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ORIGINAL ARTICLE

The use of angiogenic biomarkers in maternal blood to identify which SGA fetuses will require a preterm delivery and mothers who will develop pre-eclampsia*

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Abstract

Objective: To determine (1) whether maternal plasma concentrations of angiogenic and anti-angiogenic factors can predict which mothers diagnosed with “suspected small for gestational age fetuses (sSGA)” will develop pre-eclampsia (PE) or require an indicated early preterm delivery (≤ 34 weeks of gestation); and (2) whether risk assessment performance is improved using these proteins in addition to clinical factors and Doppler parameters.

Methods: This prospective cohort study included women with singleton pregnancies diagnosed with sSGA (estimated fetal weight <10 th percentile) between 24 and 34 weeks of gestation ($n = 314$). Plasma concentrations of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), soluble endoglin (sEng) and placental growth factor (PIGF) were determined in maternal blood obtained at the time of diagnosis. Doppler velocimetry of the umbilical (Umb) and uterine (UT) arteries was performed. The outcomes were (1) subsequent development of PE; and (2) indicated preterm delivery at ≤ 34 weeks of gestation (excluding deliveries as a result of spontaneous preterm labor, preterm pre-labor rupture of membranes or chorioamnionitis).

Results: (1) The prevalence of PE and indicated preterm delivery was 9.2% ($n = 29/314$) and 7.3% ($n = 23/314$), respectively; (2) the area under the receiver operating characteristic curve (AUC) for the identification of patients who developed PE and/or required indicated preterm delivery was greater than 80% for the UT artery pulsatility index (PI) z-score and each biochemical marker (including their ratios) except sVEGFR-1 MoM; (3) using cutoffs at a false positive rate of 15%, women with abnormal plasma concentrations of angiogenic/anti-angiogenic factors were 7–13 times more likely to develop PE, and 12–22 times more likely to require preterm delivery than those with normal plasma MoM concentrations of these factors; (4) sEng, PIGF, PIGF/sEng and PIGF/sVEGFR-1 ratios MoM, each contributed significant information about the risk of PE beyond that provided by clinical factors and/or Doppler parameters: women who had low MoM values for these biomarkers were at 5–9 times greater risk of developing PE than women who had normal values, adjusting for clinical factors and Doppler parameters (adjusted odds ratio for PIGF: 9.1, PIGF/sEng: 5.6); (5) the concentrations of sVEGFR-1 and PIGF/sVEGFR-1 ratio MoM, each contributed significant information about the risk of indicated preterm delivery beyond that provided by clinical factors and/or Doppler parameters: women who had abnormal values were at 8–9 times greater risk for indicated preterm delivery, adjusting for clinical factors and Doppler parameters; and (6) for a two-stage risk assessment (Umb artery Doppler followed by Ut artery Doppler plus biochemical markers), among women who had normal Umb artery Doppler velocimetry ($n = 279$), 21 (7.5%) developed PE and 11 (52%) of these women were identified by an abnormal UT artery Doppler

Keywords

Angiogenic/Anti-angiogenic factors, intrauterine growth restriction (IUGR), placental growth factor, pregnancy, soluble endoglin, sflt-1, soluble vascular endothelial growth factor receptor-1, umbilical artery Doppler, uterine artery Doppler

History

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mean PI z-score ($>2SD$): a combination of PIGF/sEng ratio MoM concentration and abnormal UT artery Doppler velocimetry increased the sensitivity of abnormal UT artery Doppler velocimetry to 76% (16/21) at a fixed false-positive rate of 10% ($p = 0.06$).

Conclusion: Angiogenic and anti-angiogenic factors measured in maternal blood between 24 and 34 weeks of gestation can identify the majority of mothers diagnosed with “suspected SGA” who subsequently developed PE or those who later required preterm delivery ≤ 34 weeks of gestation. Moreover, incorporation of these biochemical markers significantly improves risk assessment performance for these outcomes beyond that of clinical factors and uterine and umbilical artery Doppler velocimetry.

Introduction

The identification of fetal growth disorders, and, in particular, the small for gestational age (SGA) fetus, has been a major concern in clinical obstetrics for decades [1–15]. Most efforts have focused on the use of sonographic fetal biometric standards or reference ranges when there is an index of suspicion (i.e. when the fundal height is shorter or larger than expected) [16–33]. However, this approach is not satisfactory, because the precise diagnosis of fetal growth disorders (acceleration or deceleration) require measurement of the fetus rather than assessment of the entire uterine contents (which includes amniotic fluid and the uterine wall) through physical examination of the maternal abdomen [34–49].

Growth is defined as a time-dependent change in bodily dimensions, and therefore, its assessment needs serial examinations [1]. Therefore, many investigators have used longitudinal studies to generate standards or reference ranges to monitor fetal growth. These studies have included individual biometric parameters such as fetal head circumference [50–60], and abdominal circumference [50–54,56,58,60–63], as well as computed-estimated fetal weight [50,51] or volumetric parameters such as limb volume [64,65] or fractional limb volume [66–68]. The conceptual basis consists of comparing the trajectory of growth of a particular fetus with that of a standard. Recently, the possibility of using early biometric measurements to perform individualized growth assessment has been subject of several reports [66–71].

Once an SGA fetus has been diagnosed (using cross-sectional standards, longitudinal standards or individualized fetal growth assessment), a key question is the optimal management of such case [13,72–75]. The differential diagnosis between fetuses which are small because of growth restriction from those who are small because of “constitutional factors” has been considered a major area of interest in clinical obstetrics [13,72–75]. A recent randomized clinical trial of SGA fetuses diagnosed at term has compared induction of labor and expectant management, and there is no evidence that one approach is clearly superior to the other [76]. In the preterm fetus, a Bayesian randomized clinical trial has also shown no clear benefit of induction versus expectant management [77–83].

Mothers of SGA fetuses are at increased risk for developing pre-eclampsia (PE) [84–87], and some SGA fetuses may deteriorate and require early delivery because of suspected fetal compromise [75,88–101]. We and others previously reported that SGA [102–118], PE [102,109,110,113,119–164] and stillbirth [153,165–169] are

associated with an abnormal angiogenic to anti-angiogenic ratio by measuring placental growth factor (PIGF), soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and soluble endoglin (sEng) in the maternal circulation. Moreover, we have reported that an imbalance in angiogenic and anti-angiogenic factors measured in maternal blood are detectable before the clinical diagnosis, and that such measurements have prognostic value [102,110,119–121,123,127,129,130,132,134–138,140,143–146]. For example, maternal plasma concentrations of such biomarkers can identify women presenting to the obstetrical triage area with symptoms or signs of PE who are at subsequent risk for preterm delivery and the development of adverse outcomes [113,141,142,147,150–152].

Once the diagnosis of an SGA fetus has been made, monitoring consists of umbilical artery Doppler velocimetry and serial biometry [31,170–182]. Systematic reviews have shown that the introduction of umbilical artery Doppler velocimetry reduces perinatal death in high-risk patients [183–185]. The performance of uterine artery Doppler velocimetry can also identify patients at increased risk of obstetrical complications [186–188]; however, the likelihood ratio of a positive test to identify women with SGA fetuses who will develop PE or gestational hypertension is only 4–5 – therefore, this test has not been incorporated into clinical practice [86].

We propose that the measurement of maternal plasma angiogenic and anti-angiogenic factors can identify mothers with SGA fetuses who will develop PE or require an indicated delivery before 34 weeks of gestation. The current study was designed to test this hypothesis and determine if the information gained from the measurement of these biomarkers would have additional value to that derived from evaluation of Doppler velocimetry of the umbilical or uterine artery.

Methods

Study design

This study was a subcohort of women who were enrolled in a prospective longitudinal study conducted to identify biological markers for the prediction of PE and SGA. This subcohort included women who had sSGA fetuses based on ultrasonographic estimated fetal weight between 24 and 33 + 6/7 weeks of gestation from November 2007 to August 2011. Patients were enrolled in the Center for Advanced Obstetrical Care at Hutzel Hospital, Detroit, MI, and followed

until delivery. Inclusion criteria were: (1) singleton pregnancy; (2) ultrasound biometry below 10th percentile for gestational age; (3) first ultrasound for dating performed prior to 24 weeks of gestation; (4) no evidence of PE (hypertension and proteinuria) at the ultrasonographic examination; and (5) venipuncture was performed within 2 days of the ultrasonographic examination. Exclusion criteria were: (1) preterm labor, preterm pre-labor rupture of membranes, PE or impaired fetal growth at the time of recruitment; (2) known major fetal anomaly or fetal demise; (3) active vaginal bleeding; and (4) serious medical illness (renal insufficiency, congestive heart disease, chronic respiratory insufficiency or active hepatitis).

Maternal blood was collected by venipuncture, and Doppler examination of the UT and Umb arteries were performed, at enrollment and during examinations scheduled every 4 weeks until 24 weeks, and every 2 weeks thereafter until delivery. Ultrasonographic biometry was also performed every 3–4 weeks to assess fetal growth.

The primary and secondary outcomes of the study were development of PE and indicated preterm delivery before 34 weeks of gestation, respectively. All participants provided written informed consent and the research protocol was approved by the Institutional Review Boards of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NIH/DHHS) and Wayne State University, Detroit, Michigan.

Definitions

The diagnosis of “suspected SGA” was based on sonographic estimated fetal weight below the 10th percentile for gestational age [189] determined using fetal head circumference, biparietal diameter, abdominal circumference and femur length measurements. SGA was defined as a birth weight below the 10th percentile for gestational age [190]. Gestational age was determined by last menstrual period and confirmed by the earliest ultrasound examination. PE was defined as new-onset hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg on 2 measurements at least 4 h apart) and proteinuria after 20 weeks gestation in a previously normotensive woman [191]. Proteinuria was defined as ≥ 300 mg protein in a 24 h urine collection specimen [191,192] or, if a 24 h urine was not completed, at least 1 + protein (30 mg/dL) on 2 urine dipsticks, measured at least 4 h apart. An “indicated preterm delivery” was defined as delivery for maternal indications or concerns regarding fetal well-being. For the objectives of this study, this definition did not include deliveries occurring as a result of spontaneous preterm labor, preterm pre-labor rupture of membranes or clinical chorioamnionitis.

Sonographic examination

Fetal biometry was obtained every 3–4 weeks and the pulse wave and color Doppler ultrasound examination was performed on the Umb and both UT arteries using a 3.5- or 5-MHz curvilinear probe at every visit as previously described [108,193]. The PI of the right and left UT arteries was measured and the mean PI of the two vessels was calculated. UT artery Doppler velocimetry was defined as abnormal if the

mean PI was above the second standard deviation (SD) for gestational age using the reference range from our institution. The Doppler signal of the Umb artery was obtained from a free floating loop of the umbilical cord during the absence of fetal breathing and body movement. When three similar consecutive waveforms were obtained, the PI was measured. Umb artery Doppler velocimetry was defined as abnormal if the PI was above the second SD for gestational age using the reference range from our institution or if abnormal waveforms (absent or reversed end-diastolic velocities) were present as described by Trudinger et al. [194]. The inter- and intra-observer CV for UT Doppler measurement were 11.6 and 5.4%, and for Umb, 9.5 and 7%, respectively.

Sample collection and immunoassay

Maternal plasma was collected into tubes containing EDTA. Samples were centrifuged and stored at -70°C . Sensitive and specific immunoassays (R&D systems, Minneapolis, MN) utilizing a quantitative sandwich enzyme technique were used to determine maternal plasma concentrations of PlGF, sEng and sVEGFR-1. The inter- and intra-assay coefficients of variation (CV) were: PlGF, 6.02 and 4.8%; sEng, 2.3 and 4.6%; sVEGFR-1, 1.4 and 3.9%, respectively. The lower limits of the detection were: PlGF, 9.52 pg/mL; sEng, 0.08 ng/mL; and sVEGFR-1, 16.97 pg/mL.

Statistical analysis

Descriptive analysis of demographic and obstetrical characteristics of the study cohort was performed using SPSS Version 15.0 (SPSS, Inc., Chicago, IL). Umb artery Doppler pulsatility index (PI) and UT artery Doppler (UT) mean PI were converted to z-scores, whereas biochemical markers were converted to multiples of the expected median (MoM) for gestational age, each based on reference values from uncomplicated pregnancies with appropriately grown neonates. The unpaired *t*-test or Mann–Whitney *U* statistic was used to test for differences in the distributions of arithmetic variables, as appropriate. Logistic regression models were fitted to determine the crude and adjusted magnitude of association between biochemical markers, Doppler parameter z-scores and PE, as well as indicated preterm delivery before 34 weeks of gestation.

Multivariable models were fitted to determine whether incorporation of biochemical markers improved performance of risk assessment compared to use of clinical factors and Umb and UT artery Doppler PI z-scores, both individually and in combination [195,196]. Biochemical markers that had significant independent associations with the outcome of interest were interpreted as providing information that significantly improved risk assessment performance compared to use of the other predictors included in each model [195,197].

To describe performance in discriminating cases from non-cases, receiver-operating characteristic (ROC) curves were constructed for biochemical markers that significantly improved risk assessment performance. Statistical significance was defined either by non-overlapping 95% CIs or *p* values < 0.05 . These analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Demographic and clinical characteristics

A total of 314 women were included in this study (Figure 1). Demographic and obstetric characteristics of the study population are summarized in Table 1. The prevalence of PE was 9.2% (29/314) and 7.3% (23/314) of the women required indicated preterm delivery because of maternal and/or fetal indications at ≤ 34 weeks of gestation.

Among patients with PE, 11 (38%) were diagnosed with early onset (≤ 34 weeks of gestation) disease. The median interval from venipuncture to the diagnosis of PE was 43 days (interquartile range 24–63). Table 2 displays the indications for delivery of patients who required a preterm delivery at ≤ 34 weeks of gestation. The two most common indications

were PE [47.8% (11/23)] and abruptio placentae [30.4% (7/23)].

Differences in biomarker distributions

Patients who subsequently developed PE (Figure 2) or required an indicated delivery at ≤ 34 weeks (Figure 3) had significantly higher median plasma sVEGFR-1 and sEng MOM concentrations, and higher mean Umb and UT artery Doppler PI z-scores, but significantly lower median plasma PIGF, PIGF/sEng ratio and PIGF/sVEGFR-1 ratio MOM concentrations, than those who did not develop these complications (each $p < 0.005$).

Single-marker prognostic performance

Figure 4 shows the AUC and sensitivity at 85% specificity for each biochemical and Doppler parameter in identifying patients at risk of PE or indicated preterm delivery at ≤ 34 weeks of gestation. The AUC in identifying either outcome was greater than 80% for UT artery PI z-score (AUC 84% for PE and 93% for indicated delivery ≤ 34 weeks) and each biochemical marker (AUC 84–88% for PE and 84–87% for indicated delivery ≤ 34 weeks) except the sVEGFR-1 MoM (AUC 75% for PE and 78% for indicated delivery ≤ 34 weeks). UT artery PI z-score performed comparably to the biochemical markers in identifying patients at risk of each outcome. In addition, at a specificity of 85%, the sensitivity of biochemical markers for identifying women at risk for PE

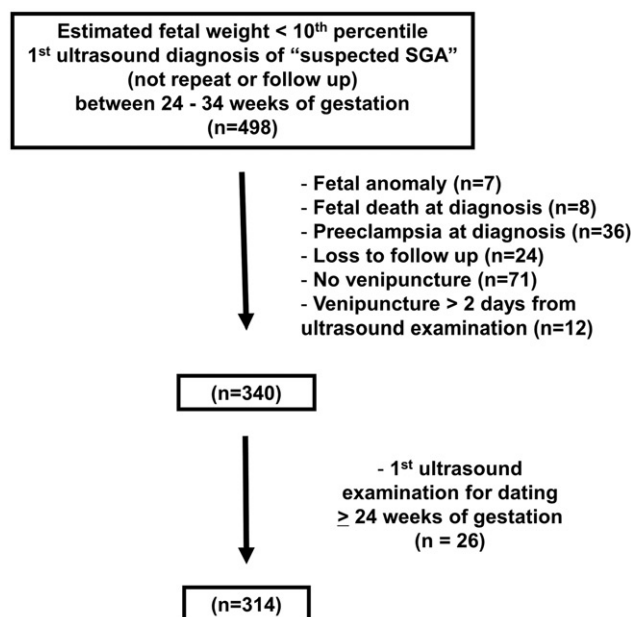


Figure 1. Flow diagram of patients enrolled in the cohort.

Table 2. Indications for preterm delivery ≤ 34 weeks of gestation ($n = 23$).

Indications*	Number (%)
Pre-eclampsia	11 (47.8%)
Abnormal umbilical artery Doppler velocimetry	6 (26.1%)
Non-reassuring fetal heart rate tracing	5 (21.7%)
Placental abruption	7 (30.4%)

*A patient may have more than one indication for delivery.

Table 1. Demographic and clinical characteristics of the study population.

	Suspected SGA without subsequent pre-eclampsia ($n = 285$)	Suspected SGA with subsequent pre-eclampsia ($n = 29$)	p
Maternal age (years)	21 (19–25)	20 (19–24)	0.08
Nulliparity	124 (44%)	15 (52%)	0.4
African American	239 (84%)	26 (90%)	0.6
Body mass index (kg/m^2)	25 (21–31)	25 (22–29)	1
Smoking	66 (23%)	8 (28%)	0.6
Gestational age at first US examination for dating	15.8 (12.4–18.9)	13.0 (11.1–18.3)	0.1
Gestational age at venipuncture (weeks)	27.1 (25.6–29.7)	27.7 (26.0–29.6)	0.6
Gestational age at diagnosis of suspected SGA (weeks)	27.1 (25.6–29.7)	27.7 (26.0–29.6)	0.6
Gestational age at delivery (weeks)	39 (37–40)	36 (33–36)	<0.001
Interval from venipuncture to delivery	80 (54–96)	53 (31–68)	<0.001
Birthweight (grams)	2825 (2420–3115)	1835 (1317–2357)	<0.001
Birthweight (percentile)	17 (7–32)	5 (1–17)	<0.001
Indicated delivery ≤ 34 weeks	12 (4.2%)	11 (38%)	<0.001
Pre-eclampsia diagnosed before 34 weeks	0	11 (38%)	–
Interval from venipuncture to diagnosis of pre-eclampsia (days)	–	43 (24–63)	–

Data are expressed as median (inter-quartile range) or number (percent); US = ultrasound; SGA = small-for-gestational age; US = ultrasonographic examination.

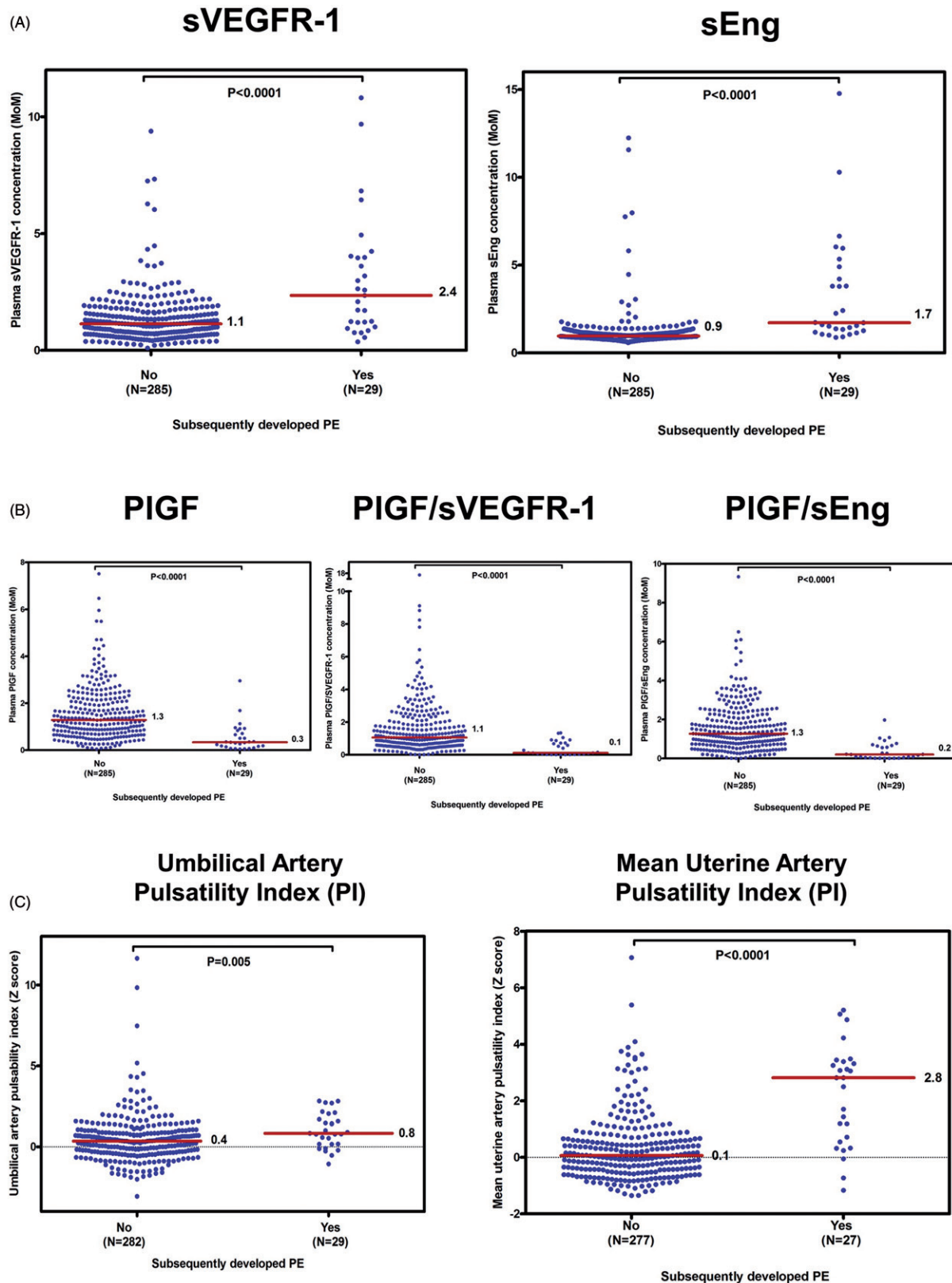


Figure 2. Median plasma concentration of angiogenic/anti-angiogenic factors and pulsatility index (PI) of the umbilical (Umb) and uterine (UT) artery Doppler velocimetry in women who did or did not subsequently develop PE. (A) Patients with “suspected SGA” who subsequently developed PE had higher median plasma multiple of the expected median (MoM) concentrations of sVEGFR-1 and sEng than those who did not. [sVEGFR-1 MoM: median 2.4, interquartile range (IQR) 1.2–4.0 versus median 1.1, IQR 0.7–1.7; $p < 0.001$; and sEng MoM: median 0.96, IQR 0.84–1.2 versus median 1.7, IQR 1.3–4.2; $p < 0.001$]. (B) Median plasma MoM concentration of placental growth factor (PIGF), the ratio of PIGF/sVEGFR-1 and the ratio of PIGF/sEng in women who did or did not subsequently develop PE. Patients with “suspected SGA” who subsequently developed PE had lower median plasma MoM concentrations of PIGF, PIGF/sVEGFR-1 and PIGF/sEng than those who did not. [PIGF MoM: median 0.34, IQR 0.16–0.71 versus median 1.3, IQR 0.71–2.1; $p < 0.001$; PIGF/sVEGFR-1 MoM: median 0.12, IQR 0.06–0.72 versus median 1.1, IQR 0.59–2.0; $p < 0.001$ and PIGF/sEng MoM: median 0.20, IQR 0.05–0.64 versus median 1.3, IQR 0.74–2.2; $p < 0.001$]. (C) Median umbilical and uterine artery PI z-scores in women who did and did not subsequently develop PE. Patients with “suspected SGA” who subsequently developed PE had higher median umbilical and uterine artery PI z-scores than those who did not. [Umbilical artery PI z-score: median 0.83, IQR 0.17–1.7 versus median 0.35, IQR –0.35 to 1.0; $p < 0.001$; Uterine artery mean PI z-score: median 2.8, IQR 0.55–3.4 versus median 0.065, IQR –0.44 to 0.68; $p < 0.001$.]

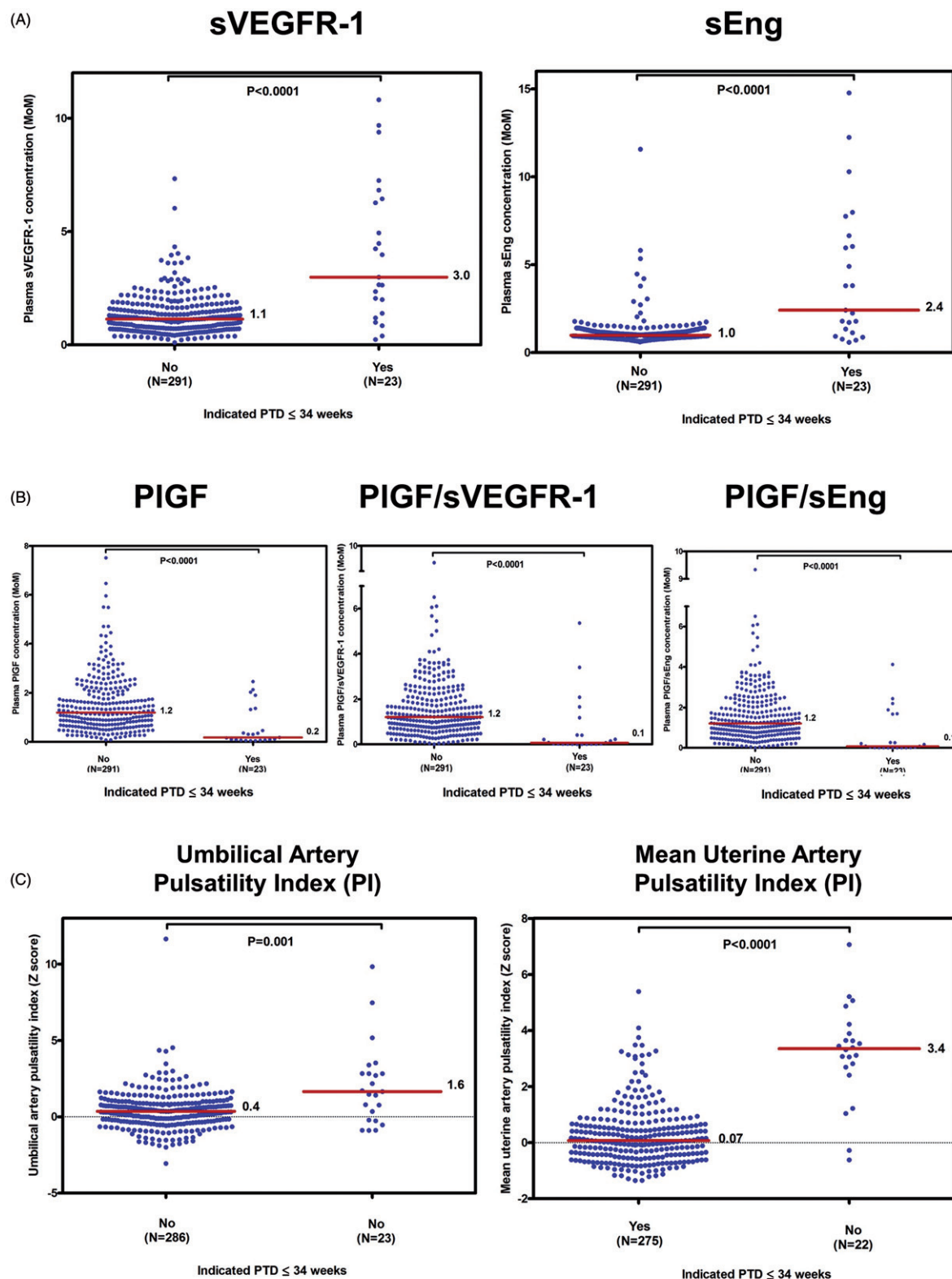


Figure 3. Median plasma concentration of angiogenic/anti-angiogenic factors as well as pulsatility index (PI) of the umbilical (Umb) and uterine (UT) artery Doppler velocimetry in women who subsequently required indicated preterm delivery at or before 34 weeks of gestation and those who did not. (A) Patients suspected to have SGA fetuses who subsequently required indicated preterm delivery had higher median plasma multiple of the medians (MoM) concentrations of sVEGFR-1 and sEng than those who did not. [sVEGFR-1 MoM: median 3.0, interquartile range (IQR) 1.4–6.4 versus median 1.1, IQR 0.72–1.7; $p < 0.001$; and sEng MoM: median 2.4, IQR 1.1–6.6 versus median 0.98, IQR 0.85–1.2; $p < 0.001$]. (B) Median plasma MoM concentration of placental growth factor (PIGF), the ratio of PIGF/sVEGFR-1 and the ratio of PIGF/sEng in women who subsequently required indicated preterm delivery and those who did not. Patients with “suspected SGA” who subsequently developed PE had lower median plasma MoM concentrations of PIGF, PIGF/sVEGFR-1 and PIGF/sEng than those who did not. [PIGF MoM: median 0.18, IQR 0.072–1.3 versus median 1.2, IQR 0.70–2.1; $p < 0.001$; PIGF/sVEGFR-1 MoM: median 0.066, IQR 0.013–0.41 versus median 1.0, IQR 0.59–1.9; $p < 0.001$ and PIGF/sEng MoM: median 0.074, IQR 0.012–1.7 versus median 1.2, IQR 0.70–2.1; $p < 0.001$]. (C) Median umbilical and uterine artery PI z-scores in women who subsequently required indicated preterm delivery and those who did not. Patients with “suspected SGA” who required indicated preterm delivery had higher median umbilical and uterine artery PI z-scores than those who did not. [Umbilical artery PI z-Score: median 1.6, interquartile range (IQR) –0.21 to 2.8 versus median 0.36, IQR –0.32 to 0.89; $p < 0.001$; and Uterine artery mean PI z-Score: median 3.3, IQR 2.4–3.9 versus median 0.071, IQR –0.44 to 0.66; $p < 0.001$.]

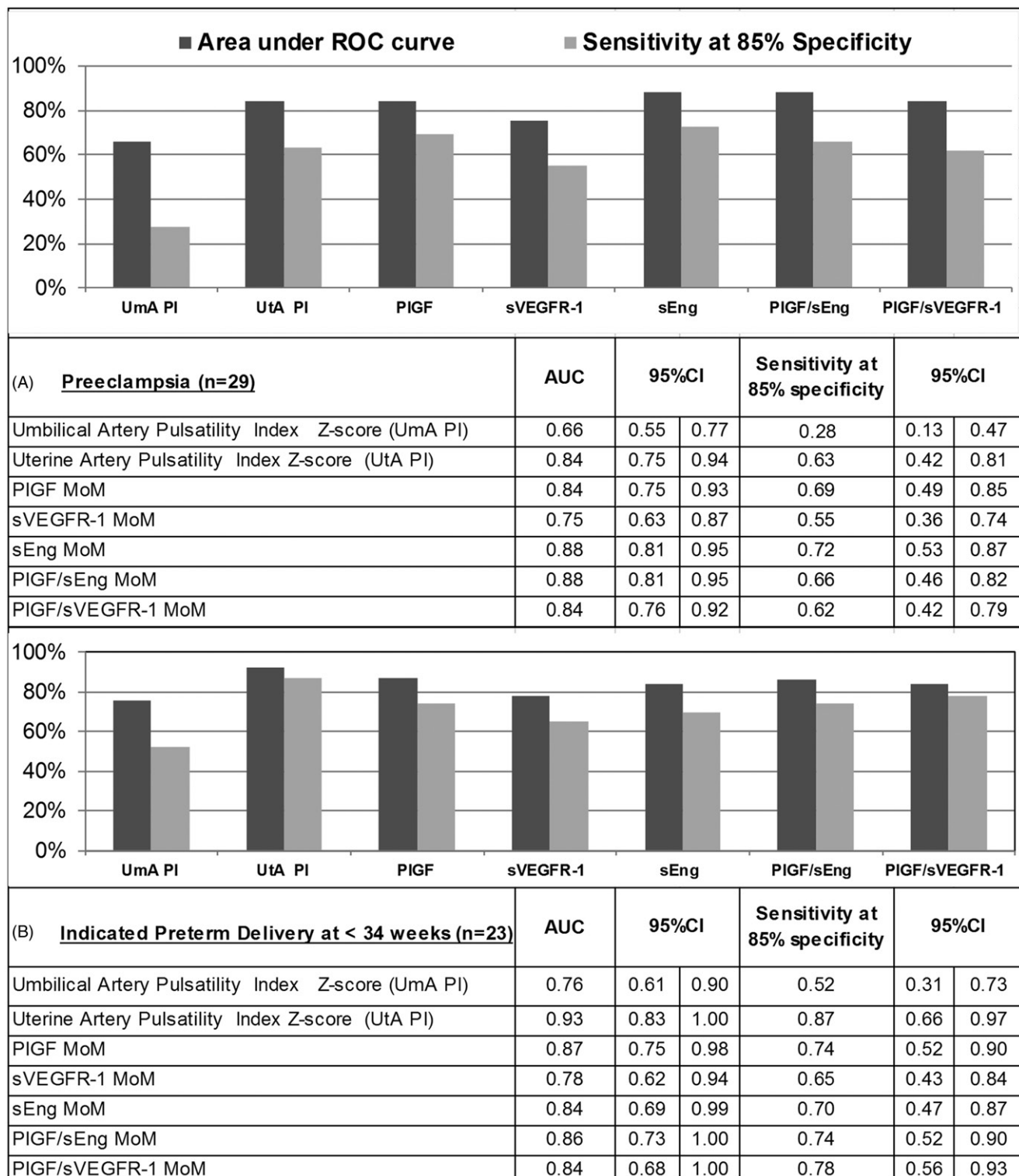


Figure 4. Area under the receiver operating characteristic curve and sensitivity at 85% specificity for the identification of patients at risk of PE (A) and indicated preterm delivery at ≤ 34 weeks of gestation (B) using individual biochemical marker and Doppler parameter.

(sensitivity 55–72%) or indicated delivery (sensitivity 65–78%) was more than double that of Umb artery PI z-score alone (sensitivity 28% for PE and 52% for indicated delivery).

Multi-marker prognostic performance

Tables 3 and 4 show the unadjusted and adjusted magnitudes of the association between each biochemical and Doppler

parameter marker (expressed either as a continuous or categorical variable) with PE and indicated preterm delivery at ≤ 34 weeks, respectively. Four columns of odds ratios are shown in each table. The first column describes unadjusted magnitudes of association between each biomarker and outcome. The remaining three columns of odds ratios are shown to describe whether inclusion of each biochemical marker improved risk assessment beyond: a combination of

Table 3. Magnitudes of association between subsequent diagnosis of pre-eclampsia and maternal plasma concentrations of angiogenic and anti-angiogenic factors, unadjusted and adjusted for clinical factors and uterine and umbilical artery Doppler velocimetry.

Biomarkers with and without cutoffs	I – Biomarkers Only			II – Biochemical markers added individually to all Clinical factors*			III – Biochemical added Individually to Doppler parameters			IV – Biochemical markers added individually to significant Clinical factors and Doppler parameters**		
	OR	95% CI		aOR	95% CI		aOR	95% CI		aOR	95% CI	
<i>Without cutoffs</i>												
PIGF MoM	0.1	0.04	0.3	0.1	0.05	0.5	0.2	0.1	0.6	0.3	0.1	0.8
sVEGFR-1 MoM	1.7	1.3	2.1	1.7	1.2	2.3	1.4	1.1	1.8	1.3	0.9	1.9
sEng MoM	1.6	1.3	1.9	2.1	1.1	3.8	1.3	1.1	1.6	1.5	0.8	2.6
PIGF/sEng MoM	0.1	0.02	0.2	0.1	0.01	0.3	0.1	0.04	0.4	0.1	0.04	0.6
PIGF/sVEGFR-1 MoM	0.1	0.02	0.2	0.1	0.02	0.4	0.2	0.1	0.5	0.2	0.1	0.7
Umbilical artery PI z-score	1.2	0.98	1.5									
Uterine artery PI z-score	2.1	1.6	2.7									
<i>With cutoffs***</i>												
PIGF < 0.5 MoM	13.2	5.6	31	31.6	7.4	134	7.1	2.7	19.0	9.1	2.5	33.3
sVEGFR-1 > 2.1 MoM	6.6	2.9	14.6	4.1	1.4	12.7	4.1	1.6	10.4	2.3	0.6	8.2
sEng > 1.3 MoM	14	5.8	33.6	17.3	5.0	60.1	7.5	2.8	19.8	5.6	1.7	18.4
PIGF/sEng < 0.45 MoM	11	4.8	25.3	17.5	4.7	64.8	5.7	2.2	15.2	5.6	1.6	19.4
PIGF/sVEGFR-1 < 0.46 MoM	9.7	4.3	22.1	10.1	3.2	32.1	4.9	1.8	12.9	5.0	1.5	17.2
Umbilical artery PI > 2SD	3.6	1.4	9.2									
Uterine artery PI > 2SD	14.2	5.9	34.1									

OR: odds ratio; 95% CI: 95% confidence interval; MoM: multiple of the medians; PIGF: placental growth factor; sVEGFR-1: soluble vascular endothelial growth factor receptor-1; sEng: soluble endoglin; SD: standard deviation.

*Clinical factors included parity, history of PE in a previous pregnancy, age, race, smoking status, pre-pregnancy body mass index-BMI and mean arterial pressure.

**Additionally adjusted for significantly associated Doppler parameters – parity, history of PE, BMI, MAP, Uterine artery PI.

***Thresholds for biochemical markers were selected at 85% fixed specificity.

Table 4. Magnitudes of association between subsequent indicated preterm delivery ≤ 34 weeks of gestation and maternal plasma concentrations of angiogenic and anti-angiogenic factors, unadjusted and adjusted for clinical factors and uterine and umbilical artery Doppler velocimetry.

Biomarkers with and without cutoffs	I – Biomarkers Only			II – Biochemical markers added individually to all Clinical factors*			III – Biochemical added Individually to Doppler parameters			IV – Biochemical markers added individually to significant Clinical factors and Doppler parameters**		
	OR	95% CI		aOR	95% CI		aOR	95% CI		aOR	95% CI	
<i>Without cutoffs</i>												
PIGF MoM	0.2	0.1	0.5	0.07	0.01	0.4	0.6	0.3	1.3	0.4	0.1	1.5
sVEGFR-1 MoM	2.3	1.7	3.1	2.6	1.6	4.3	2.0	1.4	2.8	2.2	1.2	3.9
sEng MoM	2.0	1.5	2.6	5.3	2.3	12.2	1.4	1.1	1.9	2.8	1.1	6.7
PIGF/sEng MoM	0.3	0.1	0.6	0.1	0.03	0.5	0.8	0.5	1.5	0.6	0.2	1.7
PIGF/sVEGFR-1 MoM	0.4	0.2	0.7	0.5	0.3	1.1	0.7	0.5	1.1	0.9	0.6	1.3
Umbilical artery PI z-score	1.6	1.2	2.1									
Uterine artery PI z-score	3.5	2.4	5.1									
<i>With cutoffs***</i>												
PIGF < 0.5 MoM	15.9	5.9	42.6	24.6	5.9	102.5	4.7	1.3	16.7	5.9	1.0	34.2
sVEGFR-1 > 2.1 MoM	11.8	4.7	29.5	9.4	2.9	30.3	9.5	2.5	35.8	9.1	1.5	57.3
sEng > 1.3 MoM	14	5.3	37.3	20.3	4.9	83.5	4.4	1.2	15.8	6.5	1.0	44.3
PIGF/sEng < 0.45 MoM	15.9	5.9	42.6	25.2	5.9	107.6	4.8	1.3	16.9	6.0	1.0	34.4
PIGF/sVEGFR-1 < 0.46 MOM	22	7.7	62.4	24.0	5.9	97.5	8.7	2.4	31.9	8.4	1.4	48.8
Umbilical artery PI > 2SD	10.2	4	26.2									
Uterine artery PI > 2SD	68.1	20.9	221.4									

OR: odds ratio; 95% CI: 95% confidence interval; MoM: multiple of the medians; PIGF: placental growth factor; sVEGFR-1: soluble vascular endothelial growth factor receptor-1; sEng: soluble endoglin; SD: standard deviation.

*Clinical factors included parity, history of PE in a previous pregnancy, age, race, smoking status, pre-pregnancy body mass index (BMI) and mean arterial pressure.

**Additionally adjusted for significantly associated Doppler parameters – MAP, Umbilical Artery PI, Uterine Artery PI.

***Thresholds for biochemical markers were selected at 85%.

all clinical factors (model II); a combination of both Doppler parameters (model III); or a reduced combination of factors included in either of these two models that were statistically associated with PE or indicated preterm delivery at ≤ 34 weeks (model IV), respectively. Factors with statistically significant odds ratios are interpreted as contributing significant risk information beyond other factors included in each model.

Identifying patients at risk for pre-eclampsia

Each biochemical and Doppler parameter was significantly associated with subsequent development of PE (Table 3). Patients with “abnormal” biochemical marker values (threshold defined using a fixed specificity of 85%) were 7–13 times (without multivariable adjustment) more likely to develop PE than those without such values. The odds of subsequently developing PE were 5–9 times greater among women who had “abnormal” compared to normal sEng, PIGF, PIGF/sEng or PIGF/sVEGFR-1 MoM concentrations (thresholds defined at 85% specificity), after adjusting for parity and prior history of PE, BMI, MAP and abnormal UT artery PI z-score ($>2SD$). Therefore, all of the biochemical markers, except sVEGFR-1, contributed significant risk information above that provided by a combination of clinical factors, a combination of Doppler parameters or a combination of significantly associated clinical factors and Doppler parameters. For example, women who had low PIGF/sVEGFR-1 MoM concentrations were at five-fold greater risk of PE [odds ratio (OR), 5.0; 95% confidence interval (CI), 1.5–17.2], whereas those with low PIGF MoM concentrations were at 9-fold greater risk [OR 9.1; 95% CI 2.5–33.3], when each was compared to women who had normal values, adjusting for both clinical factors and Doppler parameters (Table 3).

Identifying patients at risk for indicated delivery at ≤ 34 weeks of gestation

Similarly, each biochemical marker and Doppler parameter was significantly associated with indicated preterm delivery (Table 4); without multivariable adjustment, patients with abnormal biochemical marker concentrations (cutoff defined at 85% specificity) were at 12–22 times greater risk of early preterm delivery. Women who had low PIGF/sVEGFR-1 MoM concentration ratio or high sVEGFR-1 MoM concentrations, were at 8–9 times [OR 8.4; 95% CI 1.4–48.8 and OR 9.1; 95% CI 1.5–57.3, respectively] greater risk of indicated preterm delivery at <34 weeks than women who did not, adjusting for MAP and Doppler velocimetry findings.

Two-stage risk assessment

Since the current management of patients diagnosed with “suspected SGA” is to triage those who require intensive surveillance with Umb artery Doppler velocimetry assessment, this study evaluated potential benefits of the incorporation of angiogenic biomarkers in this clinical scenario using a two-stage selection procedure. In the first stage, Umb artery Doppler PI z-scores were used, and women with normal values were triaged to further testing. In the second stage, a combination of UT artery Doppler velocimetry abnormality

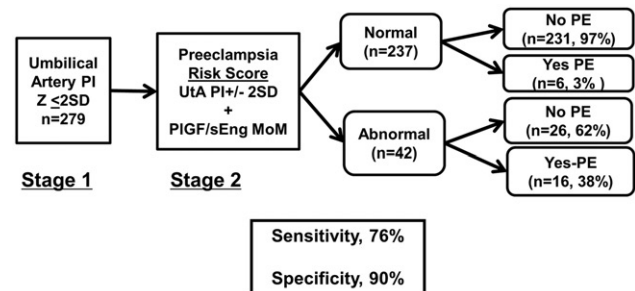


Figure 5. Risk of PE and/or indicated preterm delivery at or before 34 weeks of gestation conditional on a two-step screening approach. In the first step, Umb artery Doppler PI z-score was used to triage patients. In the second step, UT artery Doppler mean PI z-score abnormality and MoM plasma angiogenic/anti-angiogenic factor concentrations are used to identify patients that subsequently had the targeted outcome. Among patients who had normal Umb artery Doppler PI z-scores, 52% of those who subsequently developed PE had UT artery Doppler mean PI z-scores $>2SD$; adding PIGF/sEng MoM to abnormal UT artery Doppler mean PI z-score increased the sensitivity to 76% at a fixed false positive rate of 10% ($p = 0.06$).

($>2SD$) and plasma concentrations of angiogenic/anti-angiogenic factors were used to identify patients who subsequently developed adverse outcomes.

Stage 1

Umb artery Doppler velocimetry was abnormal ($>2SD$) in 10% ($n = 30$) of the study population, and 32% (9/28) (2 patients did not have information on UT artery Doppler) of these women had abnormal UT artery Doppler PI z scores ($>2SD$). Each of the nine patients with abnormal Umb and UT artery Doppler velocimetry also had an imbalance in angiogenic/anti-angiogenic factors, whereas such imbalance was found for only 16% ($n = 3/19$) of those who had abnormal Umb artery and normal UT artery Doppler velocimetry. Among these three patients with abnormal angiogenic profile, only one delivered before 34 weeks and none developed PE. There was accordingly no benefit to use biochemical markers in addition to UT artery Doppler velocimetry to identify women with abnormal Umb artery Doppler velocimetry who were at risk of PE or indicated preterm delivery at ≤ 34 weeks.

Stage 2

Among women with *normal* Umb artery Doppler velocimetry ($\leq 2SD$, $n = 279$), 10% ($n = 28$) had abnormal UT artery Doppler PI z scores. UT artery Doppler velocimetry was abnormal in 85% (11/13) of the women who subsequently required indicated preterm delivery at ≤ 34 weeks of gestation, and therefore, this biophysical marker had a high sensitivity and a reasonable positive predictive value (39%, 11/28) for this outcome. Addition of angiogenic/anti-angiogenic markers to UT artery Doppler velocimetry did not significantly improve diagnostic performance.

In contrast, the sensitivity of UT artery Doppler velocimetry in identifying women who *subsequently developed* PE, when Umb artery Doppler velocimetry was normal, was only 52% (11/21). The sensitivity of abnormal UT artery Doppler velocimetry increased to 76% (16/21) at a specificity of 90% (52% versus 76%, $p = 0.06$; Figure 5), when the PIGF/sEng concentration ratio was added.

Discussion

Principal findings of the study

Among patients diagnosed with “suspected SGA” by ultrasonographic estimated fetal weight <10th percentile for gestational age between 24 and 34 weeks of gestation: (1) women with abnormal plasma concentrations of angiogenic/anti-angiogenic factors were 7–13 times more likely to develop PE, and 12–22 times more likely to require preterm delivery ≤ 34 weeks, than women with normal plasma concentrations; (2) sEng, PIGF, PIGF/sEng or PIGF/sVEGFR-1, each contributed significant information about *the risk of PE* beyond that provided by clinical factors and/or Doppler parameters; (3) sVEGFR-1 and PIGF/sVEGFR-1, each contributed significant information about *the risk of indicated preterm delivery at ≤ 34 weeks*, beyond that provided by clinical factors and/or Doppler parameters; and (4) For the two-step risk assessment, angiogenic biomarkers did not improve risk assessment performance among patients with abnormal Umb artery Doppler velocimetry. In contrast, among patients with normal Umb artery Doppler velocimetry, the combination of PIGF/sEng with abnormal UT artery Doppler velocimetry had a sensitivity of 76% at a specificity of 90% for identifying women *destined to develop PE* (compared to a sensitivity of 52% using abnormal UT artery Doppler alone; $p=0.06$). Together, these findings indicate that angiogenic and anti-angiogenic factors measured in maternal plasma at the time of “suspected SGA” diagnosis between 24 and 34 weeks of gestation had prognostic value for subsequent development of PE or indicated early preterm delivery.

An anti-angiogenic state is present and has prognostic value in a subset of pregnancies with sSGA fetuses

An imbalance of angiogenic and anti-angiogenic factor concentrations in maternal blood was originally reported in patients with PE, both prior to [102,110,119–121,123,127,129,130,132,134–138,140,143–146] and at the time of diagnosis [122,124–126,128,131,133]. Yet, we and others have demonstrated that an anti-angiogenic state is not specific to PE, but can be observed in other obstetrical syndromes, including SGA [102–110,112,113,124], especially in patients with abnormal UT and Umb artery Doppler velocimetry findings [108]. Doppler examination of the Umb artery has been the mainstay of fetal surveillance in pregnancies with “suspected SGA” [172–174,181,198–200]. While UT artery Doppler velocimetry can identify an additional proportion of these pregnancies that are at risk of adverse perinatal outcomes [186–188], the predictive value has thus far been insufficient to warrant acceptance into clinical practice [86].

In this study, we demonstrated that plasma angiogenic/anti-angiogenic factor concentrations have prognostic value at the time of “suspected SGA” diagnosis (24–34 weeks of gestation) for the identification of patients destined to develop PE and/or require indicated preterm delivery at ≤ 34 weeks. The performance of these biomarkers alone was comparable to that of UT artery Doppler velocimetry. Thus, this approach may be useful in areas where accessible to expertise in

Doppler examination is limited. Indeed, plasma concentration of angiogenic/anti-angiogenic factors contributed significant information about the risk of PE or indicated preterm delivery at ≤ 34 weeks beyond that provided by abnormal Doppler velocimetry and/or clinical factors alone. Adjusting for these factors, patients with abnormal angiogenic/anti-angiogenic factor plasma concentrations were 5–9 and 8–9 times more likely than women with normal concentrations to subsequently develop PE or require delivery at ≤ 34 weeks, respectively.

Our findings are consistent with a prior study of 38 women with suspected SGA fetuses that showed an association between low plasma PIGF concentrations and early preterm delivery (<34 weeks of gestation) [113], and with another report that revealed an association between an anti-angiogenic state and subsequent development of PE in pregnancies with suspected sSGA. The authors concluded that the plasma angiogenic/anti-angiogenic factors concentration at diagnosis and Doppler ultrasound, both predict adverse outcome with a similar performance and that adding these biomarkers to the uterine artery Doppler did not improve predictive performance, defined as a significantly larger areas under the ROC curve [115]. In cardiovascular fields, it is recognized that the AUC test [201] can be too conservative [202], and have lower power than other statistical tests in examining incremental improvement in prognostic performance with the addition of a new marker [203]. The significant independent magnitudes of association for the studied biochemical markers did not always translate into significant improvements in AUC. In addition, the median gestational age at diagnosis of sSGA in that study was 34 (IQR 32.9–36.3) weeks as opposed to 27.7 weeks (IQR 26.0–29.6) in the current study and the median time between the diagnosis of sSGA and venipuncture was 14 (range 4–26) days as opposed to within 2 days in the current study.

Two-stage risk assessment for indicated preterm delivery

Among patients with “suspected SGA” who had abnormal Umb artery Doppler velocimetry, incorporation of these biochemical markers with UT artery Doppler PI z-score did not improve prognostic performance in identifying patients who would require indicated delivery ≤ 34 weeks of gestation, since all patients with abnormal Umb and UT arteries Doppler PI z-scores also had abnormal plasma concentrations of PIGF/sEng. Similarly, among patients who had normal Umb artery Doppler velocimetry, the incorporation of angiogenic/anti-angiogenic factors and UT artery Doppler velocimetry failed to improve prognostic performance in identifying patients who would require indicated delivery at or before 34 weeks of gestation, since 90% of these women were identified by abnormal UT artery Doppler velocimetry alone.

Two-stage risk assessment for subsequent development of PE

Among patients with normal Umb artery Doppler velocimetry, a combination of maternal plasma angiogenic/anti-angiogenic factor concentrations and UT artery Doppler

velocimetry improved the identification of patients who subsequently developed PE. This combination of tests correctly identified 76% of the women with “suspected SGA” who subsequently developed PE at a false positive rate of 10%, whereas abnormal UT artery Doppler velocimetry alone identified only 52% of these women. These findings are important since potential interventions to reverse an anti-angiogenic state are currently under extensive investigations and may be available in the near future. The use of these biomarkers may help identify patients at risk to include in the studies investigating the efficacy of these interventions.

Strengths and limitations

The strengths of this study are its prospective design and that examination of plasma angiogenic/anti-angiogenic factor concentrations were measured in combination with Doppler velocimetry in women suspected of having SGA fetuses. Limitations of this study are that: (1) women diagnosed as having severe PE or SGA and absent or reversed end-diastolic velocity of the Umb artery Doppler, were electively induced at 32–34 weeks of gestation, and, therefore, the outcomes associated with intervention and no intervention could not be compared; and (2) early preterm delivery (≤ 34 weeks of gestation) was used as a surrogate for several neonatal morbidities. Larger studies are needed to examine the association between abnormal plasma angiogenic/anti-angiogenic factor concentrations and neonatal complications among offspring of patients with SGA.

Conclusion

Among patients suspected of having SGA fetuses at less than 34 weeks of gestation, the determination of angiogenic and anti-angiogenic factors in maternal plasma can identify a majority of mothers who subsequently developed PE or those who later required early preterm delivery. Determination of these biomarkers can be obtained from a simple blood test, and thus, this approach may be useful in areas where accessible to expertise in Doppler examination is limited. Moreover, incorporation of these biochemical markers significantly improves risk assessment performance for these outcomes, beyond that of clinical factors and uterine and umbilical artery Doppler velocimetry in patients diagnosed with “suspected SGA”.

Declaration of interest

The authors report no conflicts of interest. This research was supported, in part, by the Perinatology Research Branch, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, DHHS.

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