Angiogenic Factors and the Risk of Adverse Outcomes in Women With Suspected Preeclampsia

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Background—An imbalance in circulating angiogenic factors plays a central role in the pathogenesis of preeclampsia.

Methods and Results—We prospectively studied 616 women who were evaluated for suspected preeclampsia. We measured plasma levels of antiangiogenic soluble fms-like tyrosine kinase 1 (sFlt1) and proangiogenic placental growth factor (PlGF) at presentation and examined for an association between the sFlt1/PlGF ratio and subsequent adverse maternal and perinatal outcomes within 2 weeks. The median sFlt1/PlGF ratio at presentation was elevated in participants who experienced any adverse outcome compared with those who did not (47.0 [25th–75th percentile, 15.5–112.2] versus 10.8 [25th–75th percentile, 4.1–28.6]; $P<0.0001$). Among those presenting at <34 weeks (n=167), the results were more striking (226.6 [25th–75th percentile, 50.4–547.3] versus 4.5 [25th–75th percentile, 2.0–13.5]; $P<0.0001$), and the risk was markedly elevated when the highest sFlt1/PlGF ratio tertile was compared with the lowest (odds ratio, 47.8; 95% confidence interval, 14.6–156.6). Among participants presenting at <34 weeks, the addition of sFlt1/PlGF ratio to hypertension and proteinuria significantly improved the prediction for subsequent adverse outcomes (area under the curve, 0.93 for hypertension, proteinuria, and sFlt1/PlGF versus 0.84 for hypertension and proteinuria alone; $P=0.001$). Delivery occurred within 2 weeks of presentation in 86.0% of women with an sFlt1/PlGF ratio ≥85 compared with 15.8% of women with an sFlt1/PlGF ratio <85 (hazard ratio, 15.2; 95% confidence interval, 8.0–28.7).

Conclusions—In women with suspected preeclampsia presenting at <34 weeks, circulating sFlt1/PlGF ratio predicts adverse outcomes occurring within 2 weeks. The accuracy of this test is substantially better than that of current approaches and may be useful in risk stratification and management. Additional studies are warranted to validate these findings. **(*Circulation*. 2012;125:911-919.)**

Key Words: adverse maternal and perinatal outcomes ■ hypertension ■ pre-eclampsia ■ proteinuria ■ triage ■ vascular endothelial growth factors

Pregnancy-associated hypertension is common, with an incidence rate of ≈5% of all deliveries.[1] Of the hypertensive complications associated with pregnancy, preeclampsia is the most likely to result in serious adverse events, including maternal acute renal failure, liver dysfunction, seizures and cerebral accidents, fetal growth restriction, and death of the mother or fetus. Preeclampsia is the leading indication for premature delivery of a fetus and is therefore associated with substantial neonatal morbidity and mortality, as well as considerable healthcare expenditure.[2]

Clinical Perspective on p 919

Preeclampsia is characterized by the presence of elevated blood pressures and proteinuria after 20 weeks of gestation.[3] However, clinical criteria alone may be inadequate to predict adverse outcome because a significant proportion of women may develop complications, including eclampsia and the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), without elevated blood pressures or without proteinuria.[4] Conversely, some women with preeclampsia are

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able to carry a pregnancy to nearly full term without complications. Because of the unpredictable nature of adverse outcomes in this population, women with suspected preeclampsia are often hospitalized for close observation and monitoring, including frequent laboratory testing and evaluation of fetal wellbeing.[3] The decision for preterm delivery of the fetus in the setting of preterm preeclampsia (at <34 weeks' gestation) is based on the estimated risk of an adverse outcome balanced with the considerable benefit to the fetus if pregnancy is prolonged. Expectant management is usually attempted in women thought to be at high risk for complications until 34 weeks' gestation, after which the neonatal outcomes are excellent and the benefit for the fetus is usually outweighed by the estimated risk to the mother.[3]

Despite consensus guidelines outlining the indication for delivery in patients with pregnancy-associated hypertension, risk assessment remains challenging because no sign, symptom, or laboratory test has been shown to predict adverse outcomes with high accuracy.[5,6]

The placentally released proteins soluble fms-like tyrosine kinase-1 (sFlt1) and placental growth factor (PlGF) are altered in the circulation of pregnant women with preeclampsia.[7] sFlt1 antagonizes the action of proangiogenic proteins such as vascular endothelial growth factor and PlGF, which are necessary for normal vascular endothelial homeostasis.[8] Animal studies suggest that elevated circulating antiangiogenic proteins such as sFlt1 can cause almost all complications that characterize human preeclampsia, including hypertension, proteinuria, cerebral edema, hematologic abnormalities, and fetal growth restriction.[7,9–11] In humans, angiogenic factor levels are altered in women with preeclampsia at diagnosis, as well as weeks before clinical onset.[7,9,12–14] In prospective cohort studies, the ratio of circulating antiangiogenic to proangiogenic protein levels identifies women with early-onset preeclampsia with very high sensitivity and specificity.[15,16]

We hypothesized that elevated sFlt1/PlGF ratios at initial presentation would predict adverse outcomes in pregnant women with suspected preeclampsia, especially in a prespecified group of patients presenting at <34 weeks' gestation.

Methods

Study Design

From July 2009 through October 2010, we studied women with singleton pregnancies who presented to the obstetric triage unit at the Beth Israel Deaconess Medical Center in Boston, MA, and were evaluated for possible preeclampsia. All participants evaluated were eligible to participate. The cohort represents a population of women from various ethnic and socioeconomic backgrounds. These patients either were referred by their obstetric provider because of signs of preeclampsia or self-presented with symptoms of preeclampsia. Patients were included if the triage care provider deemed an evaluation of preeclampsia necessary. Indications for evaluation included elevated blood pressure, proteinuria, or any symptoms associated with preeclampsia such as headache, visual symptoms, right upper quadrant pain, or edema. Samples were collected within 1 hour of arrival to triage and stored at −80°C for analysis. The study was approved by the Beth Israel Deaconess Medical Center ethics committee, and all patients provided informed consent.

sFlt1 and PlGF Assays

Automated assays for sFlt1 and PlGF were performed at the clinical laboratory of Charité Hospital (Berlin, Germany) with the commercially available assays on Elecsys platform (Roche Diagnostics, Penzberg, Germany) as previously described.[15,17] These automated assays take ≈20 minutes for the measurement of both the analytes. The interassay coefficient of variance for sFlt1 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. The treating physicians were unaware of the test results of sFlt1 and PlGF values.

Diagnosis and Outcomes

Ascertainment of clinical diagnoses and adverse outcomes was based on information collected from the time of presentation through the subsequent 2 weeks. Women could be re-enrolled in the study if they presented again >2 weeks after initial presentation. The diagnoses of preeclampsia, gestational hypertension, and chronic hypertension were based on modified American College of Obstetricians and Gynecologists criteria.[3] Preeclampsia was defined as a blood pressure ≥140/90 mm Hg on 2 occasions 2 hours to 2 weeks apart after 20 weeks of gestation and proteinuria of ≥300 mg/24 h or urine protein-to-creatinine ratio of ≥0.3 after 20 weeks of gestation. Gestational hypertension was defined as the presence of hypertension as defined above without proteinuria (urine levels of proteinuria below the accepted threshold for preeclampsia), and chronic hypertension was defined as the presence of hypertension before 20 weeks of gestation. Proteinuria on presentation was defined as urine dipstick with ≥2+ protein, protein-to-creatinine ratio ≥0.3, or 24-hour urine protein ≥300 mg/d (if available at the time of presentation). Adverse maternal outcomes were defined as the presence of hypertension (blood pressure ≥140/90 mm Hg on 2 occasions 2 hours to 2 weeks apart) plus one of the following: elevated aspartate aminotransferase or alanine aminotransferase (ALT; ≥80 U/L), platelet count $\leq 100\times10^9$/L, disseminated intravascular coagulation, abruption (clinical and/or pathological), pulmonary edema, cerebral hemorrhage, seizure (in a woman without underlying seizure disorder), acute renal failure (creatinine >114.4 μmol/L), or maternal death.[18] The adverse fetal/neonatal outcomes included iatrogenic delivery indicated 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.[18] Diagnoses and adverse outcomes were adjudicated by 2 study staff members before the availability of assay results.

Statistical Analysis

Baseline characteristics of patients with and without adverse outcomes were compared by use of the Wilcoxon rank-sum test and χ^2 test when appropriate. The sFlt1/PlGF ratio (value of sFlt1 measured in pg/mL divided by the value of PlGF measured in pg/mL) was used as a measure of circulating angiogenic imbalance on the basis of prior studies showing that this marker was most accurate in discriminating women with and without preeclampsia.[15,16] Because the distribution of sFlt1/PlGF ratios was skewed, we used the Wilcoxon rank-sum test to compare the median sFlt1/PlGF ratios in participants who did and did not experience adverse outcomes. One-way ANOVA with post hoc Holm-Sidak testing was used to compare the sFlt1/PlGF ratio (after natural log transformation) by diagnosis and type of adverse outcome. After dividing participants into tertiles based on sFlt1/PlGF ratio at presentation, we used single-variable logistic regression to compare the risk of developing an adverse outcome in each tertile stratified by subgroup (normotensive and nonproteinuric). We repeated each of these analyses in women presenting at <34 weeks' gestation. A post hoc multivariable logistic regression model that included gestational age at presentation, sFlt1/PlGF tertile, and all possible interaction terms found a significant interaction between gestational age at presentation and sFlt1/PlGF tertile ($P<0.001$). Therefore, the decision to examine models separately by gestational age at presentation was not only clinically but statistically justified.

To determine the value of combinations of factors for the prediction of adverse outcomes, we created single-variable and

multivariable logistic regression models using predictors of adverse outcomes. These predictors included blood pressure, laboratory parameters, and demographic information, all available during evaluation in triage. We used receiver-operating characteristic (ROC) analysis to determine the predictive value of combining factors that were independent predictors of adverse outcomes in the logistic regression models. To determine the clinical utility of the sFlt1/PlGF ratio in the prediction of adverse outcomes, we used ROC analysis to determine a cut point of sFlt1/PlGF that would correctly classify the maximum number of participants. The sensitivity, specificity, and positive and negative predictive values for this cut point were calculated. Logistic regression models with ROC analysis were used to determine the predictive value of combining factors such as systolic blood pressure, proteinuria, ALT, uric acid, and sFlt1/PlGF ratio in predicting adverse outcomes. The models were adjusted for maternal age, parity, body mass index, and smoking. Comparisons of areas under the curve (AUCs) were performed by use of a contrast matrix to take differences of the areas under the empirical ROC curves.[19]

The Pearson product-moment correlation was used to assess the relationship between sFlt1/PlGF ratio and time elapsed between presentation and delivery (both natural log transformed). Single-variable and multivariable Cox proportional hazards models were used to compare time to delivery in participants with sFlt1/PlGF ratio less than or greater than the cut point derived from ROC analysis, and Kaplan-Meier curves were used to visualize these differences.

All P values were 2 sided, and values of $P<0.05$ were considered statistically significant. All statistical analyses were performed with SAS version 9.2.

Results

Subject Characteristics

During the study period, there were 815 preeclampsia evaluations in the Beth Israel Deaconess Medical Center obstetric triage unit. Overall, 616 evaluations were studied (75% of all evaluations; Figure I in the online-only Data Supplement). Of these evaluations, 81 were repeat evaluations of women previously enrolled. One hundred seventy-six evaluations (28.6%) occurred at <34 weeks' gestation. Subject characteristics at presentation to obstetric triage, diagnoses, and adverse outcomes are shown in Table 1. Adverse outcomes occurred in 43.5% of all patients (n=268) and 33.5% of participants presenting at <34 weeks' gestation (n=59). Baseline characteristics of women who did and did not experience subsequent adverse outcomes are shown in Table 2. The subject characteristics of patients who refused participation or were excluded did not differ from patients included in the study with the exception of slightly lower body mass index among patients not included in the study.

sFlt1/PlGF Ratio and Subsequent Clinical Outcomes

As reported in previous studies,[7,16,20] the sFlt1/PlGF ratio was associated with the clinical diagnosis of preeclampsia. Participants with gestational hypertension had moderately elevated sFlt1/PlGF ratios, whereas participants with preeclampsia had markedly elevated sFlt1/PlGF ratios compared with participants who had no hypertensive disorder (Figure 1A). Similarly, in participants presenting at <34 weeks' gestation, preeclampsia was associated with dramatically elevated sFlt1/PlGF levels (Figure 1B). The individual values of sFlt1, PlGF, and sFlt1/PlGF ratio are shown in Table I in the online-only Data Supplement.

Table 1. Characteristics of Women Presenting to Obstetrical Triage With Suspected Preeclampsia

Variable	All	Presenting at <34 Weeks' Gestation
n	616	176
Baseline		
Gestational age, wk	36.6 (33.3–38.0)	30.9 (28.2–32.9)
Age, y	32 (28–35)	32 (27–35.5)
Body mass index, kg/m^2	32.7 (29.0–37.0)	32.9 (29.2–38.2)
Nulliparous, n (%)	263 (59.8)	99 (56.3)
Smoker, n (%)	52 (8.4)	15 (8.5)
Race, n (%)		
White	307 (69.9)	98 (55.7)
Black	56 (12.8)	36 (20.5)
Asian/Pacific Islander	33 (7.5)	11 (6.3)
Other	43 (9.8)	31 (17.6)
Previous preeclampsia, n (%)	47 (10.7)	36 (20.5)
Chronic hypertension, n (%)	81 (18.4)	56 (31.8)
Preexisting diabetes mellitus, n (%)	31 (7.1)	17 (9.7)
Presentation		
Highest SBP in triage, mm Hg	137 (126–147)	140 (129–150)
Highest DBP in triage, mm Hg	87 (80–94)	87 (77–97)
Proteinuria, n (%)	120 (27.3)	63 (35.8)
ALT in triage, U/L	15 (12–21)	16.0 (12–27)
Creatinine in triage, μmol/L	53 (44–62)	53 (44–62)
Uric acid in triage, μmol/L	274 (232–333)	262 (220–340)
Platelet count in triage, $\times10^9$/L	236 (194–280)	253 (206–293)
At 2 wk after presentation, n (%)		
Any hypertensive disorder	449 (72.9)	128 (72.7)
Chronic HTN	98 (15.9)	43 (24.4)
Gestational HTN	173 (28.1)	29 (16.5)
Preeclampsia	178 (28.9)	56 (31.2)
Any adverse outcome	268 (43.5)	59 (33.5)
HTN+abnormal LFTs/platelets	25 (4.1)	15 (8.5)
HTN+DIC	2 (0.3)	2 (1.1)
HTN+abruption	9 (1.5)	5 (2.8)
HTN+pulmonary edema	2 (0.3)	2 (1.1)
HTN+eclampsia	1 (0.2)	1 (0.6)
Indicated delivery	262 (42.5)	57 (32.4)
FGR/abnormal UA Doppler	25 (4.1)	12 (6.8)
Fetal death	2 (0.3)	2 (1.1)
Neonatal death	2 (0.3)	2 (1.1)

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; HTN, hypertension; DIC, disseminated intravascular coagulation; FGR, fetal growth restriction; and UA, umbilical artery. Values are median (25th–75th percentile) when appropriate. Values were missing for body mass index in 5 patients, ALT in 15 patients, serum creatinine in 12 patients, and uric acid in 21 patients. To convert creatinine to milligrams per deciliter, divide by 76.26. To convert uric acid to milligrams per deciliter, divide by 59.48.

The sFlt1/PlGF ratio at presentation was associated with subsequent adverse outcomes (Figure 2A and 2B). The median sFlt1/PlGF ratio in women who had any adverse outcome (n=268) versus no adverse outcome (n=348) was

Table 2. Subject Characteristics by Adverse Outcome

	No Adverse Outcome	Adverse Outcome	*P*
n (%)	348 (56.5)	268 (43.5)	
Gestational age, wk	35.7 (32.8–35.7)	37.0 (34.6–38.2)	<0.001
Age, y	31.0 (28.0–35.0)	32.0 (29.0–35.0)	0.27
Body mass index, kg/m^2	32.9 (29.4–37.0)	32.3 (28.4–37.2)	0.46
Nulliparous, n (%)	198 (56.9)	164 (61.2)	0.28
Smoker, n (%)	36 (10.3)	16 (6.0)	0.05
Race, n (%)			0.47
White	232 (67.7)	173 (64.8)	
Black	48 (13.8)	44 (16.5)	
Asian/Pacific Islander	22 (6.3)	22 (8.2)	
Unknown/other	46 (13.2)	28 (10.5)	
History of preeclampsia, n (%)	46 (13.2)	37 (13.8)	0.83
History of chronic hypertension, n (%)	64 (18.4)	73 (27.2)	0.009
History of diabetes mellitus, n (%)	23 (6.6)	25 (9.3)	0.21
History of renal disease, n (%)	14 (4.0)	8 (3.0)	0.49
Highest SBP in triage, mm Hg	132 (124–141)	145 (136–154)	<0.001
Highest DBP in triage, mm Hg	85 (77–90)	92 (84–99)	<0.001
Proteinuria in triage, n (%)	53 (15.2)	130 (48.5)	<0.001
ALT in triage, U/L	15 (12–21)	16 (12–23)	0.029
Creatinine >53 μmol/L, n (%)*	53.5	68.7	<0.001
Uric acid in triage, μmol/L	259 (220–309)	303 (256–369)	<0.001
Platelet count in triage, ×10^9/L	248 (205–289)	219 (187–260)	<0.001

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; and ALT, alanine aminotransferase. Values are median (25th–75th percentile) when appropriate. Values were missing for body mass index in 5 patients, ALT in 15 patients, serum creatinine in 12 patients, and uric acid in 21 patients. To convert creatinine to milligrams per deciliter, divide by 76.26. To convert uric acid to milligrams per deciliter, divide by 59.48. Values of $P<0.05$ re considered significant.

*Because the median (25th–75th percentile) ranges appear similar despite significant difference in the distribution of creatinine values in the 2 groups, we report the percentage of creatinine values greater than the median value in each group.

47.0 (25th–75th percentile, 15.5–112.2) versus 10.8 (25th–75th percentile, 4.1–28.6; $P<0.0001$). In women presenting at <34 weeks' gestation, the median sFlt1/PlGF ratio in women who experienced any adverse outcome (n=59) versus no adverse outcome (n=117) was 226.6 (25th–75th percentile, 50.4–547.3) versus 4.5 (25th–75th percentile, 2.0 to 13.5; $P<0.0001$). Although we were limited by sample size to studying the various adverse outcomes individually, our evaluation of the relatively common adverse outcomes occurring in women presenting at <34 weeks' gestation (placental abruption, elevated liver enzymes/low platelets, or small-for-gestational-age birth weight/abnormal umbilical artery Doppler) showed that they were also associated with elevated sFlt1/PlGF ratios (Figure 2C).

When participants were divided into tertiles based on sFlt1/PlGF ratio, the risk of subsequent adverse outcome was elevated in women with sFlt1/PlGF ratios in the third compared with those in the first tertile (Table 3). Although the incidence of adverse outcome was lower in women who were nonproteinuric at presentation (31.9% versus 71.0% in women with proteinuria; $P<0.001$) and in women who were normotensive at presentation (26.1% versus 58.0% in hypertensive women; $P<0.001$), the risk of adverse outcome by tertile was similar regardless of hypertension or proteinuria (Table 3). In women presenting at <34 weeks' gestation, the relationship between sFlt1/PlGF tertile and adverse outcomes was even stronger and similarly did not differ on the basis of the presence of hypertension or proteinuria (Table 3). There were no changes in the results when the model was restricted to only the first triage visit among the participants (Table II in the online-only Data Supplement).

Predictive Accuracy of sFlt1/PlGF

In single-variable logistic regression models including women presenting at <34 weeks' gestation, greater sFlt1/PlGF, systolic blood pressure, proteinuria, uric acid, and creatinine and lower platelet count were associated with higher risk of adverse outcomes ($P<0.001$ for sFlt1/PlGF, systolic blood pressure, proteinuria, and uric acid; $P=0.008$ for creatinine; $P=0.006$ for platelet count). Other subject characteristics such as maternal age, gestational age, body mass index, smoking, and history of chronic hypertension were not associated with adverse outcomes in women presenting at <34 weeks' gestation. In multivariable logistic regression models controlling for maternal age, parity, body mass index, and smoking, the factors that remained significant predictors of adverse outcomes were sFlt1/PlGF, systolic blood pressure, proteinuria, uric acid, creatinine, and lower platelet count ($P<0.001$ for sFlt1/PlGF, systolic blood pres-

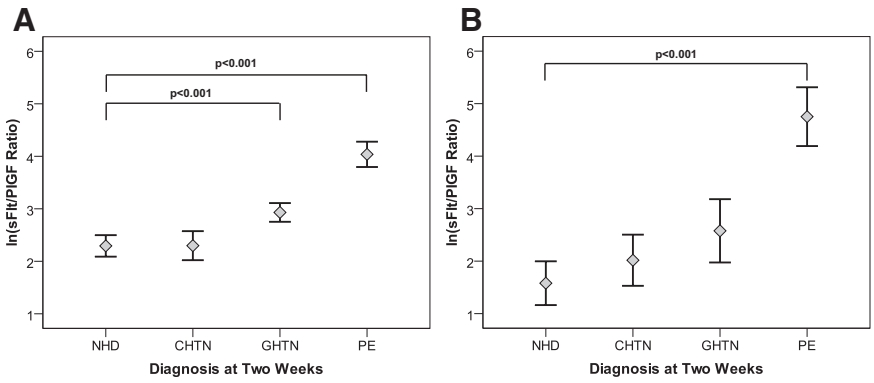

Figure 1. Ratio of soluble fms-like tyrosine kinase 1 (sFlt1) to placental growth factor (PlGF) at presentation. **A** and **B**, The distribution of natural log-transformed sFlt1/PlGF ratios at initial presentation by subsequent diagnosis. Diagnoses were ascertained 2 weeks after presentation according to American Congress of Obstetricians and Gynecologists criteria. **A**, Distribution among all participants. **B**, Distribution among participants presenting at <34 weeks' gestation. NHD indicates no hypertensive disorder; CHTN, chronic hypertension; GHTN, gestational hypertension; and PE, preeclampsia.

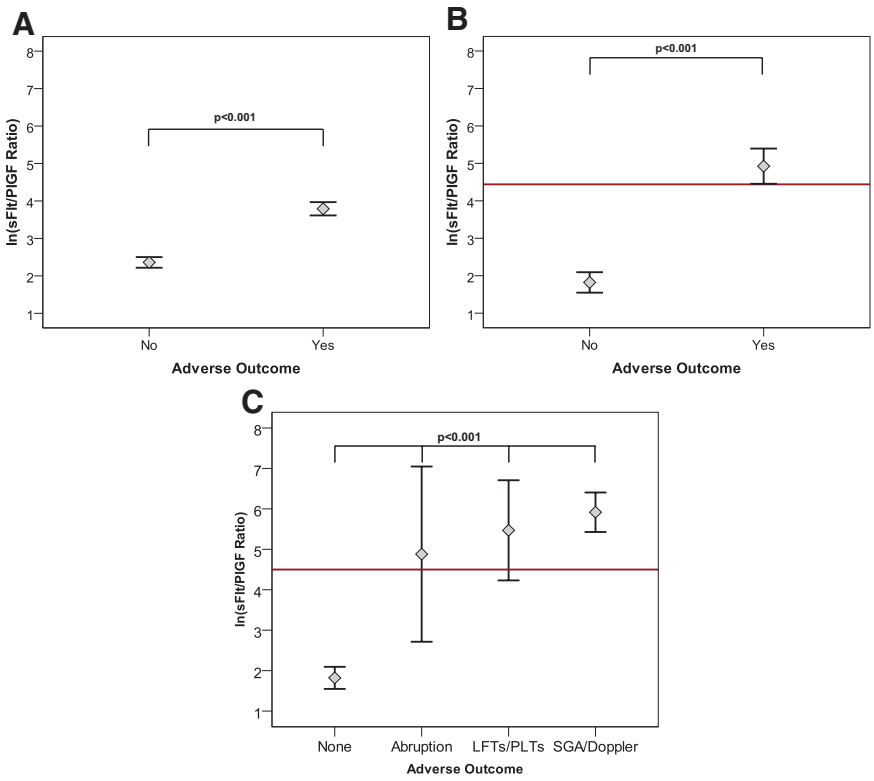

Figure 2. Ratio of soluble fms-like tyrosine kinase 1 (sFlt1) to placental growth factor (PlGF) and adverse outcomes. The distribution of natural log-transformed sFlt1/PlGF ratios at initial presentation by subsequent adverse outcomes is shown. Adverse outcomes were ascertained 2 weeks after presentation. **A**, Distribution in all participants. **B**, Distribution among only participants presenting at <34 weeks' gestation. **C**, Individual adverse outcomes in women presenting at <34 weeks' gestation. Placental abruption, elevated liver enzymes and/or low platelets (LFTs/PLTs), and small-for-gestational-age birth weight and/or absent/reversed umbilical artery Doppler (SGA/Doppler) were associated with elevated log-transformed sFlt1/PlGF ratio. The red reference line denotes an sFlt1/PlGF ratio of 85. Participants with >1 adverse outcome (n=5) were assigned to the abruption group or the SGA/Doppler group. Reassignment of these participants to other groups had no effect on the significance of the results.

sure, proteinuria, and uric acid; $P=0.013$ for creatinine; $P=0.011$ for platelet count).

In women presenting at <34 weeks' gestation, the sFlt1/PlGF ratio had superior performance to other parameters measured at the time of presentation, including systolic blood pressure, uric acid, ALT, and creatinine (as defined by greater AUC; Figure 3A). In patients presenting at <34 weeks' gestation, the combination of hypertension, proteinuria, and sFlt1/PlGF ratio was superior to hypertension and proteinuria alone for the prediction of adverse outcomes (Table 4). Uric acid, ALT, creatinine, and platelet count were not significant predictors of adverse outcome when systolic blood pressure, proteinuria, and sFlt1/PlGF were included in the model ($P=0.59$ for uric acid, $P=0.78$ for ALT, $P=0.74$ for creatinine, $P=0.78$ for platelet count). The AUC was similar when the model was restricted to only the first triage visit among the participants

Table 3. Tertile of Ratio of Soluble Fms-Like Tyrosine Kinase 1 to Placental Growth Factor and Risk of Adverse Outcome in All Participants and Those Presenting at <34 Weeks' Gestation

			Tertile 1		Tertile 2		Tertile 3	
	n	Adverse Outcomes, n (%)	Adverse Outcomes, n (%)	OR (95% CI)	Adverse Outcomes, n (%)	OR (95% CI)	Adverse Outcomes, n (%)	OR (95% CI)
All								
n			205		206		205	
S/P			≤9.7		>9.7 to <39.2		≥39.2	
All participants	616	268 (43.5)	43 (21.0)	Referent	78 (37.9)	2.3 (1.5–3.6)	147 (71.7)	9.5 (6.1–15.0)
Normotensive	280	73 (26.1)	18 (14.8)	Referent	28 (26.7)	2.1 (1.1–4.1)	27 (50.9)	6.0 (2.9–12.5)
Nonproteinuric	183	138 (31.9)	28 (16.4)	Referent	52 (32.1)	2.4 (1.4–4.1)	58 (58.0)	7.1 (4.0–12.4)
Presenting at <34 wk								
n			58		59		59	
S/P			≤4.2		>4.2 to <40.5		≥40.5	
All participants	176	59 (33.5)	4 (6.9)	Referent	9 (15.3)	2.4 (0.7–8.4)	46 (78.0)	47.8 (14.6–156.6)
Normotensive	76	11 (14.5)	2 (5.3)	Referent	2 (7.4)	1.4 (0.2–10.9)	7 (63.6)	31.5 (4.8–206.6)
Nonproteinuric	113	18 (15.9)	0 (0.0)	Referent*	5 (10.2)	Referent*	13 (61.9)	28.3 (8.0–99.7)

OR indicates odds ratio; CI, confidence interval; and S/P, ratio of soluble fms-like tyrosine kinase 1 to placental growth factor. Adverse outcomes were considered if they occurred within 2 weeks of presentation. Sample sizes and percents for each tertile represent the number of adverse outcomes and the percent of adverse outcomes from all those classified in a tertile.

*In nonproteinuric participants presenting at <34 weeks' gestation, the reference OR is the incidence of adverse outcome in tertiles 1 and 2 combined.

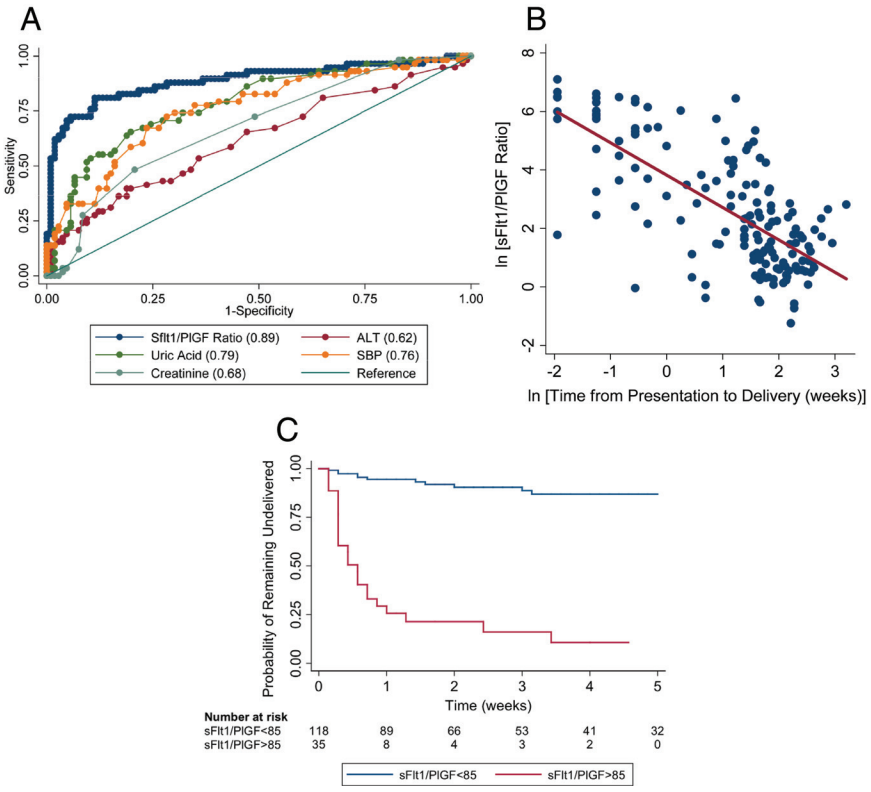

Figure 3. Predictive accuracy and correlation with duration of pregnancy of the ratio of soluble fms-like tyrosine kinase 1 (sFlt1) to placental growth factor (PlGF). **A**, Receiver-operating characteristic curves for the prediction of adverse outcomes with the sFlt1/PlGF ratio, alanine aminotransferase (ALT), uric acid, highest systolic blood pressure measured in triage (SBP), and creatinine in patients presenting at <34 weeks' gestation. All laboratory values were measured in blood collected at the time of presentation. The area under the curve (AUC) is given for each potential predictor in the legend. All AUCs were significantly different from that of the sFlt1/PlGF ratio alone ($P<0.01$). **B**, Time from presentation to delivery plotted vs the sFlt1/PlGF ratio at presentation in participants presenting at <34 weeks' gestation. $r=-0.71$, $P<0.0001$. **C**, Kaplan-Meier survival function for time to delivery in participants presenting at <34 weeks' gestation. Patients were censored at delivery or at 34 weeks' gestation.

(Figure II in the online-only Data Supplement). Exclusion of women with adverse outcomes at presentation (5%) did not substantially alter the prediction model.

ROC analysis suggested that an sFlt1/PlGF cut point of 85 would allow the maximum number of participants to be correctly classified with regard to adverse outcomes (87%). In women presenting at <34 weeks' gestation, a cut point of 85 gave a sensitivity of 72.9% and a specificity of 94.0%. Of the 126 women (71.5% of total women) who had an sFlt1/PlGF ratio <85, 16 experienced an adverse outcome (Table III in the online-only Data Supplement). This corresponded to a negative predictive value of 87.3% and a negative likelihood ratio of 0.29. Ten women with low sFlt1/PlGF ratio who experienced adverse outcomes had complicating conditions other than preeclampsia to which their adverse outcomes could have been attributed (Table III in the online-only Data Supplement). In 5 of the remaining 6 women, the adverse outcome recorded was indicated delivery, and the indication for delivery was headache. Of the 50 women with an sFlt1/PlGF ratio ≥85, 7 did not experience an adverse outcome within 2 weeks. This corresponded to a positive predictive value of 86.0% and positive likelihood ratio of 12.2. Interestingly, these 7 patients experienced adverse outcome after 3 weeks (Table IV in the online-only Data Supplement).

When we included participants presenting both before and after 34 weeks' gestation in the ROC analysis, the sFlt1/PlGF ratio had an accuracy similar to the highest systolic blood pressure measured in triage in predicting adverse outcomes (AUC=0.76 for sFlt1/PlGF versus 0.74 for systolic blood pressure; $P=0.44$). In this group, the sFlt1/PlGF ratio remained more accurate than other laboratory values in predicting adverse outcomes occurring within 2 weeks (AUC=0.76 versus uric acid [AUC=0.67; $P=0.0005$], serum ALT

Table 4. Area Under the Receiver-Operating Characteristic Curves for Each Combination of Possible Predictors

Predictors	Single-Variable AUC (95% CI)	Single-Variable *P*	Multivariable AUC (95% CI)†	Multivariable *P*
SBP+proteinuria	0.84 (0.78–0.90)	. . .	0.84 (0.78–0.90)	. . .
SBP+proteinuria+UA	0.86 (0.81–0.92)	0.24	0.86 (0.80–0.92)	0.22
SBP+proteinuria+S/P	0.93 (0.89–0.97)	0.001*	0.92 (0.88–0.96)	0.001*
SBP+proteinuria+S/P+UA	0.92 (0.88–0.96)	0.003*	0.92 (0.88–0.96)	0.002*

AUC indicates area under the curve; CI, confidence interval; SBP, highest systolic blood pressure in triage; UA, uric acid; and S/P, ratio of soluble fms-like tyrosine kinase 1 to placental growth factor. *P* values are for the comparison of each set of predictors against the combination of SBP and proteinuria alone.

*Significant at <0.05.

†Controlling for maternal age, parity, body mass index, and smoking.

[AUC=0.56; $P<0.0001$], or serum creatinine [AUC=0.60; $P<0.0001$]). When we excluded indicated delivery from the composite outcome measure among all participants, the AUC for prediction of adverse outcomes by the sFlt1/PlGF ratio substantially improved from 0.76 (95% CI, 0.72–0.80) to 0.85 (95% CI, 0.79–0.91).

sFlt1/PlGF and Remaining Duration of Pregnancy

The sFlt1/PlGF ratio was inversely correlated with the time elapsed between presentation and delivery (natural log transformed; $r=-0.51$, $P<0.0001$) in all participants. This relationship was strengthened with elimination of women presenting at 34 weeks gestation or greater ($r=-0.71$, $P<0.0001$; Figure 3B). Overall, among women presenting at <34 weeks' gestation, delivery occurred within 2 weeks of presentation in 86.0% of women with sFlt1/PlGF ratio ≥85 compared with 15.8% of women with sFlt1/PlGF ratio <85 ($P<0.001$). Time-to-event analysis confirmed a relationship between sFlt1/PlGF ratio and time to delivery at <34 weeks' gestation (hazard ratio, 15.2; 95% confidence interval, 8.1–28.7; Figure 3C). This relationship was slightly attenuated but remained significant after adjustment for gestational age at presentation, highest systolic blood pressure measured in triage, and proteinuria at presentation (hazard ratio, 9.4; 95% confidence interval, 4.7–18.7).

Discussion

This study focused on third-trimester women evaluated for suspected preeclampsia. The data indicate that a plasma sFlt1/PlGF ratio ≥85 at presentation was associated with adverse outcomes and imminent delivery within 2 weeks of presentation. Moreover, in women presenting before gestational week 34, the ratio of sFlt1/PlGF performed considerably better than other laboratory tests and clinical signs currently used to predict such outcomes. These angiogenic factor assays performed particularly well in women presenting atypically with relatively normal blood pressures or with no proteinuria. Notably, the sFlt1/PlGF ratio at presentation was inversely correlated with the remaining duration of pregnancy. These results suggest that the sFlt1/PlGF ratio may be a useful tool for the triage of women undergoing initial evaluation for suspected preeclampsia.

These data have important clinical implications. Currently, the risk stratification of patients with preeclampsia is dependent on the classification of women into 3 major categories: no preeclampsia, mild preeclampsia, or severe preeclampsia. Diagnosis of preeclampsia frequently requires hospital admission because 24 to 48 hours may be needed to evaluate serial blood pressure and laboratory values and to collect a 24-hour urine specimen for quantification of protein.[3] This information is used to guide the decision to pursue expectant management, especially when patients present at <34 weeks' gestation. However, previous studies have suggested that these criteria are inaccurate in predicting maternal and fetal complications.[5,21,22] Consistent with this, we found fair predictive value for laboratory values and signs generally considered indicative of severe preeclampsia. In contrast, the sFlt1/PlGF ratio had high predictive value in women at <34 weeks' gestation and was particularly useful among participants who presented atypically.

Accurate risk stratification of women with suspected preeclampsia has the potential to reduce unnecessary admissions, inappropriate discharges; and the considerable morbidity associated with iatrogenic preterm delivery. For example, women with nonspecific symptoms (eg, headache) may not require immediate delivery if the sFlt1/PlGF is low and other concerning signs are not present. Conversely, women presenting with atypical signs and symptoms, eg, those without hypertension and/or proteinuria, may not be currently classified as high risk for adverse outcomes but may in fact be at considerable risk if the sFlt1/PlGF ratio is high. Identifying these high-risk women at initial presentation may provide an opportunity for expedited transfer to higher-level medical facilities, administration of betamethasone, and appropriate counseling for anticipated preterm delivery. Furthermore, the sFlt1/PlGF ratio may be useful as a surrogate end point in ongoing clinical trials of therapy for preeclampsia.[23,24] Moreover, the addition of a test that accurately predicts subsequent adverse outcomes to initial preeclampsia evaluation has the potential to decrease costs associated with multiple outpatient evaluations and inpatient admissions in women with suspected preeclampsia. Although the data presented here provide compelling evidence that sFlt1/PlGF testing may be a useful adjunct to preeclampsia evaluation, this requires further testing in randomized trials.

The sFlt1/PlGF ratio did not identify women at risk of adverse outcomes related to conditions such as acute fatty liver of pregnancy, longstanding oligohydramnios, or chronic hypertension, suggesting that these markers are quite specific for preeclampsia. The predictive accuracy of the sFlt1/PlGF ratio was best in women presenting at gestational ages <34 weeks. The reason could be that obstetric care providers have a lower threshold for delivering women after 34 weeks' gestation[25,26]; thus, women presenting at late gestational ages with any suspicion of preeclampsia will likely be delivered. When indicated delivery was eliminated from the composite outcome measure, the sFlt1/PlGF ratio performed significantly better in the full cohort of women (data not shown). It is also possible that because sFlt1/PlGF ratios tend to increase slightly after 36 weeks' gestation in all women,[20] the sFlt1/PlGF ratio may not have the same discriminatory power at late gestational ages. Despite these considerations, because of the substantial risk of neonatal morbidity in women presenting at <34 weeks' gestation, accurate risk prediction has the greatest potential for benefit in this subgroup of patients.[27]

We found that the AUC was highest for the sFlt1/PlGF ratio compared with individual biomarkers alone (Table IA and IB in the online-only Data Supplement). This ratio has previously been shown to be highly correlated with preeclampsia diagnosis,[15,16] which supports prior findings that suggest that an angiogenic imbalance exists in women with preeclampsia with elevated levels of an antiangiogenic factor (sFlt1) and low levels of a proangiogenic factor (PlGF).[7,9,12] Although previous studies have shown that sFlt1 and/or PlGF levels may be useful in the diagnosis of preeclampsia,[15,16] we were unable to locate any studies that examined the association between levels of these proteins and subsequent adverse

maternal and perinatal outcomes. Our data therefore extend the findings of prior studies showing that a higher sFlt1/PlGF ratio is associated with the diagnosis of preeclampsia and more severe disease.[7,9,13,28] Previous studies examining methods for risk stratification of women with hypertensive disorders of pregnancy have generally shown these methods to have fair accuracy.[5,29] A recent prospective study used clinically available information to predict the risk of adverse outcomes in preeclampsia with an accuracy comparable to that described in the present study.[30] Several important differences exist between that study and our own, including a different study population, alternative adverse outcomes, and different time periods during which these outcomes were assessed.

Preeclampsia is a multisystem disease disorder, although its characteristic lesion, glomerular endotheliosis, occurs in the kidney. However, clinicians currently diagnose preeclampsia using clinical parameters: the appearance of de novo third-trimester hypertension and proteinuria. As demonstrated in the literature during a period when renal biopsies were performed, the clinical diagnosis was incorrect in as many as 15% to 20% of nulliparas and >30% of multiparas.[31] More recently, data from animal models have demonstrated that glomerular endotheliosis is a consequence of neutralization of vascular endothelial growth factor signaling by sFlt1 in the glomerulus.[9,32,33] These findings suggest the possibility that abnormally high sFlt1 levels may serve as a surrogate for glomerular endotheliosis, thus delineating those women in whom diagnosis is most certain and in whom higher levels of sFlt1 result in more severe endothelial abnormalities. Although this is speculative, the data here demonstrating the accuracy of the angiogenic factors in predicting adverse outcomes support this hypothesis.

Some limitations of our study merit consideration. This study was observational, and current practice limited the ability to fully test the accuracy of the sFlt1/PlGF ratio for risk stratification. Demonstrative of this is the fact that several women with low sFlt1/PlGF ratios were classified as having adverse outcomes (indicated delivery) even though they were delivered for a potentially benign reason: headache. It is unclear whether headache in these women was related to impending eclampsia or more common non–preeclampsia-related causes. A larger study with the exclusion of indicated delivery from the composite outcome measure might have partially addressed this limitation, but the fortunate rarity of devastating outcomes (such as eclampsia and death) in the developed world will continue to limit the ability of an observational study to test the true accuracy of the sFlt1/PlGF ratio. Because of the limited sample size, we were unable to examine in detail the various adverse outcomes individually. In addition, because this is an observational study, we cannot evaluate whether the alterations in these biomarkers are the cause or effect of preeclampsia.

Conclusions

The sFlt1/PlGF ratio is strongly associated with adverse outcomes and imminent delivery in women presenting with signs and/or symptoms of preeclampsia in this pilot study. The use of the sFlt1/PlGF ratio for risk stratification of women undergoing preeclampsia evaluation in the obstetric triage unit has the potential to reduce both morbidity and healthcare costs. The availability of a method like this to quickly and accurately assess risk for preeclampsia-associated adverse outcomes at initial presentation could aid in the management of patients with suspected preeclampsia. Future studies should validate our findings in large multicenter cohorts in different populations to determine the impact of clinical decisions based on results of sFlt1/PlGF testing on health outcomes and to assess the cost-effectiveness of management strategies based on sFlt1/PlGF testing.

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Disclosures

Dr Verlohren is a consultant to Roche Diagnostics. Dr Thadhani is a coinventor on patents related to the prediction of preeclampsia that have been licensed to diagnostic companies and has financial interest in Aggamin LLC. Dr Karumanchi is a coinventor of multiple patents related to angiogenic proteins for the diagnosis and therapy of preeclampsia that have been licensed to multiple companies. Dr Karumanchi reports having served as a consultant to Roche and Beckman Coulter and has financial interest in Aggamin LLC. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Preeclampsia is one of the most common medical complications of pregnancy, yet identification of patients at risk for preeclampsia-related adverse outcome remains elusive. Circulating angiogenic factors are involved in the pathogenesis of preeclampsia; thus, we hypothesized that altered angiogenic factor levels predict adverse maternal and perinatal outcomes in women being evaluated for preeclampsia. Here, we report 3 new findings. First, in women with suspicion of preeclampsia, the ratio of soluble fms-like tyrosine kinase 1 (sFlt1) to placental growth factor (PlGF) at presentation is strongly associated with subsequent maternal and perinatal adverse outcomes. Second, in women presenting at <34 weeks' gestation, the sFlt1/PlGF ratio performs better than other currently available clinical signs and laboratory tests in the prediction of adverse outcomes. Third, the sFlt1/PlGF ratio is inversely correlated with the duration of pregnancy. Estimating the timing of delivery will help risk stratify patients, determine whether patients should be transferred to a tertiary care facility for anticipated delivery, and assess whether administration of steroids is warranted. The sFlt1/PlGF ratio accurately predicts subsequent adverse outcomes and has the potential to decrease costs associated with multiple outpatient evaluations and inpatient admissions in women with suspected preeclampsia.

Angiogenic Factors and the Risk of Adverse Outcomes in Women With Suspected Preeclampsia

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SUPPLEMENTAL MATERIAL

Supplementary Table 1: Individual data for sFlt1, PlGF and ratio among all participants and excluding re-enrollments

Supplementary Table 1A

All patients included	Predictors	Median (25th-75th centile)		AUC (95% CI)
		No adverse outcome	Adverse outcome	
All Subjects	sFlt1 (pg/ml)	2,999 (1,963 - 4,958)	5,708 (3,251 - 9,003)	0.72 (0.68, 0.76)
	PlGF (pg/ml)	278 (155 - 538)	124 (70 - 228)	0.74 (0.70, 0.78)
	sFlt1/PlGF ratio	11 (4 - 29)	47 (16 - 112)	0.76 (0.72, 0.80)
<34 Weeks	sFlt1 (pg/ml)	2,102 (1,465 - 3,030)	10,399 (4,410 - 15,648)	0.87 (0.81, 0.94)
	PlGF (pg/ml)	440 (180 - 761)	51 (25 - 119)	0.87 (0.81, 0.93)
	sFlt1/PlGF ratio	4 (2 -14)	226 (50 - 547)	0.89 (0.83, 0.95)

Supplementary Table 1B

Excluding re-enrollments	Predictors	Median (25th-75th centile)		AUC (95% CI)
		No adverse outcome	Adverse outcome	
All Subjects	sFlt1 (pg/ml)	2,999 (2,003 - 5,031)	6,085 (3,270 - 9,876)	0.73 (0.69, 0.78)
	PlGF (pg/ml)	281 (158 - 538)	124 (66 - 222)	0.75 (0.71, 0.79)
	sFlt1/PlGF ratio	11 (4 - 28)	51 (15 - 126)	0.78 (0.74, 0.82)
<34 Weeks	sFlt1 (pg/ml)	2,076 (1,439 - 3,007)	10,509 (7,309 - 15,585)	0.88 (0.82, 0.95)
	PlGF (pg/ml)	440 (186 - 793)	49 (25 - 91)	0.88 (0.82, 0.94)
	sFlt1/PlGF ratio	4 (2 - 13)	248 (111 - 547)	0.90 (0.84, 0.96)

Supplementary Table 2: sFlt1/PlGF tertile and risk of adverse outcome in all participants and those presenting at less than 34 weeks gestation (excluding re-enrollments)

All			**Tertile 1 (n=178, S/P≤9.6)**		**Tertile 2 (N=179, 9.6<S/P<39.9)**		**Tertile 3 (n=178, S/P≥39.9)**	
	N	**Adverse Outcomes**	**Adverse Outcomes**	**OR (95% CI)**	**Adverse Outcomes**	**OR (95% CI)**	**Adverse Outcomes**	**OR (95% CI)**
All Participants	535	223 (41.7%)	32 (17.9%)	Ref	65 (36.3%)	2.6 (1.6, 4.2)	126 (70.8%)	11.1 (6.7, 18.2)
Normotensive	247	54 (21.9%)	12 (11.1%)	Ref	20 (21.1%)	2.1 (1.0, 4.6)	22 (50.05)	8 (3.4, 18.6)
Non-Proteinuric	377	110 (29.2%)	24 (15.6%)	Ref	40 (29.0%)	2.2 (1.3, 3.9)	46 (54.1%)	6.4 (3.5, 11.8)
Presenting at <34 Weeks			**Tertile 1 (n=52, S/P≤4.0)**		**Tertile 2 (N=53, 4.0<S/P<50.4)**		**Tertile 3 (n=53, S/P≥50.4)**	
	N	**Adverse Outcomes**	**Adverse Outcomes**	**OR (95% CI)**	**Adverse Outcomes**	**OR (95% CI)**	**Adverse Outcomes**	**OR (95% CI)**
All Participants	158	53 (33.5%)	3 (5.8%)	Ref	8 (15.1%)	2.9 (0.7, 11.6)	42 (79.3%)	62.3 (16.3, 238.4)
Normotensive	70	8 (11.4%)	2 (5.7%)	Ref*	0 (0.0%)	Ref*	6 (66.7%)	58.9 (8.2, 425.7)
Non-Proteinuric	102	15 (14.7%)	0 (0.0%)	Ref*	5 (11.4%)	Ref*	10 (58.8%)	22.9 (6.1, 85.8)

Risk of adverse outcomes in participants when only the first triage visit was included for the analyses. Adverse outcomes were considered if they occurred within 2 weeks of presentation. S/P= sFlt1/PlGF ratio.
*In normotensive and non-proteinuric participants presenting at less than 34 weeks, the reference odds ratio is the incidence of adverse outcome in tertiles 1 and 2 combined.

Supplementary Table 3: Clinical features of participants with sFlt1/PlGF ratio less than 85 at presentation who experienced adverse outcomes within 2 weeks

Study ID	GA at presentation (wks)	sFlt1/PlGF ratio	Adverse Outcome	Delivery Indication	GA at Delivery	Other Clinical Information
41	28.4	50.3	Delivery	HTN, NRFHT	28.4	Chronic HTN
153	28.0	2.4	Neonatal death	NRFHT, abruption	28.0	Chronic abruption and oligohydramnios since 16 wks, Potter's sequence
185	32.2	5.9	Delivery	Headache	32.3	Gestational HTN
227	31.0	25.8	Delivery	NRFHT	31.2	Chronic HTN
253	33.3	17.3	Delivery	Elective	35.1	Chronic HTN
303	33.4	3.0	Delivery	Headache	35.1	PE
305	33.4	58.1	Delivery	Headache	34.1	PE, Breech
399	31.1	1.3	Delivery, Elevated LFTs	AFLP	32.5	
427	33.0	11.6	Delivery	Abruption	33.2	Chronic HTN, no pathologic evidence of abruption
458	29.0	5.02	Delivery	Headache	29.0	PE
572	33.5	8.6	Delivery	Elective	34.3	Chronic HTN
598	32.0	0.95	Delivery	Headache	32.4	Chronic HTN
612	32.3	40.4	Delivery	Headache, HTN	33.1	PE
710	33.6	45.9	Delivery	Abruption	35.5	Gestational HTN, no pathologic evidence of abruption
718	33.4	38.0	Delivery	NRFHT	33.4	Chronic HTN
738	26.2	15.6	Acute Renal Failure	Labor	26.6	Chronic HTN, renal disease, diabetes, s/p gastric bypass

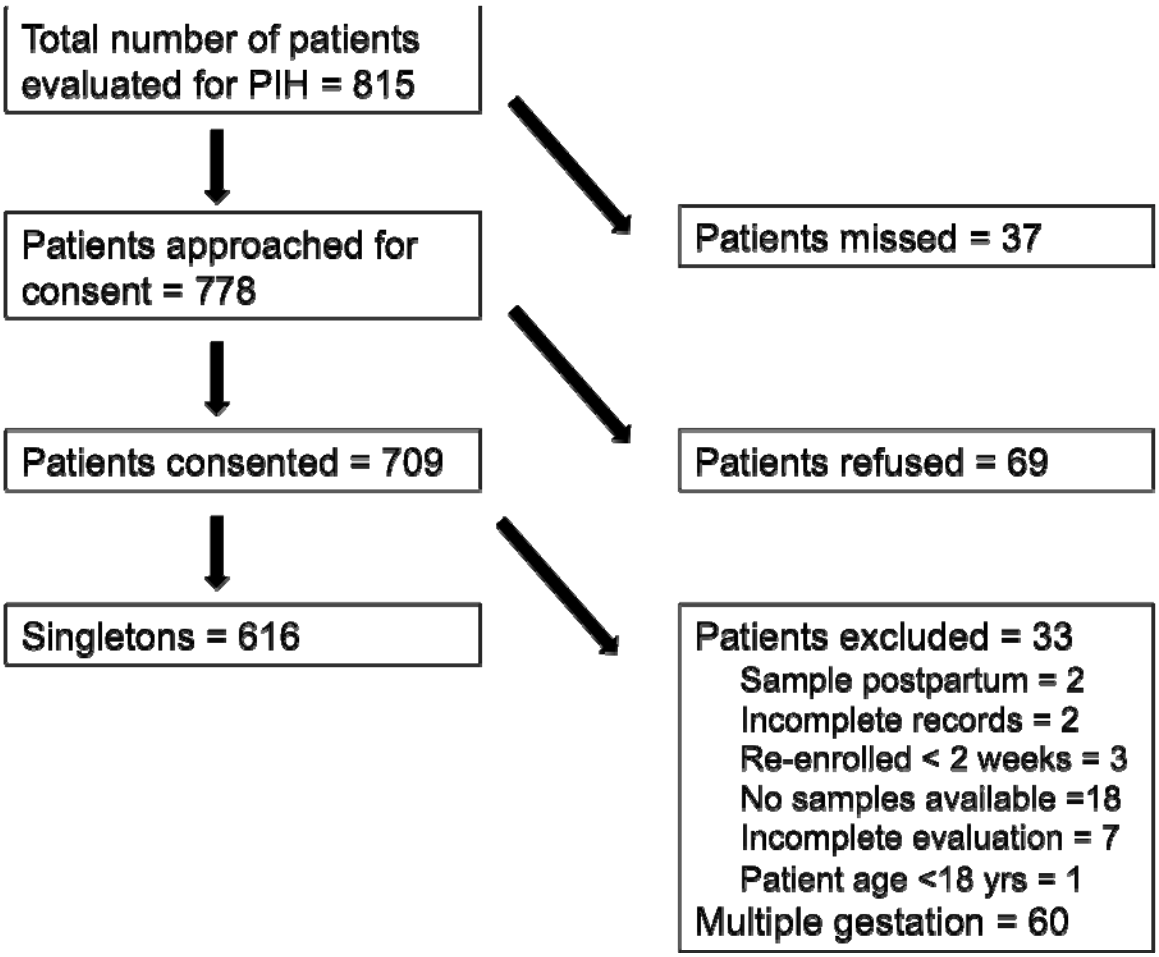
Adverse outcomes were considered if occurring within 2 weeks of presentation. GA=gestational age, HTN=hypertension, NRFHT=non-reassuring fetal heart tracing, PE= preeclampsia, LFT=liver function test, AFLP=acute fatty liver of pregnancy.

Supplementary Table 4: Clinical features of participants with sFlt1/PlGF ≥85 at presentation, but no adverse outcome occurring within in 2 weeks

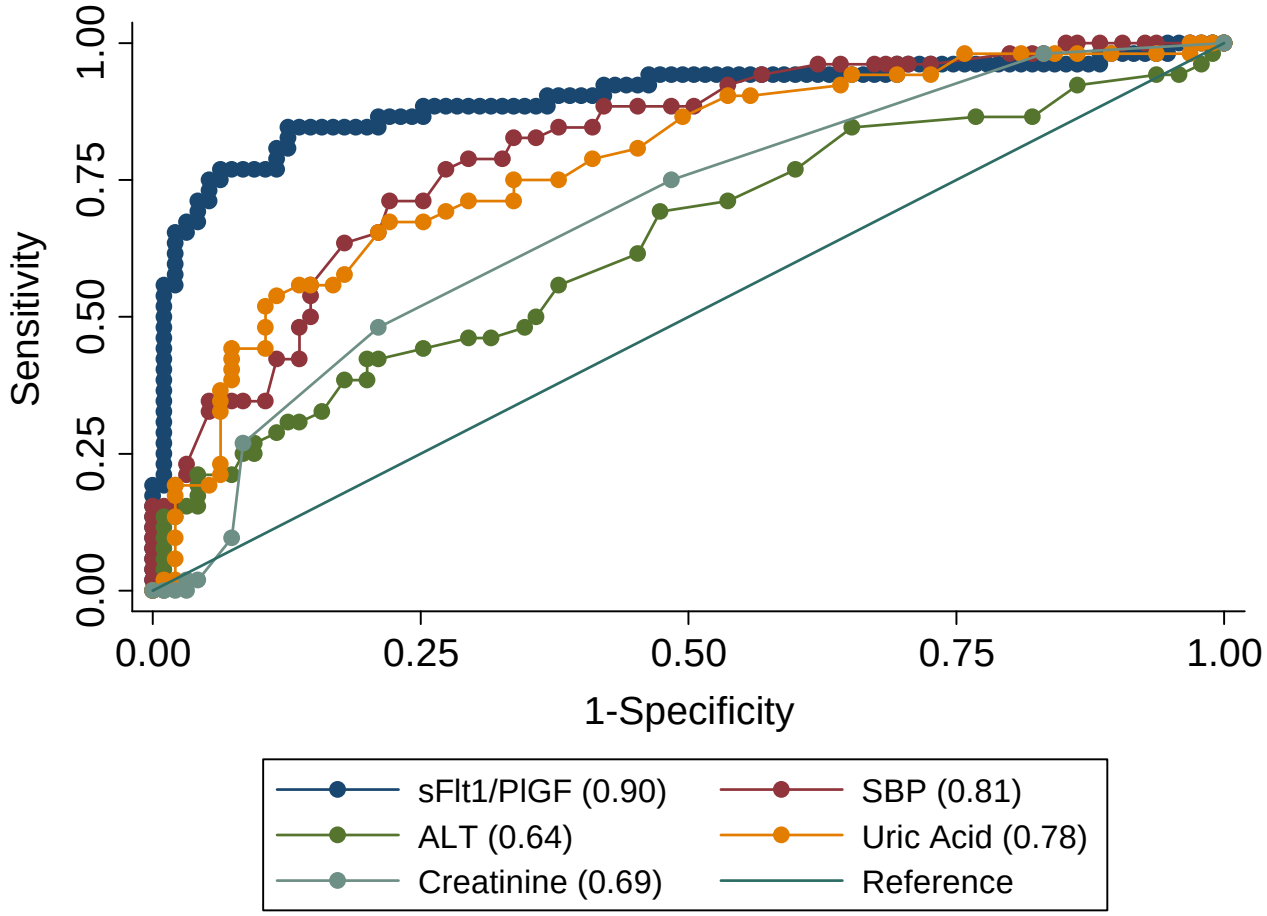
Study ID	GA at presentation (wks)	sFlt1/PlGF ratio	Clinical Diagnosis at 2 Weeks	Final Clinical Diagnosis	GA at Delivery	Other Clinical Information
42	23.5	627.8	Gestational HTN	Severe PE	27.1	Neonatal Death
238	32.2	125.0	No Hypertensive Dx	PE, Appendicitis	36.2	
306	33.3	107.6	Gestational HTN	Severe PE	37.4	Severe HTN
339	30.4	312.8	Mild PE	Severe PE	33.0	HELLP syndrome
638	29.3	210.0	No Hypertensive Dx	Severe PE	34.2	HELLP syndrome
741	32.6	147.0	Gestational HTN	Severe PE	35.5	Severe HTN
753	30.2	143.5	No Hypertensive Dx	Severe PE	34.5	Severe HTN

GA=gestational age, wks= weeks, HTN= hypertension, PE= preeclampsia, HELLP= Hemolysis, Elevated Liver enzymes and Low Platelets.

Supplementary Figure 1: Enrollment and exclusions



Supplementary Figure 2: Predictive accuracy of the sFlt1/PlGF ratio in patients less than 34 Weeks gestation (excluding re-enrollments)



Predictive accuracy is shown for various analytes amongst participants when only the first triage visit was included for the analyses. The above figure shows receiver operating characteristic curves for prediction of adverse outcomes using the sFlt1/PlGF ratio, ALT (alanine aminotransferase), uric acid, SBP (highest systolic blood pressure measured in triage) and creatinine. All laboratory values were measured in blood collected at the time of presentation. The area under the receiver operator curve (AUC) is given for each potential predictor in the legend. All AUC's were significantly different from that of the sFlt1/PlGF ratio alone ($p<0.01$).