OBSTETRICS

Gestational angiogenic biomarker patterns in high risk preeclampsia groups

Sharon E. Maynard, MD; Sybil L. Crawford, PhD; Susanne Bathgate, MD; Jing Yan, BA; Laura Robidoux, BA; Melissa Moore, PhD; Tiffany A. Moore Simas, MD, MPH, MEd

OBJECTIVE: Several conditions are associated with increased preeclampsia (PE) risk. Whether altered maternal angiogenic factor levels contribute to risk in these conditions is unknown. Our objective was to compare angiogenic biomarker patterns in high-risk pregnancies and low-risk controls.

STUDY DESIGN: We conducted a planned secondary analysis of a 2-center observational study of angiogenic biomarkers in high-risk women. A total of 156 pregnant women with a PE risk factor and 59 low-risk controls were studied. Serial maternal serum samples were collected during 3 gestational windows: 23-27 weeks, 28-31 weeks, and 32-35 weeks. Soluble fms-like tyrosine kinase 1 (sFlt1), soluble endoglin (sEng), and placental growth factor (PlGF) were measured by enzyme-linked immunosorbent assay. Geometric mean angiogenic biomarker levels and angiogenic ratio (sFlt1 + sEng):PlGF were compared with low-risk controls for each risk group, at each gestational window.

RESULTS: Gestational biomarker patterns differed in PE risk groups as compared with low-risk controls. Women with multiple gestations had markedly higher sFlt1 and sEng at all gestational windows. Women with prior PE had higher sFlt1 and angiogenic ratio, and lower PlGF, from 28 weeks onward. Women with chronic hypertension had significantly higher angiogenic ratio for all 3 gestational windows, but differences disappeared when women with PE were excluded. Obese and nulliparous women had significantly lower PlGF, but no differences in the angiogenic ratio.

CONCLUSION: High-risk groups have altered angiogenic biomarker patterns compared with controls, suggesting that altered production or metabolism of these factors may contribute to PE risk, particularly in women with multiple gestations and prior PE.

Keywords: angiogenic factors, preeclampsia, PlGF, sEng, sFlt1

Cite this article as: Maynard SE, Crawford SL, Bathgate S, et al. Gestational angiogenic biomarker patterns in high risk preeclampsia groups. Am J Obstet Gynecol 2013;208:x-ex-x-ex.

Women with chronic hypertension (cHTN), prepregnancy diabetes mellitus, obesity, multiple gestations (MGs), or preeclampsia (PE) in a prior pregnancy have a substantially increased risk of PE compared with women without such risk factors.[1] The mechanisms by which these conditions increase PE risk are unknown. Dysregulated placental production of angiogenic factors, including soluble fms-like tyrosine kinase 1 (sFlt1), placental growth factor (PlGF), and soluble endoglin (sEng), contribute to endothelial dysfunction in PE by antagonizing endothelial-protective VEGF, PlGF, and TGF-beta in the maternal circulation.[2,3] Circulating levels of these angiogenic factors are altered weeks before the onset of PE in low-risk, nulliparous women.[4,5] The angiogenic factor ratio has shown promise as a composite indicator of overall balance between circulating proangiogenic (PlGF) and antiangiogenic (sFlt1 and sEng) activity, and is more strongly predictive of PE in normal-risk women than any single biomarker.[6] However, few studies have reported angiogenic factor levels in high-risk groups.

We hypothesized that gestational angiogenic biomarker profiles differ between low-risk controls and high-risk women. These differences may provide

From the Department of Medicine, Division of Nephrology (Dr Maynard), and the Department of Obstetrics and Gynecology (Dr Bathgate), George Washington University School of Medicine and Health Sciences, Washington, DC; and the Division of Nephrology, Department of Medicine (Dr Maynard), Lehigh Valley Health Network, Allentown, PA, and the Division of Preventive and Behavioral Medicine (Dr Crawford), Department of Medicine, and the Departments of Biochemistry and Molecular Pharmacology (Ms Yan and Dr Moore), and Obstetrics and Gynecology and Pediatrics (Dr Moore Simas), University of Massachusetts Medical School, and the Departments of Obstetrics and Gynecology (Ms Robidoux and Dr Moore Simas) and Pediatrics (Dr Moore Simas), University of Massachuetts Memorial Health Care (Ms Robidoux and Dr Moore Simas), Worcester, MA.

Received Nov. 3, 2012; revised Feb. 28, 2013; accepted March 15, 2013.

Research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under award numbers UL1RR031982 and UL1TR000161 and by the Wilson Genetics Endowment of the George Washington University School of Medicine and Health Sciences.

S.E.M. is named as a coinventor on a patent filed by Beth Israel Deaconess Medical Center for the use of angiogenesis-related proteins for diagnosis and treatment of preeclampsia. All remaining authors report no conflict of interest.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Presented, in part, at the 59th Annual Scientific Meeting of the Society for Gynecologic Investigation, San Diego, CA, March 21-24, 2012.

Reprints will not be available.

 • http://dx.doi.org/10.1016/j.ajog.2013.03.017

insights into the mechanism of risk predisposition in these groups. The goal of this study was to compare gestational patterns of sFlt1, PlGF, and sEng in women with cHTN, diabetes mellitus, obesity and nulliparity, MGs, and prior PE with low-risk women.

Materials and Methods

Study population

This was a planned secondary analysis of a 2-center observational cohort study of angiogenic biomarkers in high-risk women. The purpose of the primary study was to determine the use of angiogenic biomarkers for predication of PE in high-risk women. Women presenting to the University of Massachusetts Memorial Health Care or the George Washington University Medical Faculty Associates for prenatal care between September 2007 and June 2010 were considered for enrollment. Inclusion criteria were: pregnancy at or before 27 weeks and 6 days' gestation, and eligibility into either (1) the low-risk control (LRC) cohort or (2) the high-risk cohort.

Inclusion in the high-risk cohort required the presence of at least one of the following: (1) nulliparous (no prior pregnancies beyond 20 weeks' gestation) with prepregnancy body mass index (BMI) $\geq$30 kg/m^2, (2) pregestational diabetes mellitus requiring oral hypoglycemic or insulin therapy before conception, (3) cHTN diagnosed or confirmed at screening by presence of blood pressure (BP) 140/90 mm Hg or greater on at least 2 occasions at least 4 hours apart before 20 weeks' gestation and/or use of antihypertensive medications, (4) MGs confirmed by ultrasound evaluation and/or (5) previous PE reported by subject and/or medical record review, using diagnostic criteria outlined in following section. For the purposes of this analysis, where our goal was to describe the angiogenic biomarker patterns of specific risk groups, we performed a post hoc exclusion of women with more than 1 risk factor.

Inclusion in the low-risk control cohort required prepregnancy BMI less than 26 and absence of any risk factors described above. Prior pregnancy was not an exclusion criterion for the low-risk cohort.

Exclusion criteria for both cohorts included any 1 of the following: (1) age <20 or >40 years, (2) preexisting proteinuria ($\geq$300 mg/24 hour from timed urine collection or protein:creatinine ratio $\geq$0.3), (3) prior diagnosis of systemic lupus erythematosus or antiphospholipid antibody syndrome, (4) significant concern about compliance or ability to complete study protocol, (5) use of antiretroviral medications, (6) history of organ transplantation, (7) known active illicit drug abuse or methadone maintenance, (5) expected delivery outside participating facilities, (6) inability to understand English, and/or (7) inability to provide informed consent. The institutional review boards of the University of Massachusetts Medical School and George Washington University approved the study, and all subjects provided informed consent.

Baseline demographic data and medical history were collected on enrollment by study personnel through personal interview and medical record review. Data collected included maternal age, race/ethnicity, tobacco and other substance use, medical problems, and obstetric history. Baseline data addressing absence or presence of risk factors included height, weight, number of fetuses by ultrasound, BP, and urine protein testing. Gestational age was calculated based on first trimester ultrasound or clinical dating that concurred with second trimester ultrasound.[7]

Serum sampling and immunoassay

Serum specimens were collected at three prespecified gestational windows: 23-27 completed weeks, 28-31 weeks, and 32-35 weeks' gestation. After phlebotomy, blood samples were immediately centrifuged, aliquoted, and frozen at −80°C until time of assay. Assays were performed less than 5 years after collection and each serum aliquot was thawed only once. Enzyme-linked immunosorbent assays (ELISA) for human sFlt1, PlGF, and sEng were performed in duplicate using commercial kits (R&D Systems, Minneapolis, MN) by an investigator blinded to risk group and pregnancy outcomes. Samples were repeated if there was greater than 10% variability between duplicates. Plates were repeated if the interassay variability was >15% based on an interassay standard. Interassay and intraassay variability were 4.9% and 2.5% for sFlt1, 8.3% and 1.8% for PlGF and 3.7% and 2.6% for sEng, respectively. Samples collected after the diagnosis of PE were not included in analyses.

Diagnosis of PE

PE was defined according to published guidelines[8,9] as follows. In women without cHTN, PE was defined as the new onset of hypertension and proteinuria after 20 weeks' gestation. Hypertension was either systolic BP $\geq$40 mmHg or diastolic BP $\geq$90 mmHg or greater on 2 occasions at least 4 hours apart. Proteinuria was excretion of $\geq$300 mg protein in a 24-hour urine collection, urine protein:creatinine ratio $\geq$0.30, or urine dipstick 1+ or greater on 2 occasions at least 4 hours apart, with no evidence of urinary tract infection. In women with cHTN, the diagnosis of PE required new onset proteinuria after 20 weeks' gestation. Gestational hypertension was new onset hypertension without proteinuria after 20 weeks' gestation. Although the diagnosis of PE required 2 abnormal BP readings, the onset of PE was defined as the time of the first elevated BP or urinary protein measurement leading to the diagnosis.

Statistical methods

Continuous variables were summarized using means and standard deviations, and pairwise comparisons of each high-risk subgroup with low-risk control subjects low-risk subjects were made Q2 using Wilcoxon 2-sample rank sum tests. Categoric variables were summarized using frequencies, and pairwise comparisons of each high-risk subgroup with low-risk controls were made using Fisher exact tests. In longitudinal analyses, we estimated linear mixed models[10] for each biomarker as a function of gestational window, low-risk control/high-risk subgroup, and their interaction. Each biomarker was log-transformed to handle right-skewness,

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TABLE 1
Patient characteristics and pregnancy outcome according to risk group

Characteristic	Low risk controls (LRC) (n = 59)	Hypertension (HTN) (n = 22)	Diabetes mellitus (DM) (n = 12)	Prior preeclampsia (Prior PE) (n = 42)	Obese and nulliparous (Ob&Nul) (n = 49)	Multiple gestatations (MG) (n = 31)
Maternal age, y, Mean (SD)	30.7 (5.5)	33.3 (4.9)[a]	34.1 (2.6)	30.1 (5.3)	30.3 (4.3)	34.6 (3.9)[a]
Gravity (number pregnancies), Mean (SD)	2.4 (1.4)	4.1 (2.9)[a]	2.7 (1.4)	3.2 (1.6)[a]	1.7 (0.9)[a]	2.3 (1.9)
Body mass index, kg/m^2	24.8 (2.2)	30.3 (6.3)[a]	30.4 (6.7)[a]	29.0 (5.6)[a]	38.0 (6.3)[a]	28.2 (6.9)[a]
Race/ethnicity, % (n)						
White	61.0 (36)	36.4 (8)	66.7 (8)	52.4 (22)	65.3 (32)	80.7 (25)
Hispanic	10.2 (6)	4.6 (1)	16.7 (2)	14.3 (6)	8.2 (4)	3.2 (1)
Black	23.7 (14)	54.6 (12)	16.7 (2)	33.3 (14)	24.5 (12)	12.9 (4)
Asian	5.1 (3)	4.6 (1)	0.0 (0)	0.0 (0)	2.0 (1)	3.2 (1)
Current smoker, % (n)	8.5 (5)	9.1 (2)	0.0 (0)	7.1 (3)	6.1 (3)	0.0 (0)
Gestational age at delivery, wks, Mean (SD)	39.3 (1.7)	37.9 (2.5)[a]	37.2 (3.4)[a]	37.6 (3.1)[a]	39.7 (1.2)	35.9 (2.9)[a]
Birthweight, g, Mean (SD)	3316 (529)	2990 (643)[a]	3078 (1058)	2988 (762)[a]	3492 (472)	2313 (487)[a,b]
Preeclampsia, % (n)	1.7 (1)	27.3 (6)[a]	8.3 (1)	11.9 (5)	6.1 (3)	12.9 (4)[a]
Onset <34 wks	0.0 (0)	13.6 (3)[a]	8.3 (1)	9.5 (4)[a]	0.0 (0)	6.5 (2)[a]
Onset 34-36.7 wks	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	6.5 (2)
Onset 37+ wks	1.7 (1)	13.6 (3)	0.0 (0)	2.4 (1)	6.1 (3)	0.0 (0)

DM, diabetes mellitus; *HTN*, hypertension; *LRC*, low-risk controls; *MG*, multiple gestation; *Ob&Nul*, obese and nulliparous; *PE*, preeclampsia.

[a] *P* value for difference from healthy controls < .05, using Fisher exact test or Wilcoxon rank-sum test; [b] Mean weight of all newborns.

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and estimated means were backtransformed as geometric means and 95% confidence intervals for purposes of presentation. At each gestational window, pairwise comparisons of low risk controls with each high-risk subgroup were tested, adjusting for maternal age, current smoking, and race/ethnicity; *P* values were not adjusted for multiple comparisons because each pairwise comparison was of a priori interest. Analyses were performed both including and excluding subjects who subsequently developed PE. At each gestational window, and for each risk group, pairwise comparisons of the angiogenic ratio among subjects who did vs did not develop PE were tested using linear mixed modeling of log-transformed biomarkers, adjusting for maternal age, race/ethnicity, and current smoking. Statistical significance was set at $P < .05$ for all comparisons.

RESULTS

Characteristics of the study subjects

A total of 258 women met inclusion criteria and contributed at least 1 serum specimen in the prespecified gestational windows. Of these, 43 subjects were excluded because they had more than one PE risk factor. Table 1 compares the clinical characteristics of the 156 high-risk subjects and 59 low-risk control subjects included in the analysis. Compared with low-risk control subjects, women in the high-risk groups differed with regard to both baseline characteristics and pregnancy outcomes. Specifically, women with cHTN were older, had more previous pregnancies/less nulliparity, higher prepregnancy BMI, earlier gestational age at delivery, lower birthweight, and were more likely to develop PE. Women with diabetes had a higher body mass index, and earlier gestational age at delivery. Women with prior PE had more previous pregnancies/less nulliparity, higher baseline BMI, earlier gestational age at delivery, and lower birthweight. Among women with prior PE, 35.7% classified their prior PE episode as "severe," 18% of prior PE episodes were complicated by premature delivery (<37 weeks), and 6% by severe prematurity (<34 weeks). Obese and nulliparous women had fewer prior pregnancies/more nulliparity and higher baseline BMI. Women with MGs were older, had higher baseline BMI, earlier gestational age at delivery, lower mean birthweight, and were more likely to develop PE. One woman in the low-risk control group and 19 women in the high-risk groups developed PE.

Angiogenic factors and ratio in high-risk vs low-risk pregnancies

Figure 1, A-D, compares geometric mean biomarker levels for the 5 high-risk

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FIGURE 1
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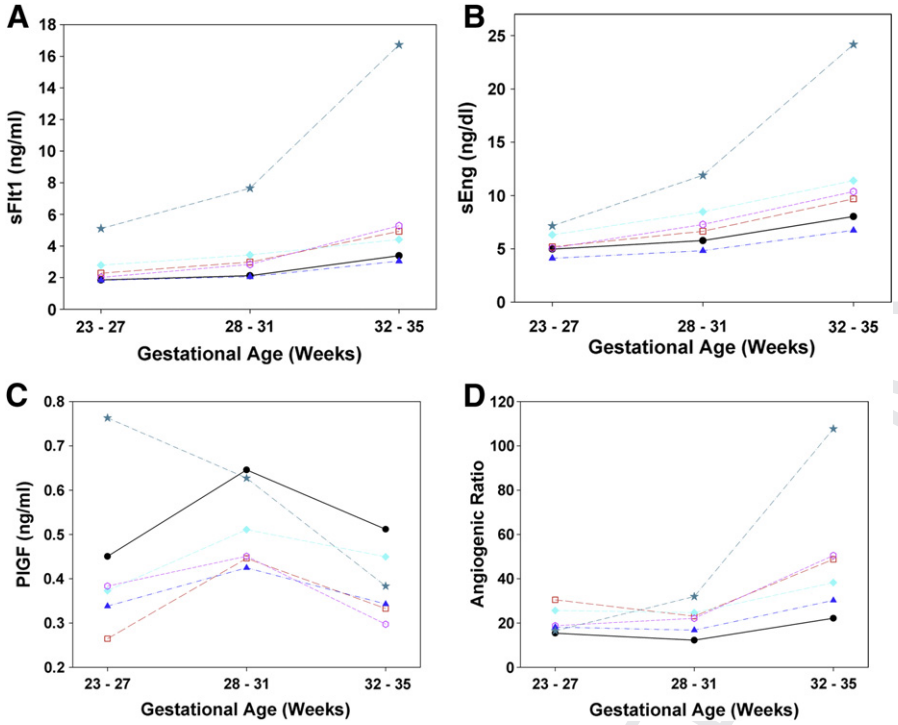

A, Maternal serum levels of sFlt1, **B,** sEng, **C,** PlGF, and **D,** the angiogenic ratio (sFlt1+sEng):PlGF by gestational age, inclusive of women who developed preeclampsia. Unadjusted geometric mean biomarker levels are shown for specimens drawn during 3 gestational age windows according to 5 high-risk groups as compared with low-risk controls. The gestational window given as number of completed weeks (ie, 23-28 weeks indicates specimen drawn between 23 weeks 0 days and 27 weeks 6 days). The key indicates which line corresponds to which group and how many specimens were contributed by how many women in each gestational age window.

Graph key	Cohort	No. of specimens/no. of women 23-27 wks	28-31 wks	32-36 wks
	Low risk controls (n = 59)[a]	55/55	52/50	47/46
	Hypertension (n = 22)	16/16	19/18	17/17
	Diabetes mellitus (n = 12)	12/12	11/11	9/9
	Prior preeclampsia (n = 42)	39/38	28/28	22/22
	Obese and nulliparous (n = 49)	46/45	42/41	41/41
	Multiple gestations (n = 31)	32/31	25/25	23/23

[a] 1 low-risk controls participant omitted in multivariate longitudinal analyses because of missing gravidity.

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groups as compared with low-risk controls for each biomarker (sFlt1, sEng, PlGF) and angiogenic ratio (sFlt1+sEng):PlGF by gestational age window. Figure 2 presents the same comparisons, excluding women who developed PE.

Multiple gestations

Women with MGs had higher sFlt1 (Figure 1, A) and sEng (Figure 1, B) levels in all gestational windows ($P < .0001$) as compared with low-risk controls (LRC) and with the other high-risk groups. PlGF levels (Figure 1, C) in the MG group were significantly higher in the 23-27 week window ($P = .0011$), and decreased through gestation; differences from the LRC group were not significant for subsequent windows. The angiogenic ratio was significantly higher in MG as compared with LRC for the 28-31 week ($P = .0004$) and the 32-36 week ($P < .0001$) windows (Figure 1, D). Exclusion of women who developed PE did not significantly affect these results (Figure 2).

Prior PE

Women with prior PE (PE) had higher sFlt1 ($P < .05$), lower PlGF ($P < .05$), and higher angiogenic ratio ($P < .02$) in the 28-31 and the 32-35 week windows as compared with LRC (Figure 1). sEng tended to be higher in these windows as well, though the difference was of borderline significance in the 32-35 week window ($P = .023$ at 28-31 weeks, $P = .051$ at 32-35 weeks). In the 23-27 week window, there were no significant differences from the LRC group for any biomarker. When women who developed PE were excluded (Figure 2), overall patterns were similar, but differences from the LRC group were no longer statistically significant in the 28-31 week window for sFlt1 ($P = .121$) and PlGF ($P = .108$). The angiogenic ratio remained significantly higher for the latter 2 gestational windows ($P = .038$ at 28-31 weeks, $P = .012$ at 32-35 weeks) after exclusion of women with PE.

Diabetes mellitus

sFlt1 and sEng were higher in women with DM as compared with LRC for the first 2 gestational windows ($P < .05$), but were not significantly different from LRC for the 32-35 week window. PlGF tended to be lower, and the angiogenic ratio tended to be higher, as compared with LRC for all 3 windows; these differences did not reach statistical significance. Exclusion of women who developed PE attenuated the differences between DM and LRC groups with regard to sFlt1 and sEng, and differences were no longer statistically significant, with the exception of sEng in the 28-31 week window ($P = .033$) (Figure 2).

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Chronic hypertension

Women with cHTN tended to have higher sFlt1 and lower PlGF as compared with LRC; differences reached statistical signficance for the 28-32 week window for sFlt1 ($P = .048$), and the 23-28 week ($P = .005$) and 32-36 week ($P = .046$) windows for PlGF. There were no significant differences in sEng levels for any gestational age. The angiogenic ratio was significantly higher in cHTN compared with LRC for all 3 gestational windows ($P = .003$, $P = .024$, and $P = .015$ for the 23-27, 28-31, and 32-35 week windows, respectively). Analyses excluding women who developed PE showed no significant differences between the cHTN and the LRC groups for any biomarker at any gestational window.

Obese and nulliparous

Unlike the other high risk groups, sFlt1 levels in women with obesity and nulliparity were not significantly different from low-risk controls (Figure 1). sEng was significantly lower only in the 23-27 week window ($P = .0033$). In contrast, PlGF was significantly lower than LRC for all 3 gestational windows ($P = .035$, $P = .008$, and $P = .014$). There were no significant differences in the angiogenic ratio at any gestational age. Results were similar after exclusion of women who developed PE (Figure 2).

Angiogenic ratio according to PE outcome

[T2] Table 2 compares the angiogenic ratio for women who did vs did not develop PE within each risk group. The angiogenic ratio was higher in women who developed PE vs those who did not among women with cHTN, DM, and prior PE, and MGs. Because of a small number of subjects with PE in each individual risk group, power was limited and statistical significance was not captured for each window/risk group, however, the ratio was >2-fold higher in women who developed PE for most comparisons. Obesity and nulliparity was the striking exception, with similar angiogenic ratio observed in women who did vs did not develop PE.

COMMENT

In this study, we show that gestational patterns of maternal serum angiogenic factors are altered in women with PE risk factors as compared with low-risk women. In particular, sFlt1 and sEng levels in women with MGs were significantly elevated as compared with singleton low-risk control women, with differences becoming more pronounced as gestation progressed. Women with cHTN, DM, and prior PE also had significantly altered levels of the individual angiogenic biomarkers and the angiogenic ratio, though differences varied by risk group and were smaller in magnitude than those seen for MGs. In contrast, women with obesity and nulliparity had sFlt1 and sEng profiles

FIGURE 2
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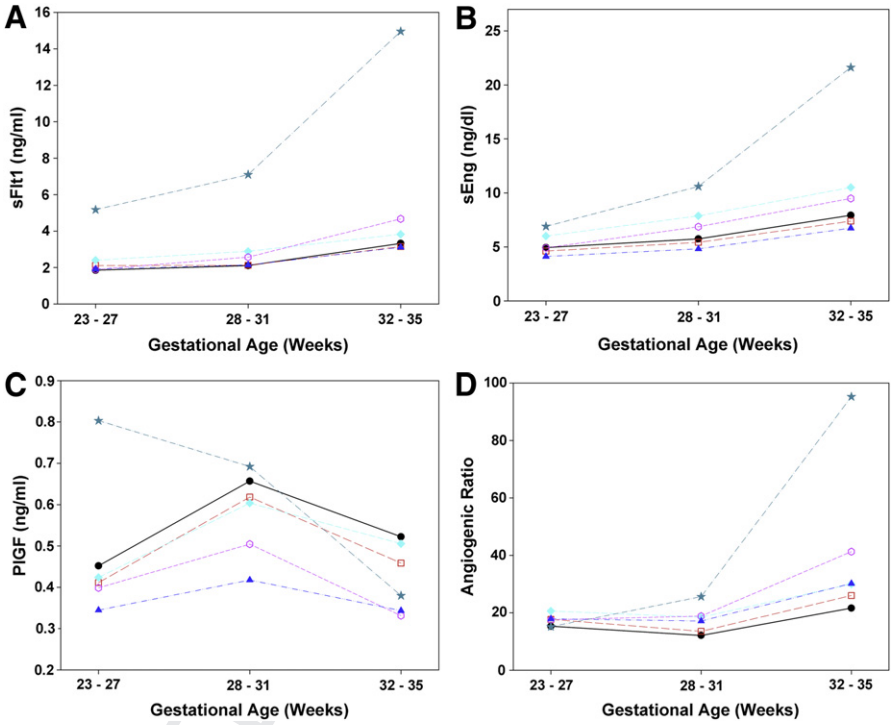

A, Maternal serum levels of sFlt1, **B,** PlGF, **C,** sEng and **D,** the angiogenic ratio of (sFlt1+sEng):PlGF by gestational age, excluding women who developed preeclampsia. Unadjusted geometric mean biomarker levels are shown for specimens drawn during 3 gestational age windows according to 5 high-risk groups as compared with low-risk controls. The key indicates which line corresponds to which group and how many specimens were contributed by how many women in each gestational age window.

Graph key	Cohort	No. of specimens/no. of women 23-27.6 wks	28-31.6 wks	32-35.6 wks
	Low risk controls (n = 58)	54/54	51/49	46/45
	Hypertension (n = 16)	10/10	14/13	14/14
	Diabetes mellitus (n = 11)	11/11	10/10	9/9
	Prior preeclampsia (n = 37)	35/34	25/25	21/21
	Obese and nulliparous (n = 46)	44/43	38/38	38/38
	Multiple gestations (n = 27)	28/27	21/21	19/19

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TABLE 2
Angiogenic ratio (sFlt1 + sEng):PlGF in women who did vs did not develop PE, by risk group

Variable	Geometric mean (95% CI) 23-27 wks' gestation	28-31 wks' gestation	32-35 wks' gestation
cHTN			
No PE (n =16)	16.16 (10.39–25.15)	12.17 (7.35–20.14)	24.83 (14.14–43.61)
PE (n = 6)	115.1 (58.91–224.9)	138.4 (52.48–364.9)	390.3 (126.6–1203)
P value for difference	< .0001	< .0001	< .0001
DM			
No PE (n = 11)	21.29 (13.10–34.60)	19.55 (9.77–39.14)	28.48 (13.35–60.78)
PE (n = 1)	280.9 (57.18–1380)	338.4 (41.53–3632)	[a]
P value for difference	.0029	.0136	[a]
Prior PE:			
No PE (n = 37)	17.73 (13.58–3.16)	18.83 (12.86–27.56)	42.23 (27.65–64.50)
PE (n = 5)	23.65 (11.28–49.58)	82.49 (29.02–234.4)	525.0 (114.0–2418)
P value for difference	.4718	.0098	.0022
Ob&Nul			
No PE (n = 46)	18.62 (14.67–23.65)	17.88 (12.91–24.78)	32.42 (22.82–46.06)
PE (n = 3)	24.75 (9.36–65.44)	12.42 (4.03–38.27)	22.48 (6.82–74.07)
P value for difference	.5769	.5438	.5654
MG			
No PE (n = 27)	15.54 (11.36–21.26)	26.99 (17.21–42.31)	102.0 (62.04–167.8)
PE (n = 4)	33.10 (14.80–74.03)	118.9 (38.62–365.9)	242.3 (72.90–805.2)
P value for difference	.0840	.0169	.1915

cHTN, chronic hypertension; *CI*, confidence interval; *DM*, diabetes mellitus; *MG*, multiple gestation; *Ob&Nul*, obese and nulliparous; *PE*, preeclampsia; *PlGF*, placental growth factor; *sEng*, soluble endoglin; *sFlt1*, soluble fms-like tyrosine kinase 1.

[a] No DM subjects with preeclampsia had a specimen in the 32-35 wk window.

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that were generally similar to controls, although PlGF was lower. These observations suggest that altered angiogenic biomarker expression and/or metabolism may contribute to PE risk, particularly in women with MGs and prior PE.

Multiple gestations

Although the impressive alterations in angiogenic biomarker patterns in multiple gestation pregnancies have been noted previously by our group[11] and others[12] using high-risk singleton comparison groups, to our knowledge only one other study has confirmed this finding using a low-risk singleton comparison group[13]: Sanchez et al reported first trimester sFlt1 levels were 60% higher in women with twin vs singleton pregnancies. In this study we extend these findings to the second and early third trimester, showing that women with MGs have sFlt1 levels that are 2.5- to 4.5-fold higher than low-risk singletons. In addition, we describe a different gestational pattern of PlGF in women with MGs, with loss of the typical midgestation peak; instead, PlGF levels fall consistently from the late second through the third trimester. PlGF levels may peak earlier in pregnancy (ie, before 23 weeks) in women with MGs. Further studies of PlGF earlier in pregnancy in multiple gestation pregnancies are needed to evaluate this. Our data indicate that marked derangements in angiogenic factor levels, likely because of increased sFlt1 and sEng production related to increased placental mass,[14] contribute to increased PE risk in women with MGs.

Prior PE

To our knowledge, no prior studies have compared angiogenic factor levels in women with prior PE and low-risk pregnant women. The mechanism underlying increased PE risk among women with prior PE is unknown. We found that women with prior PE have higher maternal serum levels of sFlt1, and lower levels of PlGF, from 28 weeks onward as compared with low-risk control pregnancies. These differences were somewhat attenuated after exclusion of women who developed PE, but the angiogenic ratio remained significantly higher for these later gestational windows. This suggests that altered production of angiogenic factors, especially later in gestation, contributes to the higher PE risk observed in these women. Unlike women with MGs, where increased placental mass is an obvious contributor to changes in maternal serum angiogenic factors, the mechanisms leading to these patterns in women with prior PE are unknown.

Diabetes and hypertension

CHTN and DM are characterized by underlying maternal endothelial dysfunction. It is tempting to speculate that these conditions predispose to PE by increasing maternal susceptibility to the endothelial stress of pregnancy. In support of this hypothesis, women with PE superimposed on cHTN have lower sFlt1 levels at the time of delivery as compared

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with low-risk women with PE.[15] An alternative hypothesis is that women with DM and cHTN have alterations in sFlt1, sEng, or PlGF production or metabolism, either by the placenta or by extraplacental sources such as peripheral blood mononuclear cells.[16] Previous studies seem to support this possibility: Powers et al reported lower PlGF levels at study entry (9-26 weeks' gestation) in diabetics as compared with women with hypertension or prior PE, but a low-risk control group was not available for comparison.[12] Verlohren et al found the sFlt1:PlGF ratio was higher in women with cHTN as compared with low-risk controls, though differences were statistically significant only after 34 weeks,[17] and individual biomarkers were not reported. Our findings are consistent with those of Verlohren, showing the angiogenic ratio (sFlt1+sEng):PlGF is higher in cHTN as compared with LRC for all gestational windows studied (ranging, 23-35 weeks). sEng levels were not significantly different from low-risk controls. Notably, biomarker differences for both cHTN vs LRC and DM vs LRC were attenuated (and generally lost statistical significance) in analyses that excluded women who developed PE. This may be due to the unusually high rate of PE (27.3%) in the cHTN group, and the small number of subjects in the DM group (n = 11 after excluding PE). Nevertheless, these findings support the second hypothesis: alterations in angiogenic biomarker production or metabolism are likely to contribute to PE risk in women with cHTN and DM. The alterations in angiogenic markers are not as pronounced as those seen in multiple gestation pregnancies, thus altered maternal susceptibility may also contribute to risk.

Obesity and nulliparity

Obesity is an independent risk factor for PE.[18,19] The mechanism underlying increased risk in obese women is unknown, though roles have been hypothesized for both a chronic inflammatory state[20] and subclinical insulin resistance.[21] Although adipocytes appear to express and secrete sFlt1,[22] circulating sFlt1 levels are not associated with obesity measures in nonpregnant adults.[23]

We found that sFlt1 and sEng levels were slightly lower in obese and nulliparous women compared with low-risk controls, though the difference reached statistical significance only for sEng in the earliest (23-28 week) gestational window. The trend toward lower sEng and sFlt1 levels was offset by significantly lower PlGF levels at all gestational windows in obese and nulliparous women, resulting in an angiogenic ratio that was not significantly different from the low-risk, nonobese control group. These results suggest that changes in PlGF production or metabolism may be an important contributor to PE risk on obese and nulliparous women. However, because the angiogenic ratio—a proposed measure of overall angiogenic balance—is unchanged, increased maternal susceptibility may be the primary mechanism of PE risk in these women.

Other studies have consistently reported lower sFlt1, sEng, and/or PlGF levels among obese women in the first,[24] second,[25,26] and third[26] trimesters. In contrast, Suwaki et al reported no difference in sFlt1 levels in overweight (BMI >25) vs normal weight women who did not develop hypertensive disorders of pregnancy, though overweight women with PE had significantly lower sFlt1 levels than did normal weight women with PE[27]; however, this study was limited by a small sample size (n = 14 overweight and 13 normal-weight).

Nulliparity itself also appears to be associated with higher sFlt1 levels.[25,28] Because nulliparity was a criteria for inclusion in the Obesity and Nulliparous subgroup in our study, the competing effects of nulliparity and obesity on sFlt1 levels may explain the absence of a significant difference in sFlt1 levels for this group.

Heterogeneity of high-risk groups

The 5 high-risk groups studied in this study vary with regard to PE risk. For example, women with prior PE have an extremely high risk of PE is subsequent pregnancies (relative risk, 7.19), with the highest risk seen in women with severe, second trimester PE.[29] Nulliparity, overweight (BMI >26), and cHTN, in contrast, each confer a relative risk of 2.4-2.9.[29] In addition, it is likely that the mechanism of PE risk differs among risk groups. These differences highlight the importance of their analysis as separate groups.

Exclusion of women who developed PE

With the notable exception of the cHTN group, exclusion of women who developed PE had a modest impact on our findings, in general diminishing the magnitude of differences observed between high-risk women and low-risk controls. It is unclear whether exclusion of women with PE is appropriate when seeking to describe biomarker differences between risk groups. The rationale for exclusion is that the higher prevalence of PE among high-risk groups introduces bias, because PE is itself associated with alterations in angiogenic factors. According to this line of reasoning, biomarker differences between groups may reflect the effect of PE, rather than the effect of the risk factor being studied. In our opinion, this logic is flawed if one believes that angiogenic factor changes are part of the pathophysiologic pathway leading to PE, rather than a downstream consequence of PE itself.[2,30] In addition, because all specimens were collected before PE onset, it is unlikely that PE itself caused the alterations in angiogenic factors. Instead, we believe that the underlying conditions (ie, cHTN) lead to altered biomarker levels, and subsequent development of PE—in which case there is no rationale for exclusion of PE cases. We present both analytic approaches, and invite the reader to make her own judgment.

Implications for PE prediction

The angiogenic ratio tended to be higher in women with PE vs no PE for all risk groups except Obese and Nulliparous. Though statistical power was limited by the small number of women who developed PE in each group, more than 2-fold differences in the angiogenic ratio

were observed for most comparisons. These results agree with those of Powers et al,[12] who concluded that angiongenic biomarker patterns are altered before PE onset in high-risk pregnancies as they are with low-risk pregnancies. It remains unclear if these differences are large enough to be clinically useful for PE prediction. We found the largest differences for women with DM and HTN, where the angiogenic ratio was >7-fold higher in women who developed PE at all windows studied. In contrast, our findings suggest that angiogenic biomarkers are unlikely to be useful for PE prediction in women with obesity and nulliparity. Normal ranges and predictive cutoffs for angiogenic biomarkers derived from low-risk populations will not be applicable to high-risk groups.

Limitations

Our study is limited by a relatively small sample size, with the number of patients in each individual risk group ranging from 12 to 59 (Table 1). In particular, the number of subjects with DM was very small (n = 12), limiting power to detect differences in PlGF and the angiongeic ratio for this group. Because of a small number of subjects who were current smokers, we were unable to examine biomarker profiles in this "positive" risk group. The decision to combine obesity and nulliparity into a single risk group precludes any conclusions regarding the relative contribution of these 2 different conditions to the observed biomarker patterns. The study population was recruited from 2 academic urban obstetric practices, and generalization to other populations may be limited. Although analyses controlled for some baseline variables, it is possible that unmeasured covariates may have contributed to the observed differences in biomarker patterns. Finally, the observational nature of the study, limited as it was to analysis of maternal serum biomarker levels, precludes any firm conclusions about pathophysiologic pathways on the basis of our findings.

In summary, we demonstrate that altered angiogenic balance, indicated by changes in sFlt1, sEng, PlGF, and/or the angiogenic ratio (sFlt1+sEng:PlGF), is present in pregnant women with MGs, prior PE, diabetes, hypertension, and obesity and nulliparity as compared with low-risk control pregnancies. This suggests that alterations in circulating angiogenic factor production or metabolism may contribute to PE risk in these groups. Whether differences in angiogenic biomarkers are due to increased placental production, extraplacental production, increased metabolism, or effects on protein binding or distribution into different body compartments remains to be determined. ■

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