race, ethnicity, smoking history, and history of chronic hypertension, renal disease, and diabetes (all P>0.09). Baseline participant characteristics are presented in Table 1 for the full cohort and are stratified by incidence of an adverse outcome.

Although the cohort’s median gestational age at presentation was 33.9 weeks (31.9–36.0), the women who experienced an adverse outcome presented at a later gestational age of 35.0 weeks (33.2–36.3) than the 32.1 weeks (28.4–33.6) in women who did not experience an adverse outcome (P=0.0003).

Women who had an adverse outcome had higher creatinine, higher uric acid, and a lower platelet count (all P<0.004) at the time of evaluation for preeclampsia. Interestingly, the groups did not differ with respect to highest systolic and diastolic blood pressures and proteinuria. Mode of delivery did not differ between the 2 groups (P=0.32), but delivery mode was unknown for 2 women in the group without an adverse outcome who were transferred back to their primary hospital. The pregnancy duration from time of evaluation for preeclampsia to delivery was shorter in women who developed an adverse outcome (3.5 days) compared with in women who did not develop an adverse outcome (14.5 days; P=0.0006). Results of the initial clinical evaluation and delivery outcomes for the full cohort and stratified by incidence of an adverse outcome in the 2 weeks after presentation for preeclampsia are presented in Table 2.

Table 4. Angiogenic Protein Levels Stratified by Hypertensive Disorder Diagnosed and Presence of Adverse Outcome Within 2 Weeks in Women <34 Weeks at Presentation

<table>
<thead>
<tr>
<th>Angiogenic Factors</th>
<th>No Hypertensive Disorder</th>
<th>Gestational Hypertension</th>
<th>P Value*</th>
<th>Preeclampsia</th>
<th>P Value†</th>
<th>No Adverse Outcome</th>
<th>Adverse Outcome</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td>23</td>
<td>22</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sFlt-1 (pg/ml)</td>
<td>4394.0 (2578.0–17 685.0)</td>
<td>4852.0 (3317.5–9692.0)</td>
<td>0.95</td>
<td>10 688.0 (8797.0–14 981.0)</td>
<td>0.14</td>
<td>8229.5 (2498.0–10 667.0)</td>
<td>11 487.0 (8902.0–15 999.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>PlGF (pg/ml)</td>
<td>245.0 (121.0–778.0)</td>
<td>192.0 (112.0–349.0)</td>
<td>0.53</td>
<td>183.0 (118.0–243.0)</td>
<td>0.11</td>
<td>244.0 (183.0–449.0)</td>
<td>118.0 (98.0–191.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>sFlt-1/PlGF ratio</td>
<td>17.9 (3.3–79.0)</td>
<td>34.2 (11.5–75.6)</td>
<td>0.61</td>
<td>82.1 (36.2–131.2)</td>
<td>0.08</td>
<td>31.7 (6.5–48.7)</td>
<td>97.7 (76.6–178.1)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range).
*Comparison between women without a hypertensive disorder and women with gestational hypertension.
†Comparison between women without a hypertensive disorder and women with preeclampsia.
‡Comparison between women with and without an adverse outcome.

Diagnosis and Outcomes
Forty-six women (58.2%) were diagnosed with preeclampsia during the 2-week period after the initial visit. In addition, 4 women (5.1%) had chronic hypertension, 12 women (15.2%) had gestational hypertension, and 12 women (15.2%) had no hypertensive disorder. Among the women diagnosed with preeclampsia, 28 women (60.9%) had mild preeclampsia, and 18 women (39.1%) had severe preeclampsia or hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome.

Among the 52 women (65.8%) who experienced an adverse outcome within 2 weeks of presentation, the most common adverse outcome was indicated delivery, which occurred in 47 women (90.4%). Six women (11.5%) developed HELLP syndrome, 13 women (25.0%) delivered at least 1 small-for-gestational-age infant, 1 woman (1.9%) had pulmonary edema, 1 woman (1.9%) experienced a fetal death, and 1 woman (1.9%) experienced neonatal death. There were no maternal deaths. Of the 47 indicated deliveries, 13 deliveries (27.7%) occurred before 34 weeks of gestation and 39 deliveries (83.0%) before 37 weeks of gestation.

Plasma Angiogenic Proteins and Adverse Outcomes
Women who ultimately were diagnosed with preeclampsia had a significantly higher median sFlt-1 level (P=0.01). Although not statistically significantly different, they also had a higher median sFlt-1/PlGF ratio (P=0.052) and a lower median PlGF level (P=0.23) compared with women without a hypertensive disorder. A similar pattern was seen when comparing sFlt-1 and PlGF levels in women with gestational hypertension with women without a hypertensive disorder;
however, none of the differences were statistically significant (all P>0.75). There were too few patients with chronic hypertension (n=4) to conduct a meaningful analysis. Similar trends were seen in women presenting <34 weeks. Levels of the angiogenic proteins stratified by hypertensive disorder are shown in Table 3 and 4.

Among women who experienced an adverse outcome within 2 weeks, the median sFlt-1 level was significantly elevated (P=0.0004), the median level of PlGF was lower (P=0.005), and the median sFlt1/PlGF ratio was higher (P=0.0003) compared with women who did not develop an adverse outcome (Table 3). Similar patterns were seen in women presenting <34 weeks (Table 4) and when comparing women who experienced the most common adverse outcomes—indicated delivery, delivering a small-for-gestational-age infant, and HELLP syndrome—with women who did not experience an adverse outcome (Figure).

In addition, nulliparous women had higher sFlt-1/PlGF ratios than did multiparous women (nulliparous: median, 70.9 [interquartile range, 41.2–105.8], versus multiparous: median, 34.8 [interquartile range, 12.1–72.2]; P=0.01) and were more likely to develop adverse outcomes. The relationship between sFlt-1/PlGF ratio and adverse outcomes remained significant even after adjustment for parity and gestational age at presentation (P=0.002 for all subjects and P=0.009 for subjects <34 weeks).

Models for Prediction of Adverse Outcomes in 2 Weeks

When evaluating predictors individually, the area under the curve was greatest for the sFlt-1/PlGF ratio and gestational age (0.75 for each), whereas highest systolic blood pressure, proteinuria, and alanine transaminase alone were no better than was a random guess at predicting which women would experience an adverse outcome within 2 weeks. The most favorable results were seen with the combination of the sFlt-1/PlGF ratio and gestational age (0.85), as well as the model that included these 2 predictors along with the highest systolic blood pressure and proteinuria (0.83). A similar pattern was seen among women <34 weeks on presentation. The area under the curve and the 95% CIs for the full cohort and for women presenting at <34 weeks of gestation are shown in Table 5.

Ratio of sFlt-1/PlGF >85 has been validated as the optimal cut-off for the diagnosis of preeclampsia in previous studies in singleton pregnancies. Among women presenting at less than 34 weeks gestation, the sensitivity of this cut-off was 61.1% (95% CI, 35.8–82.7) and the specificity was 90.9% (95% CI, 70.8–98.9). Using a cut-off of 75, the sensitivity was 77.8% (95% CI, 58.6–97.0) and specificity was 86.4% (95% CI, 72.0–100.0). Among these women, the risk ratio for developing an adverse outcome was 3.26 (95% CI, 1.30–7.24). The area under the curve and the 95% CIs for all predictors are shown in Table 5.

Figure. Angiogenic factors at presentation. Shows median angiogenic factor levels at presentation stratified by adverse outcome. Adverse outcomes were ascertained 2 weeks after presentation. None indicates no adverse outcome (N=27); Indicated del, indicated delivery, iatrogenic delivery indicated for hypertensive complications of pregnancy (N=47); SGA, small for gestational age (N=13); HELLP, hemolysis, elevated liver enzymes, and low platelets syndrome (N=6). The extremes of the box represent the interquartile range; the bottom of the box represents the 25th percentile and top of the box represents the 75th percentile. The horizontal line across the box represents the median. The ends of the whiskers correspond to the lowest and highest values within 1.5 interquartile range below and above the interquartile range.

A. sFlt-1 levels. The median (IQR) sFlt-1 for each group were as follows: none, 7495.0 (3498.0–10482.0); indicated delivery, 12286.0 (8975.0–14981.0); SGA, 14714.0 (8096.0–18205.0); HELLP, 19333.5 (5623.0–15117.0).

B. PlGF levels. The median (IQR) PlGF for each group were as follows: none, 224.0 (156.0–449.0); indicated delivery, 174.0 (99.0–229.0); SGA, 104.0 (96.0–187.0); HELLP, 141.0 (98.0–185.0).

C. sFlt-1/PlGF levels. The median (IQR) sFlt-1/PlGF for each group were as follows: none, 36.2 (7.1–71.3); indicated delivery, 71.8 (43.3–112.3); SGA, 105.1 (67.5–233.6); HELLP, 84.4 (55.6–104.1).
CI, 1.66–6.43) for a cut-off of 85 and 4.74 (95% CI, 1.89–11.85) for a cut-off of 75.

A higher sFlt-1/PlGF ratio was also associated with a shorter duration of pregnancy from time of presentation to delivery. The Spearman correlation coefficient for this association was −0.25 (P=0.03) for the full cohort and −0.36 (P=0.03) for women before 34 weeks of gestation.

**Discussion**

In this study, we examined the role of angiogenic factors as predictors of adverse outcomes in a cohort of women with twin pregnancies being evaluated for suspected preeclampsia. We found that within 2 weeks of evaluation, an adverse outcome occurred in about 65% of women, and we identified the sFlt-1/PlGF ratio as the strongest predictor of adverse outcomes alone and in combination with clinically available measurements. We also found that the sFlt-1/PlGF ratio was inversely correlated with duration of pregnancy from time of evaluation to delivery.

In our cohort, we identified that traditional clinical parameters and tests used in triage, such as blood pressure measurement, proteinuria, and abnormal liver enzymes are poor predictors of preeclampsia-related adverse outcomes among twin pregnancy; this is consistent with previous reports. The sFlt-1/PlGF ratio as a marker of angiogenic imbalance has been used in previous studies as an antiangiogenic index; hence, we used this in the present study. We found that sFlt-1/PlGF ratio alone is the single best marker for prediction of adverse outcome with sensitivity similar to gestational age. Given that the most common adverse outcome was indicated delivery, which is partially dependent on gestational age, it is not surprising that gestational age by itself is a good predictor. However, our study was performed in an urban tertiary care center where gestational age is very well documented. If this information is not available, calculation of gestational age in late pregnancy is very inaccurate. The fact that sFlt-1/PlGF is an independent marker of adverse outcome regardless of gestational age will make it a useful test in situations where accurate information about gestational age is not available, such as lack of prenatal care and no previous early ultrasound.

Other interesting finding of the study is that sFlt-1/PlGF ratio alone is a better predictor of adverse outcome than are traditional measures used for evaluation, such as blood pressure, proteinuria, and uric acid either alone or in combination. We also found that sFlt-1/PlGF ratio is inversely correlated to duration of pregnancy from evaluation to delivery. These findings are similar to those in our study evaluating the role of sFlt-1/PlGF ratio in singleton pregnancy.

Interestingly, in our study, normotensive women carrying twins had approximately 2-fold-higher circulating sFlt-1 and 3-fold-higher sFlt-1/PlGF ratio (Table 3 and Table 4) than did normotensive women with singleton pregnancies. This increased circulating antiangiogenic state may be one mechanism for the increased risk of preeclampsia noted in women carrying twin pregnancies. We also found a 2-fold-higher circulating sFlt-1/PlGF ratio in nulliparous women compared with in multiparous women. These findings are similar to previous reports in singleton pregnancies, suggesting common pathogenic mechanisms.

Our study has important clinical implications. Use of a highly sensitive test for initial evaluation will help in appropriate risk assessment of women with twin pregnancies and suspicion of preeclampsia. This will help in appropriate management of patients with high sFlt-1/PlGF ratio in the form of prompt transfer to a tertiary care center, admission

### Table 5. Area Under the Curve Predicting Adverse Outcomes at 2 Weeks

<table>
<thead>
<tr>
<th>Clinical and Laboratory Parameters</th>
<th>All Women*</th>
<th>&lt;34 Weeks*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>77</td>
<td>39</td>
</tr>
<tr>
<td>Highest SBP (mm Hg)</td>
<td>0.51 (0.37–0.65)</td>
<td>0.53 (0.34–0.72)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>0.60 (0.49–0.72)</td>
<td>0.62 (0.46–0.77)</td>
</tr>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>0.53 (0.40–0.67)</td>
<td>0.70 (0.53–0.87)</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>0.71 (0.58–0.83)</td>
<td>0.70 (0.53–0.87)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>0.70 (0.58–0.83)</td>
<td>0.75 (0.61–0.90)</td>
</tr>
<tr>
<td>sFlt-1/PlGF ratio</td>
<td>0.75 (0.64–0.86)</td>
<td>0.81 (0.66–0.96)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>0.75 (0.63–0.87)</td>
<td>0.61 (0.42–0.79)</td>
</tr>
<tr>
<td>Highest SBP + proteinuria</td>
<td>0.62 (0.49–0.75)</td>
<td>0.70 (0.53–0.87)</td>
</tr>
<tr>
<td>Highest SBP + proteinuria + uric acid</td>
<td>0.72 (0.59–0.85)</td>
<td>0.76 (0.61–0.92)</td>
</tr>
<tr>
<td>Highest SBP + proteinuria + sFlt-1/PlGF ratio</td>
<td>0.78 (0.67–0.89)</td>
<td>0.85 (0.73–0.98)</td>
</tr>
<tr>
<td>sFlt-1/PlGF ratio + gestational age</td>
<td>0.83 (0.74–0.93)</td>
<td>0.85 (0.73–0.97)</td>
</tr>
<tr>
<td>sFlt-1/PlGF ratio + uric acid</td>
<td>0.80 (0.70–0.91)</td>
<td>0.85 (0.72–0.89)</td>
</tr>
<tr>
<td>Highest SBP + proteinuria + sFlt-1/PlGF ratio</td>
<td>0.85 (0.76–0.94)</td>
<td>0.87 (0.77–0.98)</td>
</tr>
</tbody>
</table>

*Any woman with missing data for any of the predictors above was excluded from the regression model. Thus, 2 women were not included in the calculation of area under the curve.

Systolic blood pressure; sFlt-1, soluble fms-like tyrosine kinase 1; PlGF, placental growth factor.
and use of betamethasone for fetal lung maturity in anticipation of premature delivery, and appropriate counseling of patients. Similarly, patients with low sFlt-1/PIGF ratio can be managed expectantly and we can avoid iatrogenic premature delivery in women at low risk for preeclampsia-related adverse outcomes and will be useful for resource utilization.

The strengths of our study include its prospective design, well-defined patient population, high rate of enrollment, high quality, and accurate data collection. All diagnosis and outcomes were ascertained before angiogenic factor assessment, which was carried out by technicians blinded to all clinical information.

Our study has certain limitations. The study was performed in a single center and is of relatively small size. Although we were interested in all preeclampsia-related adverse outcomes, we did not find rare, but meaningful, adverse outcomes, such as pulmonary edema, disseminated intravascular coagulation, and maternal deaths. Even if we were to increase the sample size, the rarity of these outcomes in our center makes it impossible to evaluate the role of angiogenic factors in prediction of such adverse outcomes. In addition, given that this was an observational study, the true effect of measurement of sFlt-1/PIGF ratio in management of women with twin pregnancy and suspected preeclampsia remains unknown. We also noted that women who went on to develop adverse outcomes were more likely to present at a later gestational age than were women who went on to have no adverse effects. This is likely related to the fact that women with suspicion of preeclampsia are usually delivered when they reach 37 weeks of gestation to avoid maternal or fetal complications. Future studies focusing only in subjects who present preterm (<34 weeks) and very-preterm (<32 weeks) are needed to evaluate whether these markers are useful for the expectant management of preeclampsia.

In conclusion, angiogenic factor measurements in women carrying twin pregnancies provides valuable diagnostic and prognostic information in the evaluation of preeclampsia in a triage setting.

Perspectives
Incidence of preeclampsia and related adverse outcomes is high in women with twin pregnancies. There is ample evidence that angiogenic factors are elevated in women with preeclampsia and correlate with adverse outcomes. In this cohort study, we found that sFlt-1/PIGF ratio was independently associated with adverse outcomes, whereas traditional clinical assessment is of poor value. Assessment of angiogenic factors in these patients may represent a valuable addition to standard clinical assessment for the diagnosis, prognosis, and management on initial presentation. Additional studies are needed to validate these findings.

Acknowledgments
We thank Dawn McCullough, RN, for patient recruitment and data collection. This work was conducted with support from Harvard Catalyst The Harvard Clinical and Translational Science Center (NIH Award #UL1 RR 025758 and financial contributions from Harvard University and its affiliated academic health care centers).

Sources of Funding
S.R. is supported by Harvard Diversity and Community Partnership Faculty Fellowship Award. S.A.K is an investigator of the Howard Hughes Medical Institute.

Disclosures
S.V. is a consultant to Roche Diagnostics. S.A.K. is a coinventor of multiple patents related to angiogenic proteins for the diagnosis and therapy of preeclampsia. These patents have been licensed to multiple companies. S.A.K. reports having served as a consultant to Roche and Beckman Coulter and has financial interest in Aggamin LLC. The remaining authors report no conflicts.

References


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**Novelty and Significance**

**What Is New?**

- In this study, we show that women with twin gestation being evaluated for suspected preeclampsia have an abnormal angiogenic profile.

**What Is Relevant?**

- Patients with twin pregnancy are at increased risk of preeclampsia. Measurement of angiogenic factors has increasingly been evaluated in women with singleton pregnancy; however, studies in twin pregnancies are lacking.

**Summary**

- Prediction of preeclampsia and related adverse outcomes is challenging because there are no tests that are sensitive and specific enough to be used clinically.

Our data have major clinical implications and provide an important step toward the clinical utility of the angiogenic biomarkers for accurate diagnosis, prediction, and risk stratification of subjects at high risk for preeclampsia-related adverse maternal and perinatal outcomes.
Circulating Angiogenic Factors and Risk of Adverse Maternal and Perinatal Outcomes in Twin Pregnancies With Suspected Preeclampsia
Sarosh Rana, Michele R. Hacker, Anna Merport Modest, Saira Salahuddin, Kee-Hak Lim, Stefan Verlohren, Frank H. Perschel and S. Ananth Karumanchi

Hypertension. published online July 2, 2012;

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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