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MATERNAL PLASMA CONCENTRATIONS OF ANGIOGENIC/ANTI-ANGIOGENIC FACTORS ARE OF PROGNOSTIC VALUE IN PATIENTS PRESENTING TO THE OBSTETRICAL TRIAGE AREA WITH THE SUSPICION OF PREECLAMPSIA

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Abstract

Objective—To determine if maternal plasma concentrations of placental growth factor (PlGF), soluble endoglin (sEng), soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and -2 could identify patients at risk for developing preeclampsia (PE) requiring preterm delivery.

Study design—Patients presenting with the diagnosis 'rule out PE' to the obstetrical triage area of our hospital at <37 weeks of gestation (n=87) were included in this study. Delivery outcomes were used to classify patients into 4 groups: I) patients without PE or those with gestational hypertension (GHTN) or chronic hypertension (CHTN) who subsequently developed PE at term (n=19); II): mild PE who delivered at term (n=15); III): mild disease (mild PE, GHTN, CHTN) who subsequently developed severe PE requiring preterm delivery (n=26); and IV): diagnosis of severe PE (n=27). Plasma concentrations of PlGF, sEng, sVEGFR-1 and -2 were determined at the time of presentation by ELISA. Reference ranges for analytes were constructed by quantile regression in our laboratory (n=180; 1,046 samples). Comparisons among groups were performed using multiples of the median (MoM) and parametric statistics after log transformation. Receiver operating characteristic curves, logistic regression and survival analysis were employed for analysis.

Results—The mean MoM plasma concentration of PlGF/sVEGFR-1, PlGF/sEng, PlGF, sVEGFR-1 and -2, and sEng in Group III was significantly different from Group II (all $p<0.05$). A plasma concentration of PlGF/sVEGFR-1 ≤ 0.05 MoM or PlGF/sEng ≤ 0.07 MoM had the highest

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Declaration of interest

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likelihood ratio of a positive test (8.3, 95% CI 2.8–25 and 8.6, 95% CI 2.9–25, respectively), while that of PlGF ≤0.396 MoM had the lowest likelihood ratio of a negative test (0.08, 95% CI 0.03–0.25). The association between low plasma concentrations of PlGF/sVEGFR-1 (≤0.05 MoM) as well as that of PlGF/sEng (≤ 0.07 MoM) and the development of severe PE remained significant after adjusting for gestational age at presentation, average systolic and diastolic blood pressure, and a history of chronic hypertension [adjusted odds ratio (OR) = 27 (95% CI 6.4–109) and adjusted OR 30 (95% CI 6.9–126), respectively]. Among patients who presented <34 weeks gestation (n=59), a plasma concentration of PlGF/sVEGFR-1 <0.033 MoM identified patients who delivered within 2 weeks because of PE with a sensitivity of 93% (25/27) and a specificity of 78% (25/32). This cut-off was associated with a shorter interval-to-delivery due to PE [hazard ratio = 6 (95% CI 2.5–14.6)].

Conclusions—Plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in the obstetrical triage area. These observations support the value of these biomarkers in the clinical setting for the identification of the patient at risk for disease progression requiring preterm delivery.

Keywords

placental growth factor (PlGF); soluble endoglin (sEng); soluble vascular endothelial growth factor receptor-1 (sVEGFR-1); soluble vascular endothelial growth factor receptor-2 (sVEGFR-2); preeclampsia; IUGR; preterm labor; automated assay; angiogenesis; sflt-1

INTRODUCTION

Preeclampsia (PE) remains one of the leading causes of maternal mortality/morbidity worldwide [1–9]. Neonates born to mothers with preeclampsia are at risk for complications because of prematurity and small-for-gestational age [10–13]. Moreover, recent studies also suggest that patients with PE are at risk for death from cardiovascular disease and stroke later in life [14–19]. The only effective treatment for preeclampsia is delivery. Several preventive measures such as supplementation with vitamin C and vitamin E [20–26], aspirin [27–34], or calcium [35,36] also fail to consistently prevent subsequent development of PE.

One in 10 pregnant women develop some signs and symptoms observed in PE, and only about 20% of such patients are eventually diagnosed to have PE [37–39]. Women with signs and symptoms observed in PE (e.g. headache, abdominal pain, edema, etc.) are usually referred to an obstetrical triage area, where they undergo evaluation for maternal and fetal involvement. The standard work-up includes blood pressure determination, urine analysis for protein and the determination for uric acid, platelet counts and liver enzymes in peripheral blood [39–42]. However, the diagnostic performance of these tests is controversial and several studies report poor performance in the prediction of PE in women with suspected gestational hypertension [43–45]. Furthermore, many symptoms associated with PE, such as headache, epigastric pain and visual disorders, are subjective and non-specific. Consequently, many patients with signs and symptoms observed in PE are hospitalized for observation. Those who are diagnosed with PE at preterm gestations undergo long-term hospitalization or frequent monitoring as outpatients (in some cases of mild PE), if they qualify for expectant management. The lack of adequate biomarkers to predict disease progression and/or adverse maternal/perinatal outcomes [10,46] makes clinical decision-making a challenge for physicians (e.g. hospitalization, intensive surveillance, repeated blood test, induction of labor, etc.). This clinical uncertainty results in excessive utilization of the laboratory, ultrasound, antepartum testing, etc. and is a cause for additional charges to the health care system [47–51]. Therefore, a new set of tests with a better prognostic performance in the identification of patients who will develop severe PE

requiring preterm delivery or those who will develop maternal/fetal complications is highly desirable and urgently needed.

A test with a low false positive rate has the potential to reduce the costs of intensive monitoring of these patients to avoid unnecessary hospitalization, while that with a low false negative rate can potentially decrease the likelihood of complications. Early diagnosis may allow prevention of the complications of preeclampsia by timely delivery [52].

A growing body of evidence suggests that an imbalance of angiogenic/anti-angiogenic factors is involved in the pathophysiology of PE [53–75]. The changes in concentrations of the angiogenic factor placental growth factor (PlGF), and the anti-angiogenic factors soluble vascular endothelial growth factor receptor (sVEGFR)-1 and -2 as well as soluble endoglin (sEng) in maternal circulation, precede the manifestations of disease with a higher magnitude in preterm than in term PE [55,57,76–80]. Most studies examining the value of these biomarkers have focused on the prediction of disease in the first and second trimesters. The results of such studies largely suggest that an imbalance between angiogenic and anti-angiogenic factors increases the likelihood of preterm PE, but not of term PE [65,75,78,81–84]. However, not all studies have uniform results [85,86]. Yet, even if these biomarkers had excellent predictive values for preterm preeclampsia, a major issue is that there is not a clear intervention that can prevent the subsequent development of the disease. Thus, the value of such biomarkers remains either a “research procedure” or a method of risk assessment to identify patients who may benefit from more intensive surveillance[75,78,81–86]. As of yet, there are no studies evaluating the role of plasma angiogenic and anti-angiogenic factors in predicting the outcome of patients with suspected PE admitted to the obstetrical triage area.

The objective of this study was to examine if plasma concentrations of angiogenic/anti-angiogenic factors in patients presenting with the diagnosis ‘rule out PE’ to the obstetrical triage area with preterm gestations have prognostic value in the identification of patients who would require preterm delivery because of PE.

MATERIALS AND METHODS

Study design

A retrospective cohort study was conducted by searching our clinical database and bank of biologic samples. Patients who were sent to the obstetrical triage area for the diagnosis of “suspected PE” and had blood samples taken prior to medications between 20–36 weeks of gestation were included (n=87). Exclusion criteria were: 1) known major fetal or chromosomal anomaly; and 2) multiple gestations.

Delivery outcomes were reviewed and used to retrospectively classify the patients into 4 groups: Group I: patients without PE or those with gestational hypertension or chronic hypertension who subsequently developed mild PE at ≥37 weeks (n=19); Group II: mild PE or those with gestational hypertension or chronic hypertension who developed mild PE before 37 weeks and either remained stable until term or delivered preterm spontaneously (n=15); Group III: mild disease (mild PE, gestational hypertension, chronic hypertension) who subsequently developed severe PE requiring preterm delivery (n=26); and Group IV: severe PE at admission (n=27).

Reference ranges for sVEGFR-1, sEng, PlGF, sVEGFR-2, PlGF/sVEGFR-1 and PlGF/sEng were constructed from a separate set of uncomplicated pregnant women (n=180) who were enrolled in a longitudinal study and delivered at term. Pregnancies were considered to be “uncomplicated” if patients did not have major obstetrical, medical or surgical

complications during pregnancy, and delivered a term neonate whose birthweight was between the 10–90th percentile using the reference range of Alexander et al [87].

All women were enrolled at Hutzel Women's Hospital, Detroit, MI and followed until delivery. All patients provided written informed consent for the collection and use of samples for research purposes under the protocols approved by the Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services (NICHD/NIH/DHHS).

Clinical definitions

PE was defined as the new onset of hypertension that developed after 20 weeks of gestation and proteinuria [40]. Hypertension was defined as systolic ≥140 or diastolic blood pressure ≥90 mm Hg, measured at two occasions, 4 h–1 week apart. Proteinuria was defined as a urine protein of ≥300 mg in a 24-h urine collection, or two random urine specimens obtained 4 h–1 week apart containing ≥1+ by dipstick or one dipstick demonstrating ≥2+ protein [88]. Gestational hypertension was defined as hypertension without proteinuria after 20 weeks of gestation. Chronic hypertension was defined as women with hypertension before 20 weeks of gestation or those who reported a history of hypertension. Chronic hypertension with superimposed PE was diagnosed in women with a diagnosis of chronic hypertension with a sudden increase in blood pressure or proteinuria [40].

Severe hypertension was defined as systolic blood pressure of at least 160 and/or diastolic blood pressure of at least 110 mm Hg. Severe proteinuria was diagnosed by a 24-hour urine sample containing 5 gm protein or two random urine specimens with 3+ protein by dipstick. Severe preeclampsia was defined as severe hypertension with proteinuria, mild hypertension with severe proteinuria or mild preeclampsia with one of the following [40]: 1) development of neurological symptoms (severe headache, scotoma, epigastric pain); 2) oliguria (<500 mL/24 hours); 3) pulmonary edema; 4) thrombocytopenia (platelet counts <100,000/mm3 in the absence of other known causes); 5) evidence of hepatic dysfunction (increased aspartate aminotransferase level of 70 IU/L and/or increased alanine aminotransferase level of 70 IU/L); 6) HELLP (hemolysis, elevated liver enzymes, low platelet) syndrome; 7) small-for-gestational age fetuses which was defined as estimated fetal weight of <10th percentile for gestational age confirmed by neonatal birthweight; or 8) eclampsia. The diagnosis of HELLP syndrome required the presence of thrombocytopenia, evidence of hepatic dysfunction and hemolysis (lactate dehydrogenase >600 IU/L or the presence of schistocytes on the peripheral blood smear).

Sample collection and immunoassays

Venipuncture was performed on admission to the triage area and the blood was collected into tubes containing EDTA. Samples were centrifuged and stored at −70C. Maternal plasma concentrations of PlGF, sVEGFR-2, sEng and sVEGFR-1 were determined by sensitive and specific immunoassays obtained from R&D Systems (Minneapolis, MN). All immunoassays utilized the quantitative sandwich enzyme immunoassay technique, and their concentrations in maternal plasma were determined by interpolation from the standard curves. The inter- and intra-assay coefficients of variation (CV) obtained were as follows: PlGF, 6.02 and 4.8%, respectively; sVEGFR-2, 2 and 4%, respectively; sEng, 2.3 and 4.6%, respectively; and sVEGFR-1, 1.4 and 3.9%, respectively. The sensitivity of the assays were as follows: PlGF, 9.52 pg/ml; sVEGFR-2, 19.01 pg/ml; sEng, 0.08 ng/ml and sVEGFR-1, 16.97 pg/ml. The validation of these assays has been described in previous studies [89].

STATISTICAL ANALYSIS

Reference range for PlGF, sEng, sVEGFR-1 and sVEGFR-2 using the quantile regression model

Data was log transformed to achieve normality. A quantile regression model [90] was used to estimate the q-th conditional quantile function of the response (log analyte concentration), given the covariate (gestational age). To allow for a non-linear relation between the analyte level and gestational age, a moving window approach was used by iteratively fitting one model for every discrete gestational age encountered in the dataset. At each iteration, only samples having the exact same gestational age were assigned a full weight of 1.0, while data points further away (in either direction) received smaller weights decreasing as dictated by a Gaussian distribution. The processes were repeated for each of the quantile of interest: 0.025, 0.05, 0.5, 0.95, and 0.975, hence constructing a 95% and 90% confidence interval around the median. When the concentration of an analyte fell below the limit of detection of the assay, the concentration was scored to correspond to 99% of the lowest detected concentrations in all samples. This was done to avoid dealing with zero values.

Analysis of the data

Comparisons among groups were performed using multiples of the median (MoM) derived from the observed over the expected median of the analyte concentration at each gestational age. After logarithmic transformation, Analysis of Variance (ANOVA) and post-hoc tests with Bonferroni correction for multiple comparisons were performed. The contingency table and χ^2 tests were used to compare the proportion among and between groups. Receiver operating characteristic (ROC) curves were used to determine the cut-offs at which each biomarker best identified the outcomes (i.e. severe preeclampsia and delivery within 14 days). Multiple logistic regression (backward-stepwise) was applied to estimate the association between angiogenic/anti-angiogenic factor concentrations and these outcomes, while adjusting for potential confounders (gestational age at presentation, average systolic and diastolic blood pressure measured in the obstetrical triage area, and a history of chronic hypertension). Survival analysis and Cox proportional hazard models were utilized to examine the relationship between sampling-to-delivery interval and plasma MoM concentrations of PlGF/sVEGFR-1 while adjusting for the above mentioned potential confounders. Patients who delivered preterm due to causes other than PE had the interval between triage and delivery treated as a censored observation. Analysis was conducted with SPSS V.15 (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

RESULTS

Demographic and clinical characteristic of the study groups

The demographic, clinical and obstetrical characteristics of the study population are presented in Tables I and II. There was no significant difference in the mean gestational age at presentation or at blood sampling among groups (ANOVA p=0.2). The severe PE group (Group IV) had the highest mean systolic and diastolic blood pressure among groups (ANOVA p<0.001). There was no significant difference in the mean systolic and diastolic blood pressure between patients with mild PE who delivered at term (Group II) and those who subsequently developed severe PE and delivered preterm (Group III) (Table I). Fourteen patients (73.7%) in Group I delivered at term, whereas the other five delivered preterm because of: spontaneous preterm labor/delivery (n=3), chronic hypertension with oligohydramnios (n=1) and gestational hypertension with non-reassuring fetal heart rate tracing (n=1). All patients in Group II developed PE before 37 weeks. Three delivered after spontaneous preterm labor and the rest remained stable until delivery at term (Table II).

Thirteen patients had maternal complications including placental abruption (n=5), pulmonary edema (n=4), HELLP (n=2) and eclampsia (n=2). All except one were in Groups III and IV (Table II). As expected, the mean gestational age at delivery and the mean birthweight in Groups III and IV were lower than in Groups I and II ($p<0.001$).

The demographic and clinical characteristics of uncomplicated pregnant women (n=180) whose samples (n=1,046) were used to constructed reference ranges for plasma concentrations of angiogenic/anti-angiogenic factors are displayed in Table III. The majority of patients (83%) were African-American and approximately one-third were nulliparous. Plasma samples were collected from each patient in the following eight intervals: 1) 6–9.9 weeks; 2) 10–14.9 weeks; 3) 15–19.9 weeks; 4) 20–23.9 weeks; 5) 24–27.9 weeks; 6) 28–31.9 weeks; 7) 32–36.7 weeks; 8) 37 weeks of gestation or more.

Maternal plasma concentrations of angiogenic/anti-angiogenic factors are associated with the severity and the subsequent development of preeclampsia requiring preterm delivery

Figures 1–6 display the mean plasma concentration of each analyte and their ratios in MoM units. This allows comparison among groups without adjustment for gestational age at venipuncture. The mean MoM plasma concentration of angiogenic/anti-angiogenic factors tested in this study was significantly different between patients with mild PE who subsequently developed severe PE, requiring preterm delivery (Group III), and those who remained stable until term (Group II) (each $p<0.05$; Figure 1–6). There was no significant difference in the mean MoM plasma concentration of any angiogenic/anti-angiogenic factors tested in this study between patients with mild PE who subsequently developed severe PE requiring preterm delivery (Group III) and those presented with severe preeclampsia (Group IV) (each $p>0.05$). The mean interval from diagnosis of mild preeclampsia to diagnosis of severe preeclampsia requiring preterm delivery was 17+18 days (median 10 days, inter-quartile range 6–19 days).

Table IV displays the MoM cut-offs (derived from ROC curves) for each angiogenic/anti-angiogenic factor and the diagnostic performance of each analyte for the identification of patients who developed severe preeclampsia (Group III +IV). A plasma concentration of PlGF/sEng ≤0.07 MoM had a sensitivity of 76% (40/53) and a specificity of 91% (31/34), while that of PlGF ≤0.396 MoM had a sensitivity of 94% (50/53) and a specificity of 71% (24/34) for the identification of patients who developed severe PE (Group III + IV). A plasma concentration of PlGF/sVEGFR-1 ≤ 0.05 MoM or PlGF/sEng ≤ 0.07 MoM had the highest likelihood ratio of a positive test (8.3, 95% CI 2.8–25 and 8.6, 95% CI 2.9–25, respectively), while that of PlGF ≤0.396 MoM had the lowest likelihood ratio of a negative test (0.08, 95% CI 0.03–0.25). The probability that a patient will develop severe PE after a positive test for PlGF/sVEGFR-1 ratio and the PlGF/sEng ratio was similar (93%), whereas that of a negative test for PlGF was 11% (Table IV).

The association between low plasma concentrations of PlGF/sVEGFR-1 (≤0.05 MoM) as well as that of PlGF/sEng (≤ 0.07 MoM) and the development of severe PE remained significant after adjusting for gestational age at presentation, average systolic and diastolic blood pressure in the triage area, and a history of chronic hypertension [adjusted odds ratio (OR) = 27 (95% CI 6.4–109) and adjusted OR 30 (95% CI 6.9–126), respectively].

Among 42 patients who had a plasma PlGF/sVEGFR-1 concentration below 0.05 MoM, three patients (7%) did not subsequently develop severe PE; two (0.003 MoM, 0.015 MoM) from Group II and one (0.027 MoM) from Group I. The first patient was referred to the triage area complaining of a headache and with a new-onset hypertension (142/99, 146/100 mmHg) at 32 4/7 weeks. She was hospitalized for 3 days, and her 24-hour urine sample showed a total protein of 512 mg. Two days after discharge, she returned to the hospital with

a diagnosis of spontaneous preterm labor and went on to deliver a neonate with a birthweight of 1790 grams (13th percentile). The second patient had underlying chronic hypertension and diabetes class B, and was referred to the obstetrical triage area at 35 6/7 weeks for borderline high blood pressure (117/71, 147/77 mmHg) and edema in the lower extremities. Her 24-hour urine collection showed a total protein of 944 mg. She developed preterm prelabor rupture of membranes the next day and subsequently delivered by C-section for non-reassuring fetal heart rate tracing. The last patient was referred to the obstetrical triage area at 33 weeks for high blood pressure (169/100, 157/87 mmHg) and a urine dip-stick was negative for protein. She delivered spontaneously the next day with an appropriate-weight-for gestational-age neonate. These 3 patients also had very high plasma concentrations of sEng (2.1, 7.6 and 11.2 MoM) and sVEGFR-1 (7.5, 10 and 18 MoM).

Among 45 patients who had a plasma PlGF/sVEGFR-1 concentration above 0.05 MoM, fourteen (31%) subsequently developed severe PE; 10 from Group III and 4 from Group IV. Five of these fourteen (36%) were presented after 34 weeks of gestation and among the other 9 patients, only 2 had plasma concentrations of these biomarkers above the 5th centile (0.532 MoM and 0.579 MoM respectively).

The first patient had an elevated blood pressure from 15 weeks of gestation onward, and thus, was diagnosed to have chronic hypertension. She was sent to the obstetrical triage area with a blood pressure of 142/87 mmHg at 27 3/7 weeks. She was hospitalized for 3 days and her 24-hour urine collection showed a total protein of 752 mg, which increased to 1280 mg in one month. At 36 3/7 weeks, the patient was hospitalized and diagnosed to have severe PE because of headache, high blood pressure (175/85), and an estimated fetal weight of less than the 5th percentile. A male baby, weight 2110 grams (4th percentile), was delivered by Cesarean section for an arrest of dilatation after induction of labor. Another patient presented at 26 weeks with new-onset high blood pressure of 152/67 mmHg. She was hospitalized for 3 days and her 24 hour urine collection showed a total protein of 525 mg, and the estimated fetal weight was < 10th %. Her blood pressure was stable until 36 6/7 weeks when she underwent induction of labor for headache, excessive weight gain and intrauterine growth restriction. A female baby, weight 2390 grams (6th percentile), was delivered by Cesarean section due to non-reassuring fetal heart rate tracing. The duration from blood sampling to delivery was 64 and 74 days, respectively.

Figures 7–12 display plasma concentrations of each analyte according to gestational age at presentation in each group of patients on the reference ranges derived from 180 uncomplicated pregnant women. By examining plasma angiogenic/anti-angiogenic factor concentrations in each group, the earlier the gestational age at blood sampling, the better the discrimination of patients in Groups III and IV from those in Groups I and II.

Among patients who presented to the obstetrical triage area before 34 weeks of gestation, forty-six percent (27/59) delivered within 2 weeks due to PE. Table V displays the MoM cut-offs (derived from ROC curves) for each angiogenic/anti-angiogenic factor and the diagnostic performance of each analyte for the identification of patients who delivered within 2 weeks because of PE. A plasma concentration of PlGF/sVEGFR-1 ≤0.033 MoM identified patients who delivered within 2 weeks because of PE with the largest area under the ROC curve (0.88), a sensitivity of 93% (25/27) and a specificity of 78% (25/32).

The association between low plasma concentrations of PlGF/sVEGFR-1 (< 0.033 MoM) and delivery within 2 weeks due to PE remained significant after adjusting for gestational age at presentation, average systolic and diastolic blood pressure and a history of chronic hypertension [adjusted OR 45 (95% CI 8.4–236)]. This cut-off was also associated with a shorter interval-to-delivery due to PE after adjusting for the above-mentioned potential

confounders [PlGF/sVEGFR-1 >0.033 MoM: n= 27, censored 5; median survival 41 days, inter-quartile range (IQR) 22–85 days vs. PlGF/sVEGFR-1 ≤ 0.033 MoM: n= 32, censored 3, median survival 6 days, IQR 3–10 days; p<0.001; hazard ratio = 6 (95% CI 2.5–14.6); Figure 13].

Development of grading criteria using plasma concentrations of angiogenic/anti-angiogenic factors

Upon examination of the distribution of PlGF/sVEGFR-1 and PlGF/sEng in each group, plasma concentrations of these biomarkers between patients in Groups I+II and those in Groups III+IV were overlapping in the middle part of the scattergram. Thus, the decision was made to divide these biomarkers into 3 zones including: Zone 1, which included the majority of patients in Groups I and II; Zone 2, the overlapping zone; and Zone 3, which included the majority of patients in Groups III and IV.

The cut-off points for Zone 3 (PlGF/sVEGFR-1 ≤ 0.05 MoM or PlGF/sEng ≤ 0.07 MoM) were derived from the ROC curves for the identification of patients in Groups III and IV (see Table IV) because these cut-offs had a high specificity (91%) for severe PE in preterm gestations. The cut-off points for Zone 1 (PlGF/sVEGFR-1 ≥ 0.35 MoM or PlGF/sEng ≥ 0.30 MoM) was derived from the ROC curves for the identification of patients in Groups I and II before 34 weeks of gestation because these cut-offs had a high specificity (95%) for patients without severe PE. Plasma concentrations of PlGF/sVEGFR-1 ≥ 0.35 (rounded up from 0.3602 MoM which was derived from the ROC curve) and PlGF/sEng ≥ 0.30 (rounded up from 0.3008 MoM which was derived from the ROC curve) had a similar sensitivity of 78% (14/18) and a similar specificity of 95% (39/41) for the identification of patients in Groups I and II (Figures 14 and 15).

Table VI displays the rate of preterm delivery of patients presenting to the obstetrical triage area before 34 weeks with a diagnosis of “suspected preeclampsia” according to the proposed 3-zone classification of plasma MoM concentrations of PlGF/sVEGFR-1 and PlGF/sEng ratio. Among patients suspected to have PE and presented to the obstetrical triage area before 34 weeks with plasma concentrations of PlGF/sVEGFR-1 ≤0.05 MoM or PlGF/sEng ≤0.07 MoM (Zone 3), the rate of preterm delivery before 34 weeks was 79% (26/33). In contrast, among patients with plasma concentrations of PlGF/sVEGFR-1 ≥0.35 MoM (Zone 1), the rate of preterm delivery before 34 weeks was 6.3% (1/16). The only patient who delivered before 34 weeks in this group was referred to the obstetrical triage area for a headache, vaginal bleeding and high blood pressure (146/78 mmHg) at 33 3/7 weeks. She had a plasma PlGF/sVEGFR-1 concentration of 0.828 MoM and subsequently delivered spontaneously the next day. The rate of preterm delivery before 34 weeks in Zone 2 was 30% (3/10). The rates of preterm delivery (before 34 weeks, within 7 or 14 days) were correlated with the proposed 3-zone classification of plasma concentrations of PlGF/sVEGFR-1 and PlGF/sEng ratio (each p<0.001; Chi-square for trend; Table VI).

Table VII displays the disposition of patients with “suspected PE” before 34 weeks of gestation from the obstetrical triage area according to the proposed 3-zone classification of plasma angiogenic/anti-angiogenic factor concentrations. Half of the patients (8/16) who were hospitalized had plasma PlGF/sVEGFR-1≥ 0.35 MoM or plasma PlGF/sEng ≥0.30 MoM (Zone I). Six of these were in Groups I and II, while the other two were in Groups III and IV. However, these two patients were stable until delivery at 36 4/7 and 37 weeks or 64 and 74 days after venipuncture (mentioned above).

Two patients were discharged from the obstetrical triage area although plasma concentrations of PlGF/sVEGFR-1 or PlGF/sEng were in Zone 3. The first patient was referred to the obstetrical triage area at 28 3/7 weeks because of an elevated blood pressure.

Her blood pressure in the obstetrical triage area was 139–153/70–96 mmHg and proteinuria of 1+. She was discharged and the total protein in 24-hour urine sample was later found to be 1958 mg. She was induced 1 week later for worsening maternal disease (high blood pressure and central nervous system symptoms). Another patient was referred to the obstetrical triage area for headache and elevated blood pressure at 26 3/7 weeks. However, her blood pressure in the triage was 132/87 mmHg with a urine protein of 1+ and she was discharged. The total protein in her 24-hour urine sample was later found to be 1855 mg. This patient developed HELLP syndrome at 32 3/7 weeks.

Table VIII displays obstetrical characteristics and maternal plasma MoM concentrations of PlGF/sVEGFR-1 as well as PlGF/sEng of 13 patients who had maternal complications. Ten patients (4 with abruptio placentae, 2 with HELLP syndrome, 3 with pulmonary edema and 1 with eclampsia) had plasma PlGF/sVEGFR-1 in Zone 3. Two patients were in Zone 2 (eclampsia and pulmonary edema) and one patient was in Zone 1 (gestational hypertension with placental abruption). This last patient had the longest interval from blood sampling to delivery (85 days).

DISCUSSION

Principal findings of this study

1) Patients who presented to the obstetrical triage area with a diagnosis of “suspected PE” and subsequently were diagnosed with mild PE or severe PE requiring preterm delivery had significantly different mean plasma MoM concentrations of angiogenic/anti-angiogenic factors from those who did not require delivery until term; 2) plasma concentrations of sEng, PlGF, PlGF/sEng and PlGF/sVEGFR-1 performed very well in the identification of patients who developed severe PE requiring preterm delivery (area under the ROC curve of 87%–90%); 3) among patients who presented before 34 weeks gestation, a plasma concentration of PlGF/sVEGFR-1 of ≤0.033 MoM was associated with a shorter interval-to-delivery than those above the cutoff (PlGF/sVEGFR-1 of >0.033); 4) this biomarker was useful to identify patients who required delivery within 2 weeks; 5) plasma concentrations of the PlGF/sVEGFR-1 ratio or the PlGF/sEng ratio may assist clinicians in the management and disposition of patients suspected to have PE who present to the hospital with the suspected diagnosis of PE; and 6) we propose that the ratios reported herein are of clinical value in obstetrical practice.

The clinical challenge of evaluating the patient with suspected PE

Preeclampsia/eclampsia are, generally, retrospective diagnoses which can be confidently made after delivery. Some patients will present with mild gestational hypertension, and it is not possible to ascertain whether they will remain stable or progress to have proteinuria and other signs of multiple organ involvement. This is the rationale for obtaining baseline protein determination and other tests, such as a platelet count, liver function test, etc. Yet, these tests are valuable if an abnormality is detected (e.g. thrombocytopenia, abnormal liver function test, etc.). It is unclear if such tests (when results are within the normal range) have prognostic value to identify the patient with worsening disease who will require a preterm delivery for maternal or fetal indications.

The plasma concentrations of angiogenic and anti-angiogenic factors have been shown to be altered in patients with preterm PE and the abnormalities in the absolute concentrations of these biomarkers, and their ratios have been shown to precede the clinical diagnosis of PE by several weeks (see below for details). This set of observations was the basis for the hypothesis tested in this study; namely, that the concentration of these biomarkers in peripheral blood would identify the patient in whom the diagnosis of PE is uncertain when

she is admitted to the obstetrical triage area. Therefore, we tested whether the biomarkers and their ratios could predict preterm delivery for maternal or fetal indications.

The rationale for the study design

We divided patients into four groups based on clinical severity of PE and gestational age at delivery (term or preterm). Severity was chosen because morbidity/mortality of mothers with PE increases as a function of these criteria [91–94]. Preterm delivery was chosen because neonatal morbidity/mortality is largely dependent on gestational age at delivery [11–13,95]. These assumptions were confirmed because all patients except one (who had severe maternal complications) belonged to Groups III (mild PE who required preterm delivery) and IV (severe PE).

We found that plasma concentrations of angiogenic/anti-angiogenic factors in the obstetrical triage area are of prognostic value for the identification of patients who developed severe PE or those who required delivery within 2 weeks because of worsening PE. Such observations are consistent with results of previous studies reported by our group using a cross-sectional approach [56]. We reported that plasma concentrations of sVEGFR-1 at the time of diagnosis of PE are positively correlated with the clinical severity of PE [56], the degree of proteinuria [56] and abnormalities of uterine artery and umbilical artery Doppler velocimetries [96,97]. Moreover, we have also found a negative correlation between the maternal plasma concentration of sVEGFR-1 and gestational age at diagnosis of PE, platelet count, neonatal birth weight, adjusted neonatal birth weight for gestational age, as well as gestational age at delivery [56]. Similar findings were demonstrated for maternal plasma PlGF [80,98,99], maternal plasma sEng [100,101] and their ratio [54,102–105].

Several investigators have reported that plasma concentrations of angiogenic/anti-angiogenic factors in PE change prior to the clinical manifestation of the disease [55,57,65,68,71,74,75,78,82,83,106–108]. An elevation of plasma sVEGFR-1 concentration began 6–10 weeks prior to the clinical manifestations, and the increase was more pronounced 2–5 weeks before the clinical diagnosis [55,76]. Similarly, a lower plasma PlGF/sEng ratio in PE than women with normal pregnancies was observed 20 weeks before the clinical diagnosis of preterm PE and 10 weeks before the diagnosis of term PE [65].

Patients who developed preterm and term PE had a significantly lower plasma PlGF/sVEGFR-1 ratio 20 and 14 weeks before clinical diagnosis [55,57,65]. Since plasma concentrations of angiogenic/anti-angiogenic factors in PE change several weeks prior to the clinical diagnosis, we reasoned that determination of these biomarkers in the obstetrical triage area could be of value in the assessment of progression of disease (prognosis) or clinical severity, which could be overlooked by traditional clinical criteria. The scientific basis and rationale for the selection of criteria for severity of PE is not clearly established, and the possibility that biomarkers (such as the plasma concentration of angiogenic/anti-angiogenic markers) may be superior to traditional clinical signs or symptoms and other laboratory tests may need to be considered.

The diagnostic and prognostic properties of angiogenic/anti-angiogenic factor concentrations are in contrast to that of a spot urine protein/creatinine ratio or serum uric acid. Although severe proteinuria is often used to diagnose severe PE, many factors such as patient posture, activity and body temperature can affect the amount of protein excreted in the urine [109]. Moreover, once the diagnosis of proteinuria is made, the magnitude of changes in the degree of proteinuria do not appear to alter solid clinical endpoints, although it may change the classification of the disease to mild or severe [110–112].

A spot urine protein/creatinine ratio has a high sensitivity to exclude patients with PE at the time of clinical presentation, but not to make a positive diagnosis [113,114]. This is the reason why most clinicians prefer to quantitate protein excretion with a 24-hour urine collection. To complicate matters, different cut-off values for urine protein/creatinine ratio have been proposed, and this may reflect a high variability of protein excretion in a clinical setting [109,114,115]. Similarly, although the mean serum uric acid concentration is higher in women with PE than in normal pregnant women, an elevation of uric acid is a poor predictor of the development of PE as well as the subsequent development of maternal and fetal complications [43].

A quantile regression model was used in the current study because the distribution of angiogenic/anti-angiogenic factor concentrations, especially in the 3rd trimester, is close to, but not normally distributed even if logarithmic transformation is employed. Moreover, the standard deviation tends to increase with advancing gestational age. These findings are similar to those reported in Japanese population by Ohkuchi et al. [116]. The median is a better measure of central tendency than the mean if the distribution of the data is asymmetric (i.e. not normally distributed) [90]. In the current study, we used MoM cut-off instead of a particular percentile cut-off (derived from median regression analysis) because the purpose of the study was not to differentiate patients with PE from those without this disorder, but rather to identify those who were at risk for worsening disease or who would require preterm delivery. Indeed, the majority of patients with PE (who eventually had a term or preterm delivery) had plasma MoM concentrations of angiogenic/anti-angiogenic factors below the reference range of normal pregnant women.

The clinical significance of the findings of the study

In the current study, a PlGF/sVEGFR-1 ratio of ≤ 0.05 MoM and a PlGF/sEng ratio of ≤ 0.07 MoM were associated with PE requiring preterm delivery with a likelihood ratio for a positive test of 8.3 and 8.6, respectively. This association remained significant [adjusted odds ratio (OR) = 27 (95% CI 6.4–109) and adjusted OR 30 (95% CI 6.9–126), respectively] after adjustment for gestational age at presentation, average systolic and diastolic blood pressure in the obstetrical triage area and a history of chronic hypertension, parameters which are associated with the development of PE among patients with gestational hypertension [43].

The soluble form of VEGFR-1 is able to bind VEGF in the circulation and decrease the amount of free VEGF, which functions to stabilize endothelial cells in mature blood vessels [117–119]. sVEGFR-1 is also essential in maintaining the integrity of the basement membrane, especially in the fenestrated endothelium of the kidney, liver and brain [73,120–124]. In addition, the soluble form of Eng can bind to TGF-β and interfere with its nitric oxide-mediated vasodilation effect [54]. Thus, it is biologically plausible that plasma concentrations of angiogenic/anti-angiogenic factors reflect the severity of the pathophysiologic state of PE and its multiple organ involvement [2,41,42].

Although plasma concentrations of the PlGF/sVEGFR-1 ratio of ≤0.05 MoM or the PlGF/sEng ratio of ≤0.07 MoM had a high likelihood ratio for a positive test for the identification of patients in Groups III and IV, 14 false negative cases were noted and 5 of these were presented after 34 weeks of gestation. Among the other 9 patients, only 2 had plasma concentrations of these biomarkers above the 5th centile. The interval from blood sampling to delivery, however, was 64 and 74 days, respectively. Therefore, it seems that the concentrations of these angiogenic/anti-angiogenic factors are likely to perform better if the outcome was evaluated in a shorter period of time (i.e. within 4–5 weeks from first assessment) or if repeated or sequential testing was performed. Alternatively, other

mechanisms of diseases may be operative in these patients [125–159]. However, this requires further study.

It is noteworthy that all three false positive cases (a plasma PlGF/sVEGFR-1 ratio below 0.05 MoM in Groups I and II) were delivered spontaneously shortly after blood sampling. We have demonstrated that an imbalance of angiogenic/anti-angiogenic factor concentrations is not a unique characteristic of patients with PE since this perturbation can occur in isolated fetal growth restriction with abnormal uterine artery Doppler velocimetry [96], unexplained fetal death [160–163], twin-to-twin transfusion syndrome [164] and viral-induced hydrops fetalis [165,166] or Ballantynes syndrome [167]. Moreover, a subset of patients with spontaneous preterm labor with intact membranes also had an abnormal angiogenic/anti-angiogenic profile in maternal circulation prior to the diagnosis of preterm labor/delivery [168]. However, the magnitude of the changes of these angiogenic/anti-angiogenic factor concentrations in patients destined to have a spontaneous preterm labor are different from those who subsequently will have a normal delivery at term; yet, the magnitude of the abnormality is milder and is detected prior to the diagnosis of preterm labor/delivery (<5 weeks for PlGF, sVEGFR-1, sVEGFR-2 and > 5 weeks for sEng) than that observed in patients with PE [168]. It is possible that when the maternal angiogenic/anti-angiogenic factor ratio is very low (which reflects the condition of the placenta), pregnant women would need to deploy adaptive mechanisms which could be increasing maternal blood pressure to maintain perfusion to the fetus and placenta, or alternatively, resort to the onset of labor to allow the fetus to exit a hostile intrauterine environment. However, the precise molecular mechanisms employed under each of these circumstances remains to be elucidated.

It is noteworthy that the likelihood ratios of a positive test were higher when the ratios of angiogenic/anti-angiogenic factor concentrations were used as biomarkers for the identification of severe PE requiring preterm delivery than those of individual concentrations of angiogenic/anti-angiogenic factors. In contrast, individual angiogenic or anti-angiogenic factor concentrations seem to have a lower likelihood ratio of a negative test for the prediction of preterm delivery than the use of ratios of these factors (see Table IV for a detailed comparison).

Since plasma concentrations of a PlGF/sVEGFR-1 ratio of ≤0.05 MoM and a PlGF/sEng ratio of ≤ 0.07 MoM performed better in diagnosing rather than excluding patients who would develop severe PE (higher specificity than sensitivity), the decision was made to establish another cut-off point of the PlGF/sVEGFR-1 ratio of ≥ 0.35 MoM and the PlGF/sEng ratio of ≥0.30 MoM, which had a high specificity for the identification of patients with and without PE before 34 weeks of gestation who remained stable until term (or to exclude severe PE).

We have demonstrated that plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in identifying patients with severe PE requiring preterm delivery. Recently, automated assay systems for PlGF and sVEGFR-1 have been developed and the results could be obtained within 18 minutes [169]. Moreover, studies have recently demonstrated a very high correlation between these automated assay systems and the conventional ELISA assay method used in our study [116,169]. Plasma sVEGFR-1, PlGF and their ratio determined by an electrochemiluminescence immunoassay correlated with clinical severity, gestational age at onset and at delivery of PE in nested case-controlled studies [170,171]. Such results are consistent with those found using ELISA methods. The concentrations of angiogenic/anti-angiogenic factors in maternal blood may be useful in assisting physicians in the clinical assessment of patients presenting to the obstetrical triage

area before 34 weeks with a diagnosis of "suspected preeclampsia" if the results of these assays are available for clinical decision-making in the obstetrical triage area.

In our study, the rate of preterm delivery (before 34 weeks or delivery within 7 or 14 days) was correlated with the proposed 3 zone criteria of plasma MoM concentrations of the PlGF/sVEGFR-1 ratio and the PlGF/sEng ratio. When the PlGF/sVEGFR-1 ratio was ≥35% of the median (Zone 1), the rate of preterm delivery before 34 weeks was 6.3%. When this ratio was 5–35% (Zone 2) and ≤5% (Zone 3) of the median, the rate of preterm delivery was 30% and 79%, respectively.

When examining the disposition of patients with "suspected PE" before 34 weeks of gestation from the obstetrical triage area according to the proposed 3 zone criteria, these biomarkers could assist physicians or mid-level providers in triaging these patients. For example, this approach may reduce the number of hospital admissions of patients suspected to have PE when the plasma angiogenic/anti-angiogenic factor concentrations are in Zone 1. Alternatively, hospitalization may be limited to patients who are in Zone 3. The management of patients in Zone 2 remains uncertain at this point because patients in this zone may have disease progression or remain stable. It is possible that additional biomarkers will be required to assist in the assessment of these patients, or that serial tests may prove to be informative. This was not performed in this study because of its retrospective nature.

Strengths and limitations of the study

This is the first study to demonstrate an association between plasma concentrations of angiogenic/anti-angiogenic factors and the prognosis of PE in terms of progression to severe PE or preterm delivery in patients who presented to the obstetrical triage area before 34 weeks for suspected PE. Moreover, a new approach to integrate these biomarkers into clinical practice has been proposed. We believe that this simple approach may assist clinicians in managing the uncertainty presented by patients with the clinical diagnosis "rule out PE".

All proposed cut-off values have been standardized and reported as multiples of the median. This allows independent replication of these results in other populations that may have a different median or distribution of the concentration of angiogenic/anti-angiogenic factors [172,173]. Limitations of the study are: 1) a high number of patients with a diagnosis of severe PE and adverse outcomes were included in this study – this may reflect a selection bias towards preferential enrollment of patients with severe disease in this retrospective cohort study; and 2) the management for PE was based on clinical findings, and most patients who were diagnosed to have severe PE, either stable or not stable, were induced at 34 weeks of gestation. This practice precludes the assessment of the natural history between the behavior of biomarkers and clinical progression or preterm delivery.

Conclusion

We propose that maternal plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in the obstetrical triage area. These observations strengthen the clinical value of these biomarkers in obstetrics. Prospective studies are desirable to confirm the observations reported herein and to evaluate other outcomes, such as maternal and neonatal morbid events. Importantly, our findings suggest that determination of PlGF, sVEGFR-1 and sEng can have clinical value, even if there is no treatment to reverse PE at this time. Their potential value would be to improve the efficiency with which healthcare providers can manage patients at risk. This is a clinical challenge which presents itself on a daily basis in busy obstetrical units.

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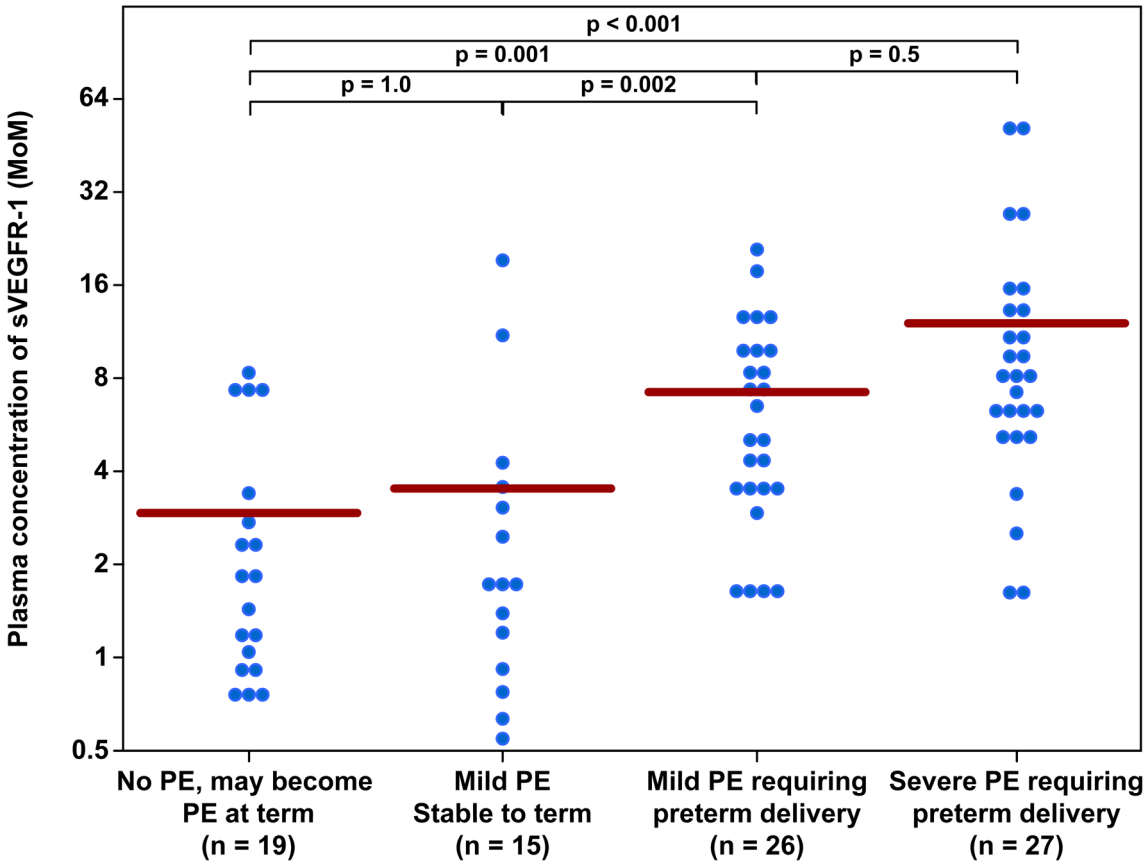

Figure 1.

Plasma concentration of sVEGFR-1 in Multiple of Median (MoM) unit. The mean MoM plasma concentration of sVEGFR-1 was significantly higher in patients with mild preeclampsia who subsequently developed severe preeclampsia than those who remained stable until term (p=0.002). Comparisons among groups were performed after logarithmic transformation.

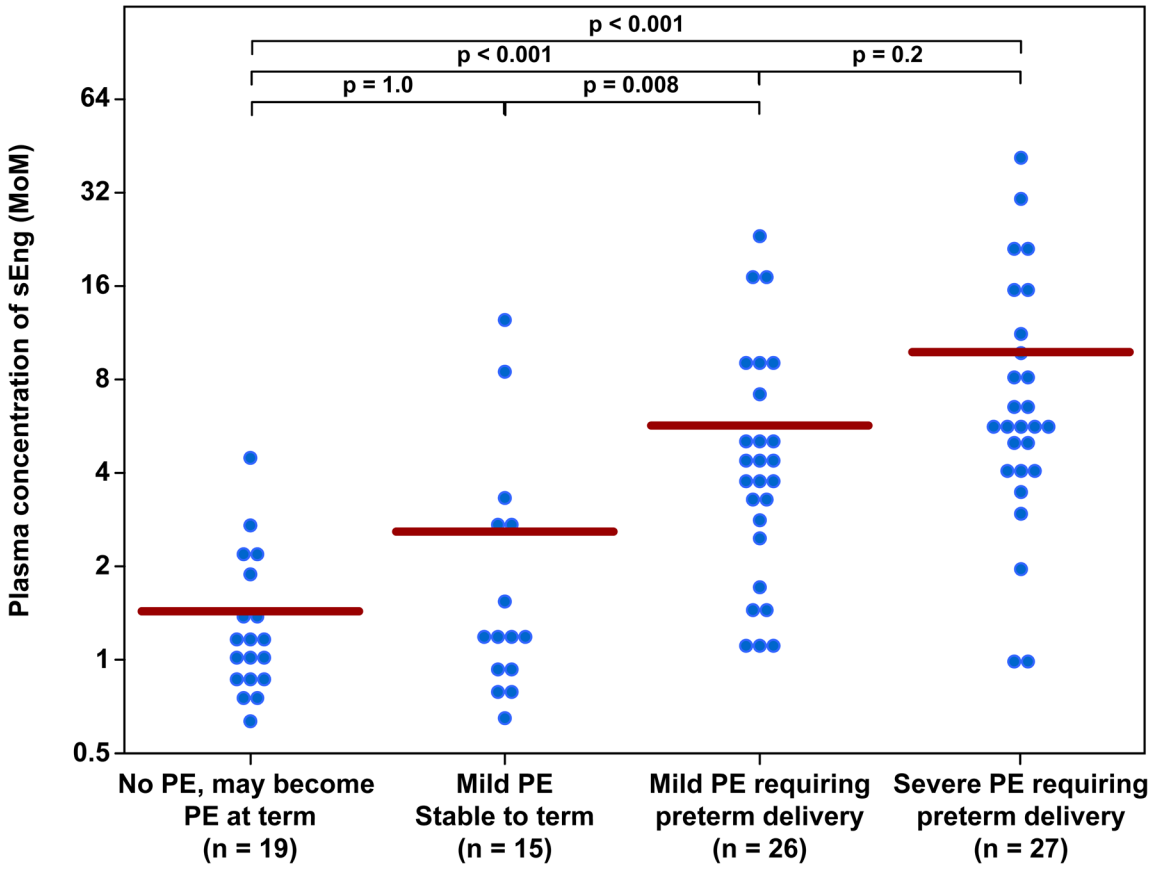

Figure 2.

Plasma concentration of sEng in Multiple of Median (MoM) unit. The mean MoM plasma concentration of sEng was significantly higher in patients with mild preeclampsia who subsequently developed severe preeclampsia than those who remained stable until term (p=0.008). Comparisons among groups were performed after logarithmic transformation.

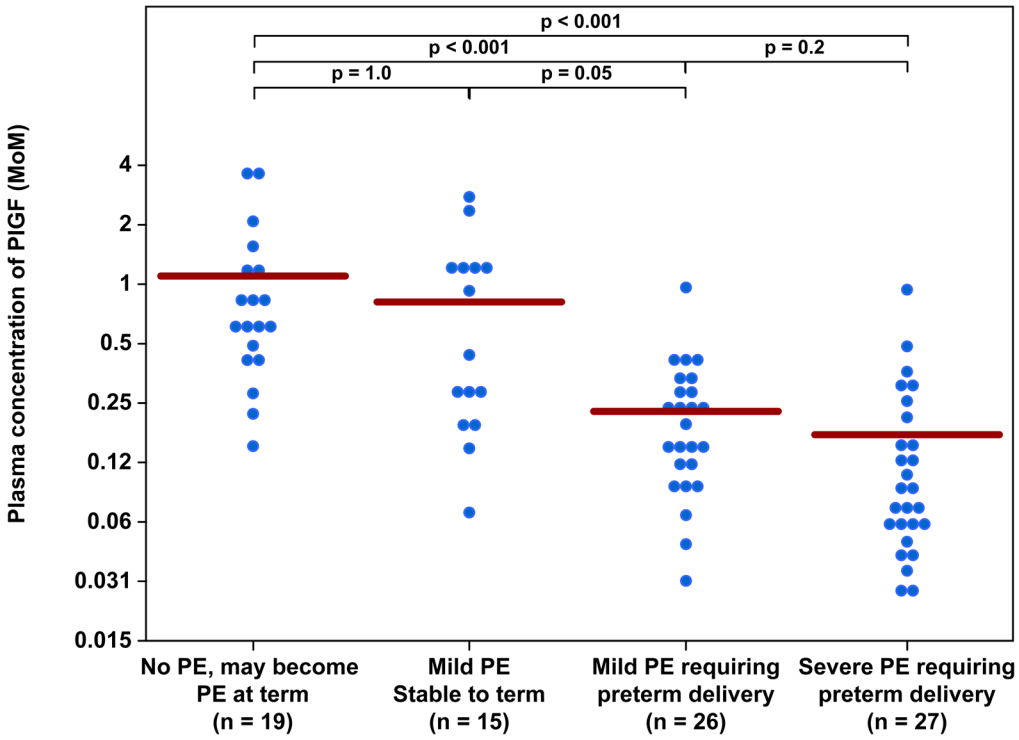

Figure 3.

Plasma concentration of PlGF in Multiple of Median (MoM) unit. The mean MoM plasma concentration of PlGF was significantly lower in patients with mild preeclampsia who subsequently developed severe preeclampsia than those who remained stable until term (p=0.005). Comparisons among groups were performed after logarithmic transformation.

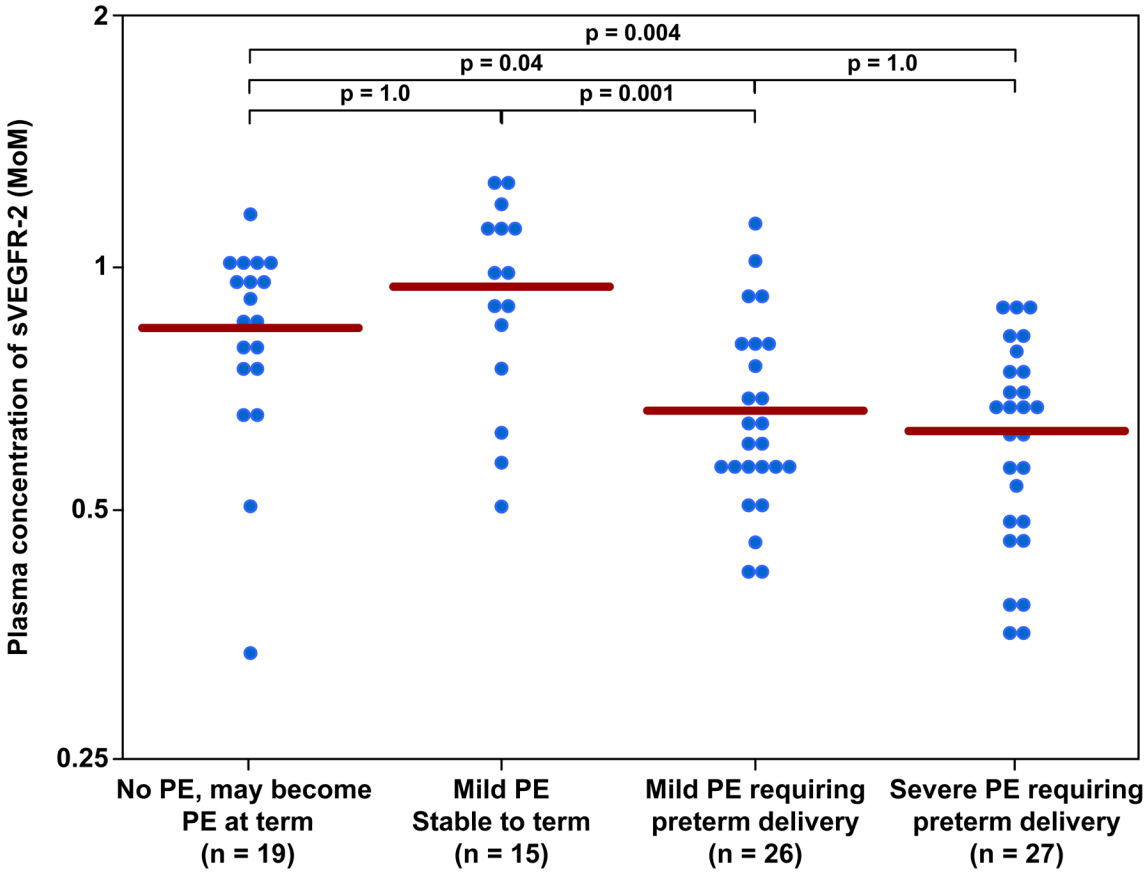

Figure 4.

Plasma concentration of sVEGFR-2 in Multiple of Median (MoM) unit. The mean MoM plasma concentration of sVEGFR-2 was significantly lower in patients with mild preeclampsia who subsequently developed severe preeclampsia than those who remained stable until term (p=0.001). Comparisons among groups were performed after logarithmic transformation.

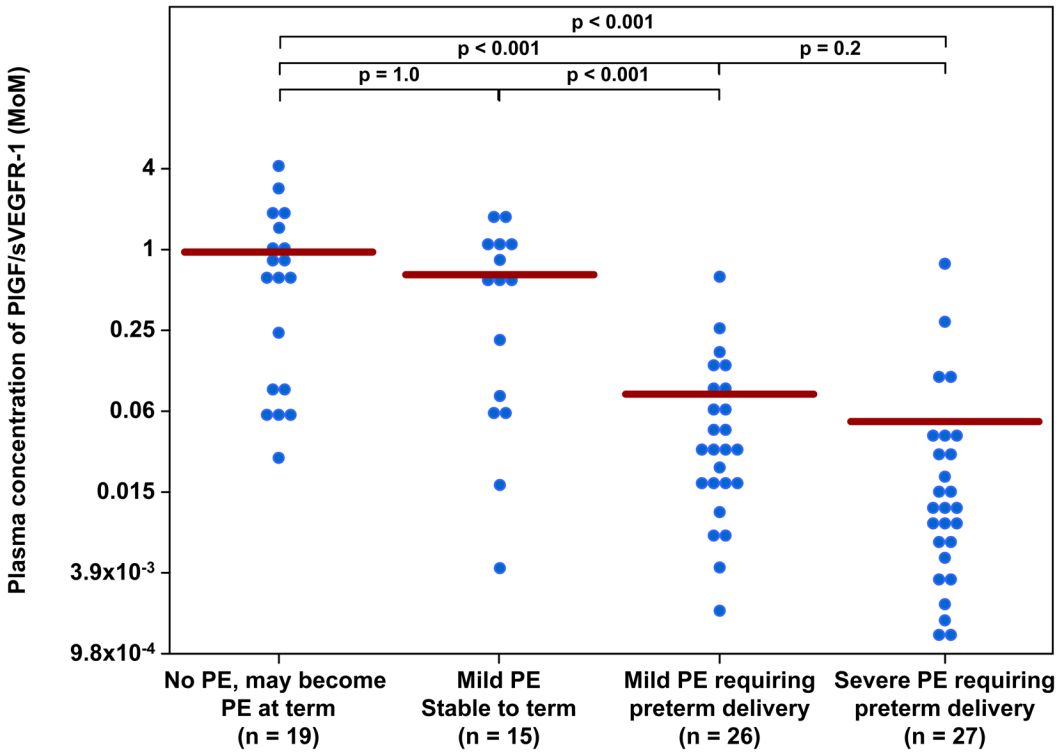

Figure 5.

Plasma concentration of PlGF/sVEGFR-1 ratio in Multiple of Median (MoM) unit. The mean MoM plasma concentration of PlGF/sVEGFR-1 ratio was significantly lower in patients with mild preeclampsia who subsequently developed severe preeclampsia than those who remained stable until term ($p<0.001$). Comparisons among groups were performed after logarithmic transformation.

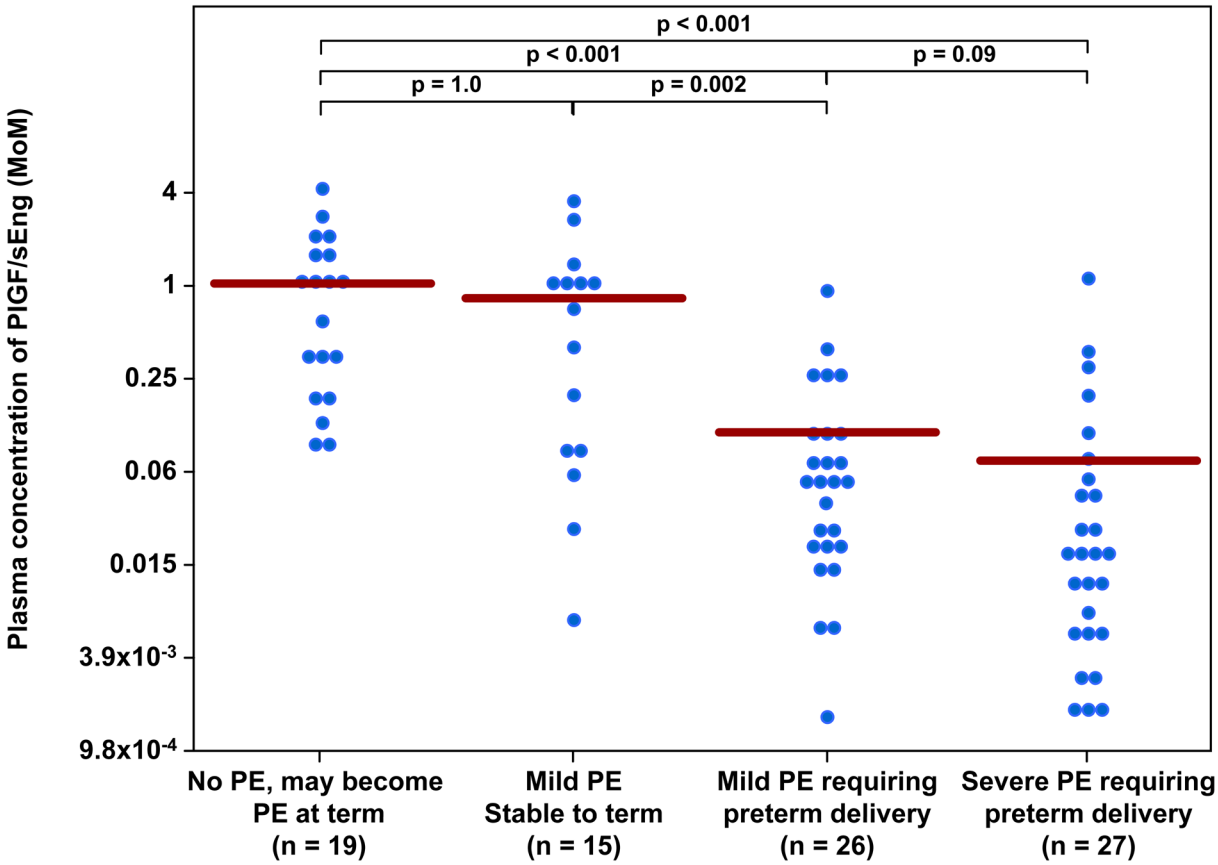

Figure 6.

Plasma concentration of PlGF/sEng ratio in Multiple of Median (MoM) unit. The mean MoM plasma concentration of PlGF/sEng ratio was significantly lower in patients with mild preeclampsia who subsequently developed severe preeclampsia than those who remained stable until term (p=0.002). Comparisons among groups were performed after logarithmic transformation.

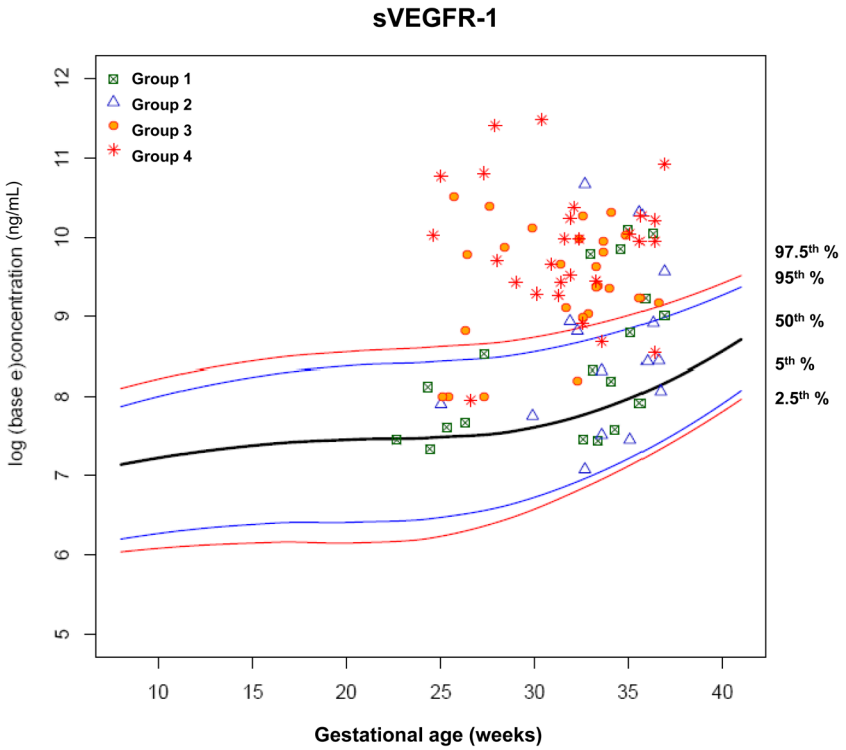

Figure 7.

Plasma concentrations of sVEGFR-1 (ng/mL) in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) derived from quantile regression of 1,046 samples obtained from 180 uncomplicated pregnant women.

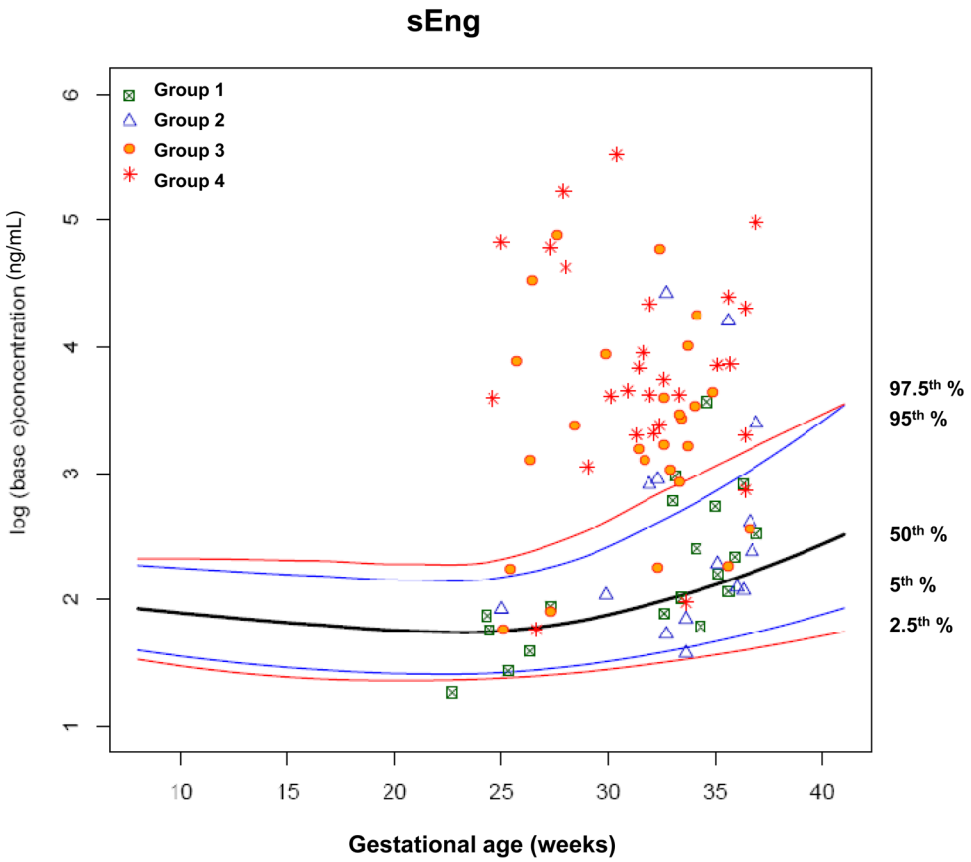

Figure 8.

Plasma concentrations of sEng (ng/mL) in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) derived from quantile regression of 1,046 samples obtained from 180 uncomplicated pregnant women.

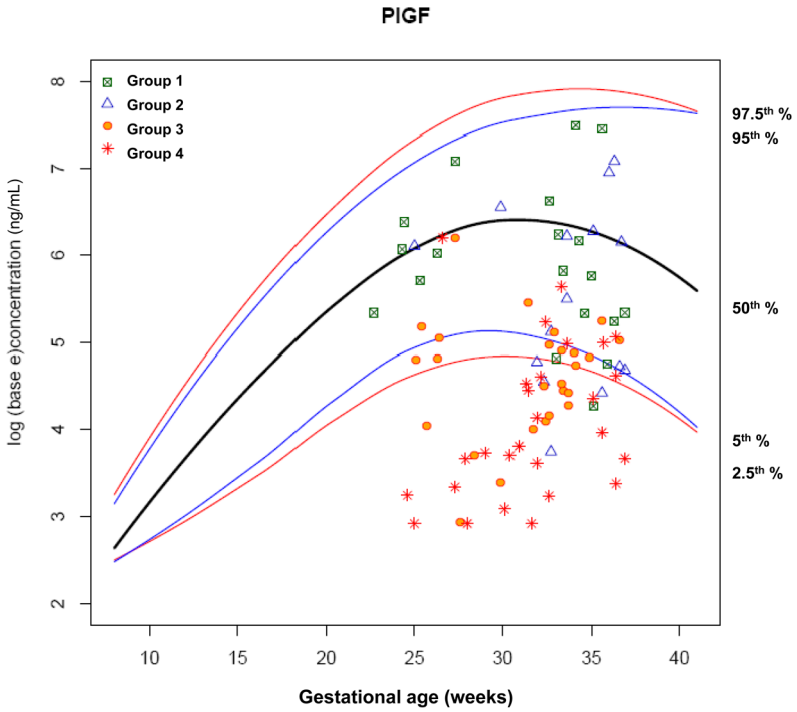

Figure 9.

Plasma concentrations of PlGF (ng/mL) in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) derived from quantile regression of 1,046 samples obtained from 180 uncomplicated pregnant women.

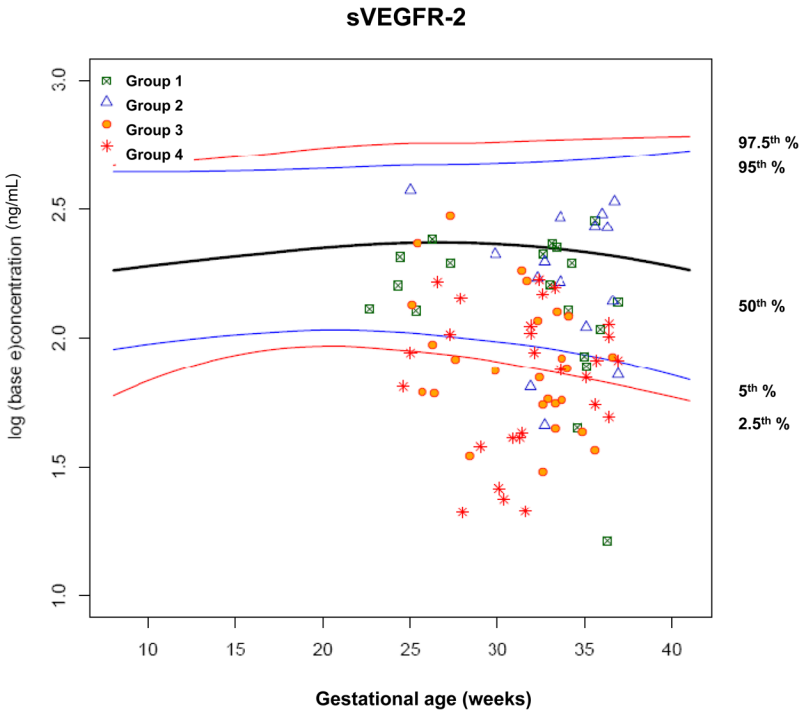

Figure 10.

Plasma concentrations of sVEGFR-2 (ng/mL) in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) derived from quantile regression of 1,046 samples obtained from 180 uncomplicated pregnant women.

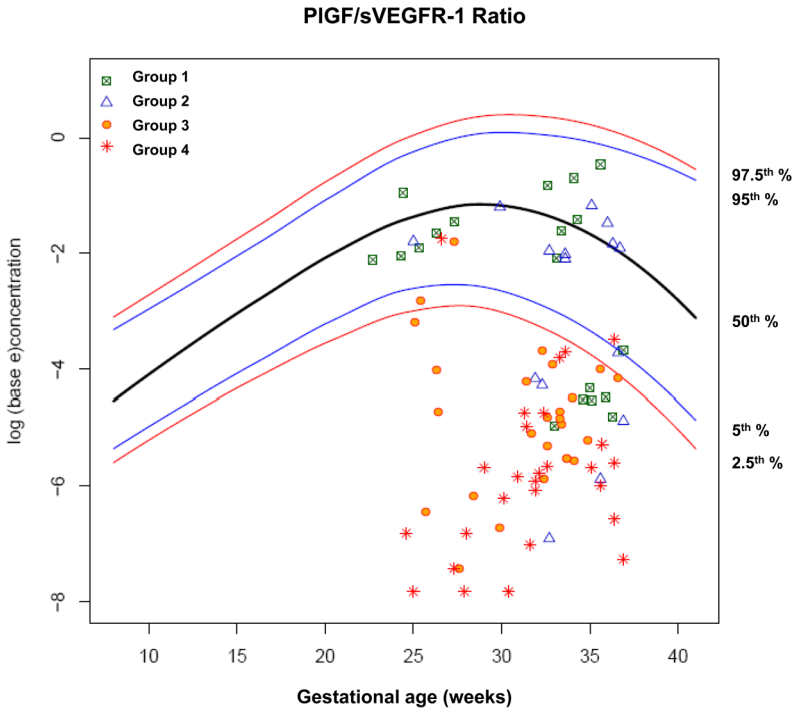

Figure 11.

Plasma concentrations of PlGF/sVEGFR-1 ratio in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) derived from quantile regression of 1,046 samples obtained from 180 uncomplicated pregnant women.

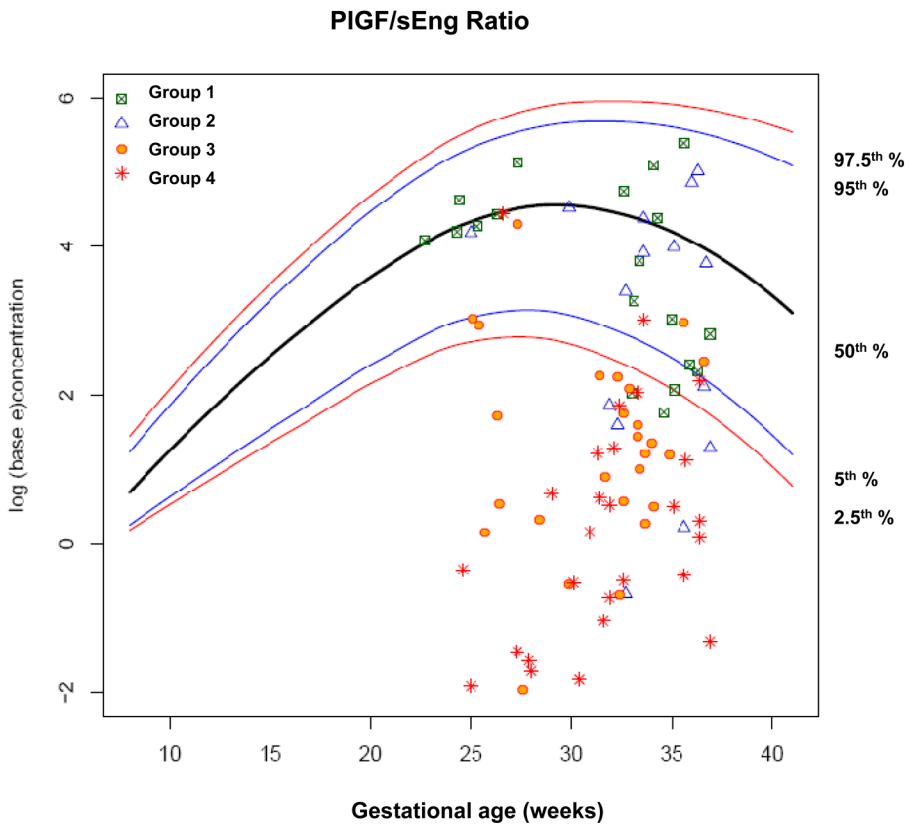

Figure 12.

Plasma concentrations of PlGF/sEng ratio in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) derived from quantile regression of 1,046 samples obtained from 180 uncomplicated pregnant women.

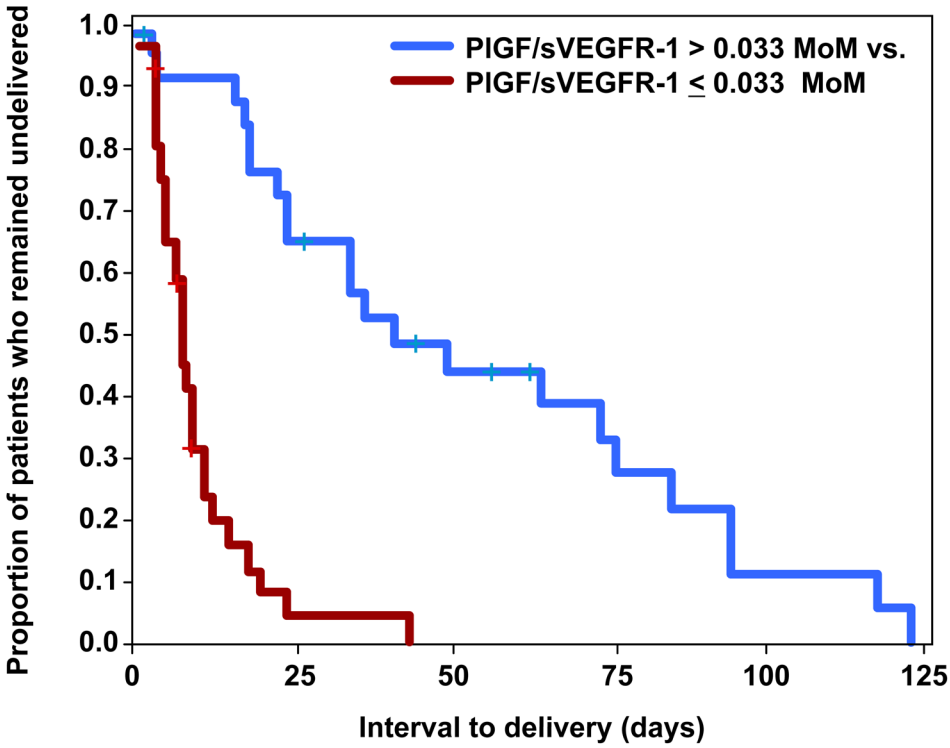

Figure 13.

Survival curve of patients who had plasma concentration of PlGF/sVEGFR-1 ratio ≤ 0.033 MoM and >0.033 MoM. This cut-off was associated with a shorter interval-to-delivery due to preeclampsia [PlGF/sVEGFR-1 >0.033 MoM: n= 27, censored 5; median survival 41 days, interquartile range (IQR) 22–85 days vs. PlGF/sVEGFR-1 ≤ 0.033 MoM: n= 32, censored 3, median survival 6 days, IQR 3–10 days; p<0.001; hazard ratio = 6 (95% CI 2.5–14.6)].

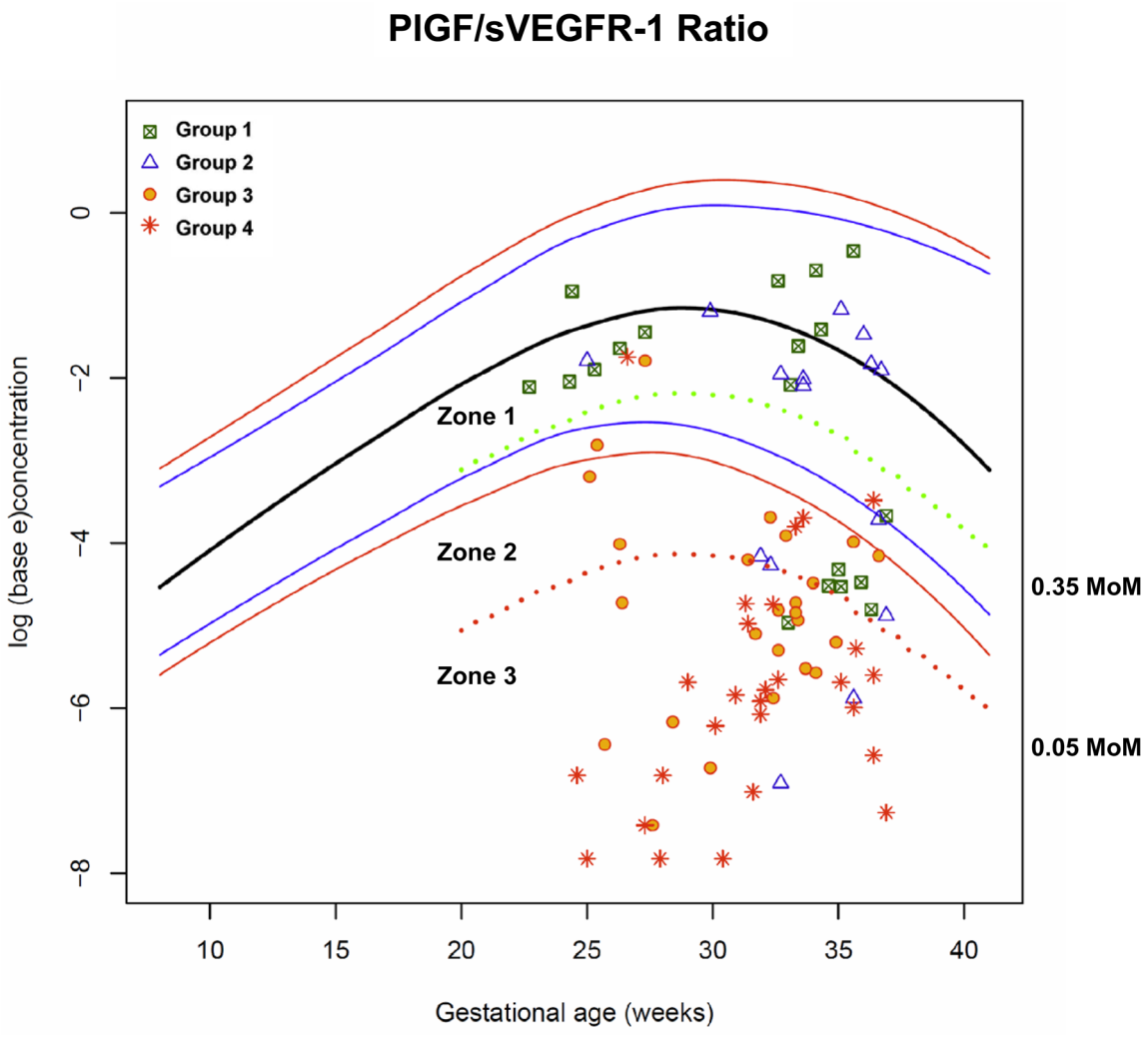

Figure 14.
Plasma concentrations of PlGF/sVEGFR-1 ratio in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) and the cut-offs (dash line) according to the 3-zone classification.

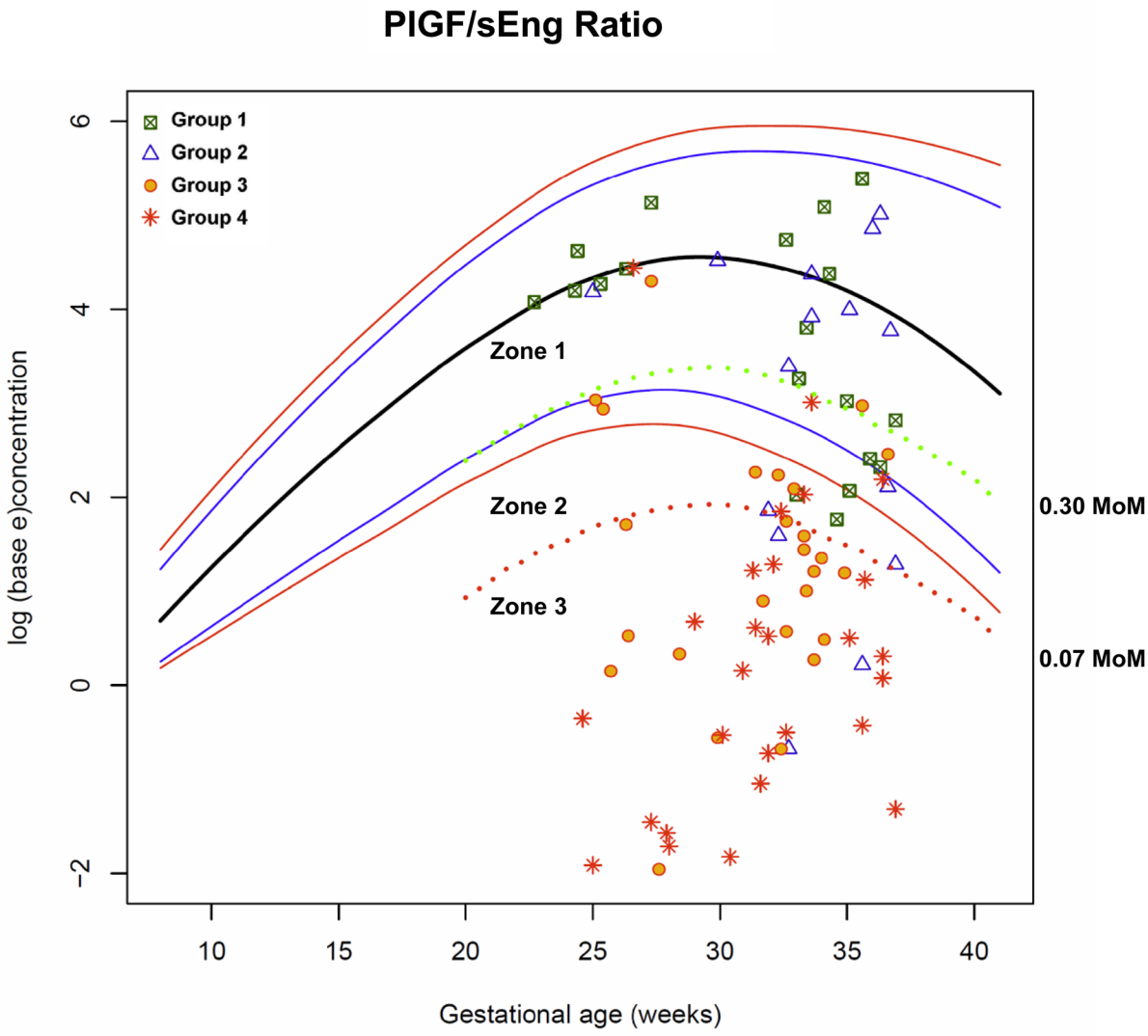

Figure 15.
Plasma concentrations of PlGF/sEng ratio in patients from each study group plotted against a reference range (2.5th, 5th, 50th, 95th, and 97.5th percentile) and the cutoffs (dash line) according to the 3-zone classification.

Table I

Demographic and clinical characteristics of the study population

	No PE, may become mild PE at term (n=19)	Mild PE and stable until term delivery (n=15)	Mild PE became severe PE requiring preterm delivery (n=26)	Severe PE (n=27)	p
Age (years)	26±6.5	28±7.7	24±5.1	27±6.7	0.2
African American	17 (89.5%)	14 (93.3%)	23 (88.5%)	24 (88.9%)	0.9
Nulliparous	6 (31.6%)	6 (40%)	13 (50%)	12 (55.6%)	0.7
Smoking	4 (21.1%)	7 (46.7%)	4 (15.4%)	6 (22.2%)	0.1
BMI (Kg/m2)	38.6 ± 10.3	36.9±10.7	33.6±8	6.8±12.6	0.5
Previous preeclampsia	2 (10.5%)	1 (6.7%)	7 (26.9%)	6 (22.2%)	0.3
Chronic hypertension	9 (47.3%)	5 (33.3%)	7 (26.9%)	13 (48.1%)	0.2
Pre-gestational diabetes	--	3 (20%)	--	1 (3.7%)	0.02
GA at blood sampling (weeks)					
Mean ± SD	31 ± 4.8	34 ± 3.2	31 ± 3.5	31 ± 3.5	0.2
Median (inter-quartile range)	33 (26–35)	33 (32–36)	32.5 (27–33)	31.8 (29–35)	
GA <34 weeks	10 (52.6%)	8 (53.3%)	21 (80.8%)	20 (74.1%)	
Average blood pressure in triage area					
Systolic (mmHg)	134 ±15	144 ±112	152 ±20	165 ±22	<0.001
Diastolic (mmHg)	79 ± 11	85 ± 12	94 ± 11	96 ± 11	<0.001
Diagnosis in the Triage Area					
- No hypertensive disease	5 (26.3%)	--	1 (3.8%)	--	<0.001
- Gestational hypertension	5 (26.3%)	1 (6.7%)	3 (11.5%)	--	
- Chronic hypertension	9 (47.7%)	2 (13.3%)	4 (15.4%)	--	
- Chronic hypertension with superimposed preeclampsia	--	3 (20%)	3 (11.5%)	13 (48.1%)	
- Mild preeclampsia	--	9(60%)	15 (57.7%)	--	
- Severe preeclampsia	--	--	--	14 (51.9%)	

	No PE, may become mild PE at term (n=19)	Mild PE and stable until term delivery (n=15)	Mild PE became severe PE requiring preterm delivery (n=26)	Severe PE (n=27)	p
Diagnosis at delivery					
- No hypertensive disease	3 (15.8%)	--	--	--	<0.001
- Gestational hypertension	4 (21.1%)	--	--	--	
- Chronic hypertension	7 (36.8%)	--	--	--	
- Chronic hypertension with superimposed preeclampsia	2 (10.5%)	5 (33.3%)	7 (26.9%)	13 (48.1%)	
- Mild preeclampsia	2 (10.5%)	7(46.7%)	--	--	
- Severe preeclampsia	1 (5.3%)	3 (20%)	19 (73.1%)	14 (51.9%)	

Table II

Obstetrical and neonatal outcomes of the study population

	No PE, may become PE at term (n=19)	Mild PE and stable until term delivery (n=15)	Mild PE became severe PE requiring preterm delivery (n=26)	Severe PE (n=27)	p
Obstetrical outcomes					
Interval to delivery (days)					
Mean+ SD	43± 37	25±24	17±18	6 ± 14	<0.001
Median (inter-quartile range)	33 (13–62)	21 (5–36)	10 (6–19)	3 (2–5)	
Labor					
Spontaneously	4 (21.1%)	3 (20%)	1 (3.8%)	1 (3.7%)	0.03
Induced	13 (68.4%)	4 (26.7%)	16 (61.5%)	14 (51.9%)	
No labor	2 (10.5%)	8 (53.3%)	9 (34.6%)	12 (44.4%)	
Delivery					
Cesarean section	7 (36.8%)	11 (73.3%)	15 (57.7%)	19 (70.4%)	0.2
Induced for preeclampsia (either at term or preterm)	4 (21.1%)	10 (66.7%)	25 (96.2%)	26 (96.3%)	<0.001
Maternal complications					
Abruption	1 (5.3%)	--	--	4 (14.8%)	0.08
Pulmonary edema	--	--	2 (7.7%)	2 (7.7%)	0.4
HELLP	--	--	1 (3.8%)	1 (3.7%)	0.7
Eclampsia	--	--	1 (3.8%)	1 (3.8%)	0.7
Neonatal outcomes					
GA at delivery (weeks)					
Mean ± SD	37 ±2.3	37 ± 1.5	33 ± 2.9	32 ± 3.4	<0.001
Median (inter-quartile range)	38 (36–39)	37 (37–38)	34 (32–35)	32 (30–36)	
Delivery < 34 weeks	2 (10.5%)	1 (6.7%)	10 (38.5%)	17 (63%)	
Delivery < 37 weeks	5 (26.3%)	3 (20%)	26 (100%)	25 (92.6%)	
Birthweight (grams)	3120 ± 637	2627 ± 665	1907 ± 714	1566 ± 630	<0.001

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	No PE, may become PE at term (n=19)	Mild PE and stable until term delivery (n=15)	Mild PE became severe PE requiring preterm delivery (n=26)	Severe PE (n=27)	p
< 10%	1 (5.3%)	8 (53.3%)	9 (34.6%)	15 (55.6%)	
<5%	1 (5.3%)	4 (26.7%)	5 (19.2%)	7 (25.9%)	
Apgar at 5 minutes <7	--	1 (6.7%)	1 (3.8%)	5 (18.5%)	0.09

Table III

Demographic and clinical characteristics of uncomplicated pregnant women whose samples were used to construct the reference range (n=180)

Age (years)	23 ± 4
African American	149 (82.8%)
Nulliparous	63 (35%)
Smoking	24 (13.3%)
BMI (Kg/m2)	27.7 ± 6.8
GA at 1st US (weeks)	12.6 ± 4.1
GA at delivery (weeks)	39.6 ± 1.0
Birthweight (grams)	3350 ± 304

Table IV

Diagnostic performance of plasma concentrations of angiogenic/anti-angiogenic factors for the identification of patients who subsequently develop severe preeclampsia (Group III and IV)

	Cut-off (MoM) derived from ROC	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Likelihood Ratio of a positive test	Posterior probability (95% CI)	Likelihood Ratio of a negative test	Posterior probability (95% CI)
sVEGFR-1	≥ 3.46	84 (76–93)	81 (43/53)	79 (27/34)	3.9 (2.0–7.7)	86% (76–92)	0.24 (0.13–0.43)	27% (17–40)
sEng	≥ 2.693	87 (79–95)	81 (43/53)	88 (30/34)	6.9 (2.7–17)	91% (81–96)	0.21 (0.12–0.38)	25% (16–37)
PlGF	≤0.396	87 (79–95)	94 (50/53)	71 (24/34)	3.2 (1.9–5.4)	83% (75–89)	0.08 (0.03–0.25)	11% (4–28)
sVEGFR-2	≤ 0.74	81 (71–91)	74 (39/53)	79 (24/34)	3.6 (1.8–7.1)	85% (74–92)	0.33 (0.21–0.54)	34% (25–46)
PlGF/sVEGFR-1	≤0.05	88 (81–96)	74 (39/53)	91 (31/34)	8.3 (2.8–25)	93% (81–97)	0.29 (0.18–0.46)	31% (22–42)
PlGF/sEng	≤0.07	90 (83–97)	76 (40/53)	91 (31/34)	8.6 (2.9–25)	93% (82–97)	0.27 (0.17–0.44)	30% (21–41)

Prevalence of severe preeclampsia requiring preterm delivery = 60.9% (53/87)

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Table V

Diagnostic performance of plasma concentrations of angiogenic/anti-angiogenic factors in patients who presented at <34 weeks for the identification of those who subsequently delivered within 14 days due to preeclampsia

GA at blood sampling <34 weeks	Cut-off (MoM) derived from ROC	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Likelihood Ratio of a positive test (95% CI)	Posterior probability (95% CI)	Likelihood Ratio of a negative test (95% CI)	Posterior probability (95% CI)
sVEGFR-1	≥3.97	86 (76–96)	93 (25/27)	75 (24/32)	3.70 (2.0–6.8)	76% (63–85)	0.10 (0.03–0.38)	8% (2–24)
sEng	≥2.96	84 (74–95)	96 (26/27)	75 (24/32)	3.85 (2.1–7.1)	76% (64–86)	0.05 (0.01–0.34)	4% (1–22)
PlGF	≤0.152	85 (75–95)	82 (22/27)	84 (27/32)	5.21 (2.29–12)	81% (66–91)	0.22 (0.10–0.49)	16% (8–29)
sVEGFR-2	≤0.74	82 (72–93)	82 (22/27)	72 (23/32)	2.90 (1.6–5.2)	71% (58–81)	0.26 (0.11–0.59)	18% (9–33)
PlGF/sVEGFR-1	≤0.033	88 (79–97)	93 (25/27)	78 (25/32)	4.23 (2.2–8.2)	78% (65–87)	0.09 (0.02–0.36)	7% (2–23)
PlGF/sEng	≤0.05	87 (77–96)	85 (23/27)	84 (27/32)	5.45 (2.4–12)	82% (67–91)	0.18 (0.07–0.44)	13% (6–27)

Prevalence of patients who subsequently delivered within 14 days due to severe preeclampsia = 45.8% (27/59)

Table VI

Rate of preterm delivery in patients presenting to the obstetrical triage area at < 34 weeks with a diagnosis of "suspected preeclampsia" according to the 3-zone classification of plasma angiogenic/anti-angiogenic factor concentrations

Preterm Delivery	Prevalence % (n/N)	PlGF/sVEGFR-1 (MoM)			PlGF/sEng (MoM)		
		≥0.35 (n=16)	0.051–0.35 (n=10)	≤0.05 (n=33)	≥0.30 (n=16)	0.071–0.30 (n=10)	≤0.07 (n=33)
Delivery < 34 weeks	50.8% (30/59)	6.3% (1/16)	30.0% (3/10)	78.8% (26/33)	6.3% (1/16)	36.4% (4/10)	75.8% (25/33)
Delivery <34 weeks due to PE	44% (26/59)	0 (0/16)	30.0% (3/10)	69.7% (23/33)	0 (0/16)	30.0% (3/10)	69.7% (23/33)
Delivery within 14 days	52.5% (31/59)	6.3% (1/16)	20.0% (2/10)	84.8% (28/33)	6.3% (1/16)	40.0% (4/10)	78.8% (26/33)
Delivery within 14 days due to PE	45.8% (27/59)	0 (0/16)	20.0% (2/10)	75.8% (25/33)	0 (0/16)	30.0% (3/10)	72.7% (24/33)
Delivery within 7 days	39% (23/59)	6.3% (1/16)	20.0% (2/10)	60.6% (20/33)	6.3% (1/16)	40.0% (4/10)	54.5% (18/33)
Delivery within 7 days due to PE	33.9% (20/59)	0 (0/16)	20.0% (2/10)	54.5% (18/33)	0 (0/16)	30.0% (3/10)	51.5% (17/33)

PE: preeclampsia; MoM: multiple of the medians

Table VII

Disposition of patients with “suspected preeclampsia” before 34 weeks of gestation from the triage areaaccording to the 3-zone classification of plasma angiogenic/anti-angiogenic factor concentrations

	PlGF/sVEGFR-1 (MoM)			PlGF/sEng (MoM)		
	≥0.35 (n=16)	**0.051–0.35 (n=10)**	**≤0.05 (n=33)**	**≥0.30 (n=16)**	**0.071–0.30 (n=10)**	**≤0.07 (n=33)**
Home	8 (50%)	2 (20%)	2 (6.1%)	8 (53.3%)	2 (18.2%)	2 (6.1%)
Hospitalized	8 (50%)	8 (80%)	31 (93.9%)	8 (50%)	8 (80%)	31 (93.9%)

p<0.005 for both PlGF/sVEGFR-1 and PlGF/sEng

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Table VIII

Obstetrical characteristics and plasma concentrations of PlGF/sVEGFR-1 and PlGF/sEng ratio in patients with severe maternal complications

Complications	Group	Diagnosis in triage	Diagnosis at delivery	Gestational age at blood Draw (weeks)	Gestational age at delivery (weeks)	Birthweight (grams)	%	PlGF/R1 (MoM)	PlGF/sVEGFR-1 (MoM)	Apgar at 1/5 minute	Interval to delivery (days)
Placental Abruption	I	GHTN	GHTN	26.3	38.3	3265	50	0.667	0.983	9/9	85
Placental Abruption	IV	CHTN +SPE	CHTN +SPE	27.9	28.3	810	11.2	0.001	0.002	7/9	3
Placental Abruption	IV	CHTN +SPE	CHTN +SPE	30.1	30.3	1120	12.5	0.006	0.006	0/0	1
Placental Abruption	IV	CHTN +SPE	CHTN +SPE	30.4	30.7	1250	22	0.001	0.001	6/8	2
Placental Abruption	IV	Severe PE	Severe PE	32.1	32.9	1585	15.1	0.011	0.040	5/6	5
HELLP	III	Mild PE	Severe PE	27.6	28.4	715	6.8	0.001	0.001	3/6	6
HELLP	IV	Severe PE	Severe PE	31.6	31.7	1660	33.9	0.003	0.003	6/7	1
Pulmonary edema	III	CHTN +SPE	CHTN +SPE	26.3	28.9	941	24.4	0.062	0.064	5/8	18
Pulmonary edema	III	Mild PE	Severe PE	25.7	26.6	750	28.2	0.005	0.014	6/7	6
Pulmonary edema	IV	Severe PE	Severe PE	32.1	32.9	1585	15.1	0.011	0.040	5/6	5
Pulmonary edema	IV	Severe PE	Severe PE	35.7	36	2235	7	0.032	0.054	6/8	2
Eclampsia	III	Mild PE	Severe PE	36.6	36.6	3125	58.7	0.116	0.232	6/8	0
Eclampsia	IV	Severe PE	Severe PE	36.9	37.1	1670	1	0.005	0.005	7/8	2

PE: Preeclampsia; GHTN: Gestational hypertension; CHTN: Chronic hypertension; SPE: superimposed preeclampsia; %: Percentile