ORIGINAL ARTICLE

# Circulating Angiogenic Factors and the Risk of Preeclampsia

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## ABSTRACT

### BACKGROUND

The cause of preeclampsia remains unclear. Limited data suggest that excess circulating soluble fms-like tyrosine kinase 1 (sFlt-1), which binds placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), may have a pathogenic role.

### METHODS

We performed a nested case–control study within the Calcium for Preeclampsia Prevention trial, which involved healthy nulliparous women. Each woman with preeclampsia was matched to one normotensive control. A total of 120 pairs of women were randomly chosen. Serum concentrations of angiogenic factors (total sFlt-1, free PlGF, and free VEGF) were measured throughout pregnancy; there were a total of 655 serum specimens. The data were analyzed cross-sectionally within intervals of gestational age and according to the time before the onset of preeclampsia.

### RESULTS

During the last two months of pregnancy in the normotensive controls, the level of sFlt-1 increased and the level of PlGF decreased. These changes occurred earlier and were more pronounced in the women in whom preeclampsia later developed. The sFlt-1 level increased beginning approximately five weeks before the onset of preeclampsia. At the onset of clinical disease, the mean serum level in the women with preeclampsia was 4382 pg per milliliter, as compared with 1643 pg per milliliter in controls with fetuses of similar gestational age ($P<0.001$). The PlGF levels were significantly lower in the women who later had preeclampsia than in the controls beginning at 13 to 16 weeks of gestation (mean, 90 pg per milliliter vs. 142 pg per milliliter, $P=0.01$), with the greatest difference occurring during the weeks before the onset of preeclampsia, coincident with the increase in the sFlt-1 level. Alterations in the levels of sFlt-1 and free PlGF were greater in women with an earlier onset of preeclampsia and in women in whom preeclampsia was associated with a small-for-gestational-age infant.

### CONCLUSIONS

Increased levels of sFlt-1 and reduced levels of PlGF predict the subsequent development of preeclampsia.

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Preeclampsia affects about 5 percent of pregnancies, resulting in substantial maternal and neonatal morbidity and mortality.[1,2] Although the cause remains unclear, the syndrome may be initiated by placental factors that enter the maternal circulation and cause endothelial dysfunction resulting in hypertension and proteinuria.[3-6]

We and others have recently shown that soluble fms-like tyrosine kinase 1 (sFlt-1), a circulating antiangiogenic protein, is increased in the placenta[5,7] and serum[5,8,9] of women with preeclampsia. This protein acts by adhering to the receptor-binding domains of placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), preventing their interaction with endothelial receptors on the cell surface and thereby inducing endothelial dysfunction. Decreased concentrations of circulating free PlGF and free VEGF have been noted during clinical preeclampsia[5] and even before its onset.[10,11] We have recently demonstrated that exogenous sFlt-1 administered to pregnant rats induces hypertension, proteinuria, and glomerular endotheliosis.[5] Moreover, a 50 percent reduction in VEGF production in renal podocytes in mice with podocyte-specific heterozygosity for VEGF resulted in glomerular endotheliosis and massive proteinuria.[12] Hypertension and proteinuria have been reported in patients with cancer who were treated with VEGF-signaling inhibitors.[13,14] Taken together, these observations suggest that excess sFlt-1 may have a pathogenic role in preeclampsia.[5]

In order to describe the gestational patterns of circulating sFlt-1, free PlGF, and free VEGF in normotensive and preeclamptic pregnancy, we performed a nested case–control study within the Calcium for Preeclampsia Prevention (CPEP) trial, using archived serum samples obtained before labor. Concentrations were analyzed in a cross-sectional fashion within intervals of gestational age and according to the time before the onset of preeclampsia. We hypothesized that the sFlt-1 level would be elevated before the onset of clinical disease, with reciprocal decreases in the levels of free PlGF and VEGF.

## METHODS

### PARTICIPANTS AND SPECIMENS

The CPEP trial was a randomized, double-blind clinical trial conducted from 1992 to 1995 to evaluate the effects of daily supplementation with calcium or placebo on the incidence and severity of preeclampsia.[15,16] Healthy nulliparous women with singleton pregnancies were enrolled between 13 and 21 weeks of gestation at five participating U.S. medical centers and were followed until 24 hours after delivery, with the use of a common protocol and identical data-collection forms. Written informed consent was obtained from all the participants. Gestational age was determined by means of ultrasonographic examination. Serum specimens were requested from women before enrollment in the trial, at 26 to 29 weeks of gestation, at 36 weeks if they were still pregnant, and when hypertension or proteinuria was noted. "End-point specimens" were obtained at or after the onset of signs of preeclampsia but before labor and delivery.[15,16]

For the present study, we selected women with complete outcome information, serum samples obtained at less than 22 weeks of gestation, and a liveborn male infant. This group had previously been selected for a study of fetal DNA and preeclampsia, in which fetal and maternal DNA were differentiated through the amplification of a gene on the Y chromosome. Analysis of previous work[5] revealed no significant differences in the sFlt-1 or PlGF concentrations between 10 women with preeclampsia who delivered a male infant and 8 women with preeclampsia who delivered a female infant (sFlt-1 concentration, 6120 pg per milliliter vs. 5404 pg per milliliter; P=0.78; PlGF concentration, 99 pg per milliliter vs. 125 pg per milliliter; P=0.42).

Of the 4589 women enrolled in the CPEP trial, we excluded 253 who were lost to follow-up, 21 whose pregnancy ended before 20 weeks, 13 who had missing data on maternal or perinatal outcome, 4 who had no data on smoking history, 9 in whom the presence of hypertension had not been verified by the team that reviewed each chart, and 32 who had a stillbirth, leaving 4257 women. Of these women, 2156 had a male infant. After the exclusion of 1 woman whose infant had a chromosomal abnormality, 381 women with gestational hypertension, and 43 without a base-line serum specimen, 1731 women remained. Preeclampsia developed in 175 of these women, whereas 1556 remained normotensive throughout pregnancy.

Calcium supplementation did not affect the serum concentrations of angiogenic factors. Specimens collected at 8 to 20 weeks of gestation were considered the base-line specimens and were obtained before the administration of calcium or placebo. At 21 to 32 weeks, the mean serum sFlt-1 level in the controls who were given placebo was 866 pg

per milliliter, as compared with 889 pg per milliliter in the controls who were given calcium supplements (P=0.57); the mean PlGF level was 765 pg per milliliter and 746 pg per milliliter, respectively (P=0.78); and the mean VEGF level was 13.0 pg per milliliter and 12.5 pg per milliliter, respectively (P=0.80). At 33 to 41 weeks, the concentrations of these factors also did not differ significantly between these two groups.

Since calcium supplementation had no effect on the risk or severity of preeclampsia[16] or on the concentrations of angiogenic factors, the women with preeclampsia and the controls were chosen without regard to whether they had received calcium supplementation or placebo. For each woman with preeclampsia, one normotensive control was selected, matched according to enrollment site, gestational age of the fetus at the collection of the first serum specimen (within 1 week), and storage time of the samples at −70°C (within 12 months). A total of 120 of 159 matched pairs were randomly chosen for the analysis of all 655 serum specimens that were obtained before labor. Of the 240 women included in the study, 21 (8.8 percent) contributed one serum specimen, 52 (21.7 percent) contributed two specimens, 139 (57.9 percent) contributed three, 27 (11.2 percent) contributed four, and 1 (0.4 percent) contributed five. The mean gestational age of the fetus at the collection of the first serum specimen was 112.8 days among the women with preeclampsia and 113.6 days among the controls; the mean duration of freezer storage was 9.35 years and 9.39 years, respectively. Since most women had three or fewer serum specimens, we analyzed the data largely in a cross-sectional manner. Among the few women with more than one specimen per period, the earliest specimen was selected for analysis.

Preeclampsia was defined as a diastolic blood pressure of at least 90 mm Hg and proteinuria of at least 1+ (30 mg per deciliter) on dipstick testing, each on two occasions 4 to 168 hours apart. Severe preeclampsia was defined as the HELLP syndrome (hemolysis, elevated liver-enzyme levels, and a low platelet count), eclampsia, or preeclampsia with either severe hypertension (diastolic blood pressure ≥110 mm Hg) or severe proteinuria (urinary protein excretion ≥3.5 g per 24 hours or findings of ≥3+ [300 mg per deciliter] on dipstick testing). Detailed definitions have been published previously.[15,16] The time of onset of preeclampsia (the end point) was defined as the time of the first elevated blood-pressure or urinary protein measurement leading to the diagnosis of preeclampsia. A small-for-gestational-age infant was defined as an infant whose birth weight was below the 10th percentile according to U.S. tables of birth weight for gestational age that accounted for race, parity, and the sex of the infant.[17] Because the study used data and specimens that were collected as part of the CPEP trial and could not be linked to identifiable women, the Office of Human Subjects Research of the National Institutes of Health granted it an exemption from the requirement for review and approval by the institutional review board.

### PROCEDURES

Assays were performed by personnel who were unaware of the outcome of the pregnancy. Specimens were randomly ordered for analysis. Enzyme-linked immunosorbent assays (ELISAs) for human sFlt-1 (also called soluble VEGF receptor 1), free PlGF, and free VEGF were performed in duplicate, as previously described, with the use of commercial kits (R&D Systems).[5] The minimal detectable doses in the assays for sFlt-1, PlGF, and VEGF were 5, 7, and 5 pg per milliliter, respectively, with interassay and intra-assay coefficients of variation of 7.6 and 3.3 percent, respectively, for sFlt-1; 10.9 and 5.6 percent, respectively, for PlGF; and 7.3 and 5.4 percent, respectively, for VEGF.

### STATISTICAL ANALYSIS

Chi-square tests were used for the comparison of categorical variables, and t-tests were used for the comparison of continuous variables. Although the arithmetic mean concentrations are reported in the text and figures, statistical testing was conducted after logarithmic transformation. All P values are two-tailed. The Wilcoxon rank-sum test was also applied to the comparisons within the gestational-age intervals and provided P values indicating similar significance. Odds ratios were adjusted with the use of logistic-regression analysis.

## RESULTS

### CHARACTERISTICS OF THE WOMEN

Of the 120 women with preeclampsia, 80 had mild preeclampsia and 40 had severe preeclampsia, including 3 with the HELLP syndrome and 3 with eclampsia. As compared with the controls, the women with preeclampsia had greater body-mass index (the weight in kilograms divided by the square of the height in meters) and higher blood pressure at

the time of enrollment in the CPEP trial; a larger proportion of their current pregnancies were complicated by preterm delivery or resulted in small-for-gestational-age infants (Table 1).

### DIFFERENCES IN sFlt-1, PlGF, AND VEGF LEVELS

We first confirmed that the serum levels of sFlt-1, free PlGF, and free VEGF were altered in women with clinical preeclampsia.[5,8] Among 23 pairs of women with preeclampsia and controls with fetuses of similar gestational age, specimens drawn after the onset of preeclampsia ("end-point specimens") had higher levels of sFlt-1 and lower levels of PlGF and VEGF than specimens from controls (mean sFlt-1 level, 4382 pg per milliliter vs. 1643 pg per milliliter; P<0.001; mean PlGF level, 137 pg per milliliter vs. 669 pg per milliliter; P<0.001; and mean VEGF level, 6.4 pg per milliliter vs. 13.9 pg per milliliter; P=0.07).

### GESTATIONAL CHANGES IN THE sFlt-1 LEVEL

To evaluate gestational patterns, we performed a cross-sectional analysis of serum obtained within gestational-age intervals of four to five weeks (Fig. 1A). The sFlt-1 concentrations in controls remained constant until 33 to 36 weeks of gestation, when they increased by approximately 145 pg per milliliter per week until labor and delivery. In the women in whom preeclampsia later developed, but before the onset of preeclampsia, the concentrations began to increase at 21 to 24 weeks of gestation, with a steeper increase at 29 to 32 weeks. When we compared the sFlt-1 concentrations at different stages of pregnancy in specimens from women in whom preeclampsia developed, we found that among women with specimens drawn when their fetuses were in the same gestational-age interval, those who already had clinical preeclampsia had significantly higher concentrations than those who did not.

The concentrations of sFlt-1 were increased in women who later had preeclampsia beginning 11 to 9 weeks before the onset of preeclampsia (Fig. 1B). Samples drawn within five weeks before the onset of preeclampsia revealed a more rapid increase in the sFlt-1 levels. By one week before the onset of clinical signs, the concentrations approached those observed in end-point specimens. The increases in the sFlt-1 concentration at four weeks, three weeks, two weeks, and one week before the onset of preeclampsia occurred with little change in the mean gestational age of the fetus, and the increase observed between eight weeks and five weeks before the onset of preeclampsia appeared to be about one and a half times the increase that would be expected on the basis of advancing gestational age alone. Five weeks before the onset of preeclampsia, the average concentration in women in whom preeclampsia developed was similar to the concentrations in controls at term, but after this point the sFlt-1 concentration in the women with preeclampsia was higher. More than five weeks before the onset of preeclampsia, no substantial differences were observed between controls and women in whom preeclampsia later developed (Fig. 1A). An increase in the sFlt-1 concentration also occurred in normal pregnancies, but it occurred later during

**Table 1. Characteristics of Women Who Later Had Preeclampsia and Controls at Enrollment in the CPEP Trial and Characteristics of Their Infants.***

| Characteristic | Women with Preeclampsia (N=120) | Controls (N=120) | P Value |
|---|---|---|---|
| Age — yr | 20.8±4.5 | 20.2±3.6 | |
| Height — cm | 161.0±6.7 | 163.0±6.9 | 0.03 |
| Weight — kg | 71.0±19.4 | 66.8±17.1 | |
| Body-mass index | 27.3±6.8 | 25.1±6.1 | 0.008 |
| Systolic blood pressure — mm Hg | 109±9 | 106±9 | 0.001 |
| Diastolic blood pressure — mm Hg | 62±8 | 59±7 | 0.007 |
| Primigravida — no. (%) | 97 (80.8) | 95 (79.2) | |
| Gestational age at enrollment — wk | 17.7±2.6 | 17.7±2.7 | |
| Gestational age at delivery — wk | 38.5±2.6 | 39.3±2.5 | 0.03 |
| Current smoker — no. (%) | 9 (7.5) | 13 (10.8) | |
| Calcium treatment — no. (%) | 61 (50.8) | 55 (45.8) | |
| Private health insurance — no. (%) | 8 (6.7) | 13 (10.8) | |
| Ever married — no. (%) | 25 (20.8) | 24 (20.0) | |
| Race or ethnic group — no. (%)† | | | |
| Non-Hispanic white | 24 (20.0) | 35 (29.2) | |
| Hispanic white | 21 (17.5) | 14 (11.7) | |
| Black | 69 (57.5) | 68 (56.7) | |
| Other or unknown | 6 (5.0) | 3 (2.5) | |
| Infant's birth weight — g | 3100±796 | 3255±595 | |
| Delivery at <37 wk — no. (%) | 26 (21.7) | 9 (7.5) | 0.002 |
| Small-for-gestational-age infant (<10th percentile) — no. (%) | 18 (15.0) | 4 (3.3) | 0.002 |

* Plus–minus values are means ±SD. P values are given only for significant differences. CPEP denotes Calcium for Preeclampsia Prevention.
† Race or ethnic group was self-reported.

gestation and was less pronounced. Figure 1C depicts longitudinally the increase in the sFlt-1 concentration for individual women; the increase occurred during late gestation in the controls but earlier and to a greater extent in the women with preeclampsia.

### GESTATIONAL CHANGES IN PlGF AND VEGF LEVELS

The gestational pattern in the PlGF level is shown in Figure 2A. The PlGF concentrations in the controls increased during the first two trimesters, peaked at 29 to 32 weeks, and decreased thereafter. The PlGF concentrations in the women with preeclampsia followed a similar pattern but were significantly lower than those in the controls from 13 to 16 weeks onward; at 13 to 16 weeks, the mean level was 90 pg per milliliter in the women who later had preeclampsia and 142 pg per milliliter in the controls (P=0.01). Figure 2B shows the PlGF concentrations in the women in whom preeclampsia developed according to the number of weeks before the onset of preeclampsia. The PlGF concentrations began to decrease 11 to 9 weeks before the onset of preeclampsia, with substantial reductions during the 5 weeks before the onset of hypertension or proteinuria. At about five weeks before the onset of preeclampsia, the average PlGF concentration in women who later had preeclampsia was similar to the average PlGF concentration in controls at term, but after this point, the PlGF concentration in the women with preeclampsia was lower. By one week before the onset of preeclampsia, the concentrations approached those found in women with established preeclampsia (Fig. 2B). When specimens obtained within 5 weeks before the onset of preeclampsia were excluded, the differences between the controls and the women who later had preeclampsia were less pronounced at 25 to 28 weeks and 29 to 32 weeks of gestation; no differences were observed at 33 to 36 weeks (Fig. 2A).

The VEGF concentrations were low throughout pregnancy and did not differ between controls and women who later had preeclampsia, with two exceptions. In specimens obtained at 37 to 41 weeks of gestation, the VEGF levels were lower in the women with preeclampsia (6.7 pg per milliliter, vs. 9.9 pg per milliliter in the controls; P=0.02). In specimens obtained at 21 to 32 weeks, the VEGF levels were lower only if the specimen was obtained within 5 weeks before the onset of preeclampsia (5.1 pg per milliliter, vs. 12.8 pg per milliliter in the controls; P=0.002).

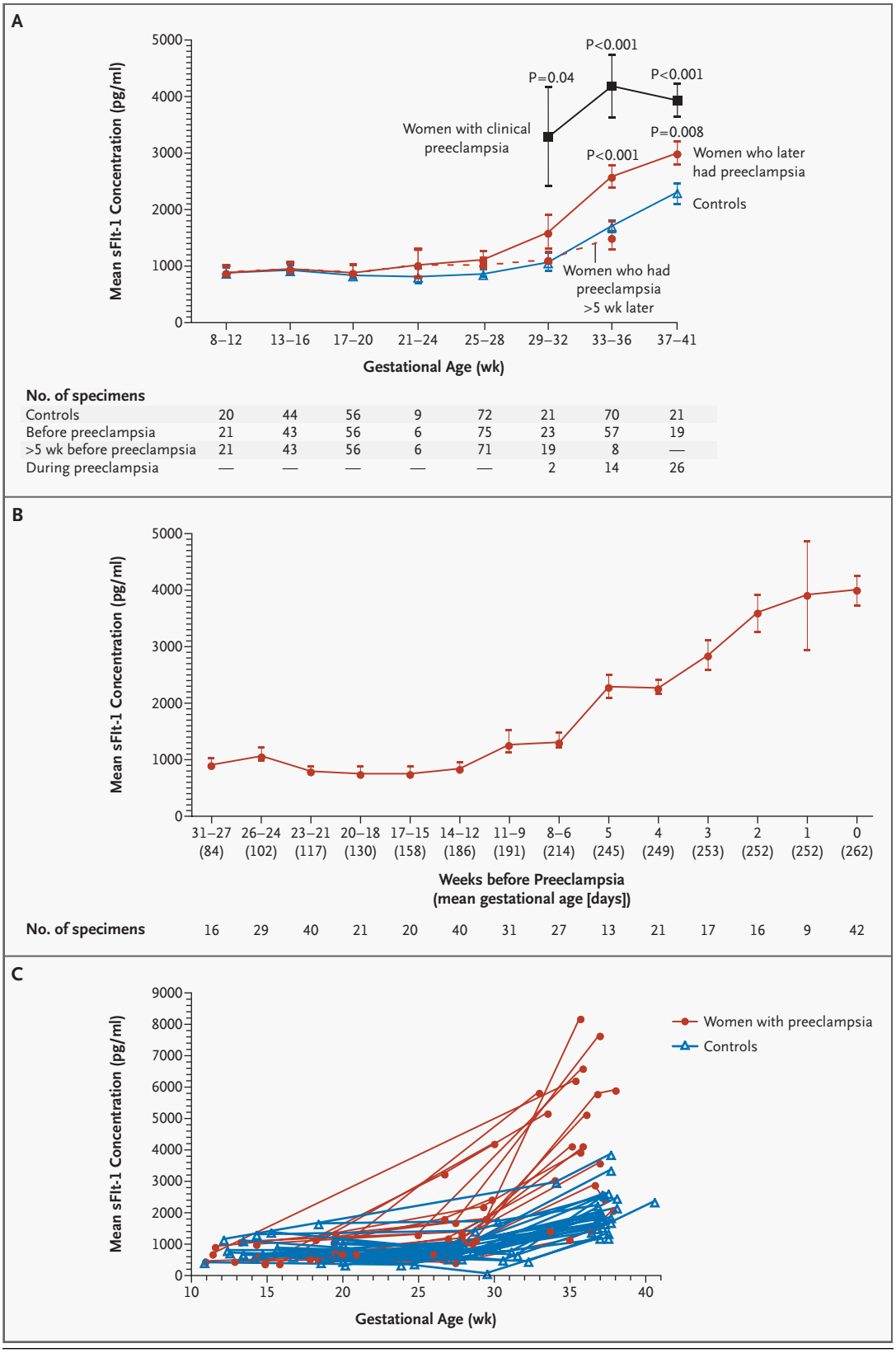
### RELATIONSHIP TO BODY-MASS INDEX AND SEVERITY OF PREECLAMPSIA

Since obesity is an important risk factor for preeclampsia, we examined whether alterations in the body-mass index could account for the elevated sFlt-1 levels and diminished free PlGF levels observed in preeclamptic pregnancy. We performed linear regression analyses of log-transformed sFlt-1 levels and log-transformed free PlGF levels in relation to the body-mass index in control women. Body-mass index was not related to the free PlGF levels at 8 to 20 weeks, 21 to 32 weeks, or 33 to 41 weeks, nor to the sFlt-1 levels at 8 to 20 weeks or 33 to 41 weeks (data not shown); it was inversely related to the sFlt-1 levels at 21 to 32 weeks (estimated beta coefficient for the association with the log-transformed sFlt-1 level, −0.02; P=0.02). Higher sFlt-1 levels and lower PlGF levels in preeclamptic pregnancy, therefore, cannot be explained by greater body-mass index in women in whom preeclampsia develops.

Before the onset of preeclampsia, there were par-

**Figure 1 (facing page). Concentrations of Soluble fms-like Tyrosine Kinase 1 (sFlt-1).**

Panel A shows the mean sFlt-1 concentrations before and after the onset of clinical preeclampsia according to the gestational age of the fetus. I bars represent standard errors. The P values given are for the comparisons, after logarithmic transformation, with specimens from controls obtained during the same gestational-age interval; the differences, after logarithmic transformation, between the specimens obtained at 33 to 41 weeks from women who already had clinical preeclampsia and those obtained at 33 to 41 weeks from women in whom preeclampsia later developed were also significant (P=0.004 for the comparison at 33 to 36 weeks and P=0.02 for the comparison at 37 to 41 weeks). Panel B shows the mean sFlt-1 concentration according to the number of weeks before the onset of preeclampsia. I bars represent standard errors. Panel C shows longitudinal plots of the mean sFlt-1 concentrations within individual women according to the gestational age of the fetus. A total of 24 controls and 19 women with preeclampsia (in whom end-point specimens were obtained at ≤38 weeks) are included. The controls included were those who had a specimen collected at the latest points in pregnancy; among women in whom specimens were obtained on the same day of gestation, the controls were selected randomly. Because all but one control had specimens obtained at 38 weeks of gestation or earlier, only women with preeclampsia who had end-point specimens obtained at 38 weeks or less were included. Of the two controls with sFlt-1 levels above 3000 pg per milliliter, one had 2+ (100 mg per deciliter) proteinuria on two occasions without hypertension, and the other had one measurement of diastolic hypertension without proteinuria.



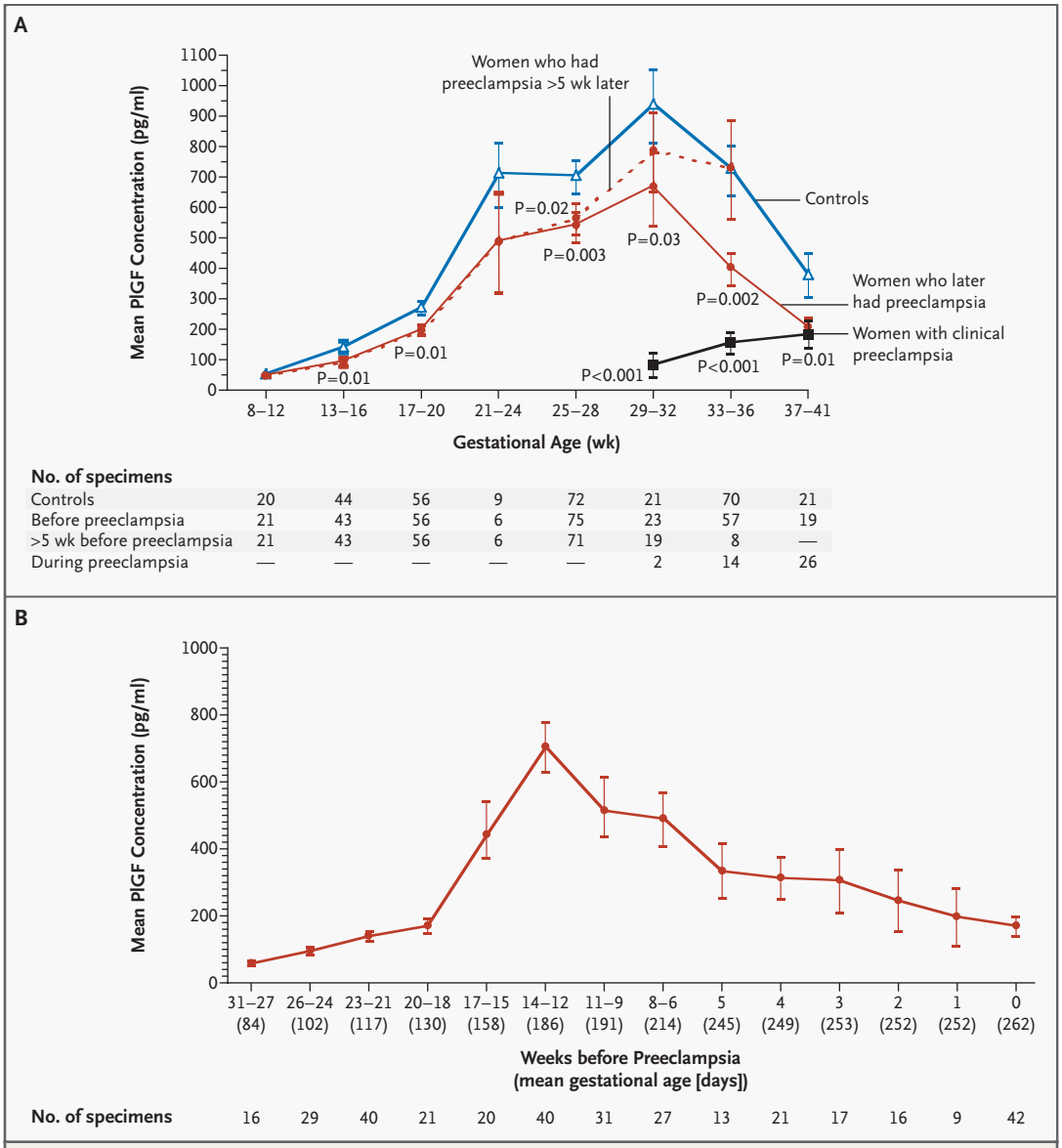



**Figure 2. Concentrations of Placental Growth Factor (PlGF).**

Panel A shows the mean free PlGF concentrations before and after the onset of preeclampsia according to the gestational age of the fetus. I bars represent standard errors. The P values given are for the comparisons, after logarithmic transformation, with specimens from controls obtained during the same gestational-age interval; the differences, after logarithmic transformation, between the specimens obtained at 29 to 36 weeks from women who already had clinical preeclampsia and those obtained at 29 to 36 weeks from women in whom preeclampsia later developed were also significant (P=0.05 for the comparison at 29 to 32 weeks and P=0.003 for the comparison at 33 to 36 weeks). Panel B shows the mean PlGF concentration according to the number of weeks before the onset of preeclampsia. I bars represent standard errors.

ticularly large differences between the concentrations of sFlt-1 and PlGF in controls and those in women who later had early-onset preeclampsia or who had preeclampsia and a small-for-gestational-age infant. Figure 3 shows the concentrations between 21 and 32 weeks of gestation (Fig. 3A) and 33 to 41 weeks of gestation (Fig. 3B). Alterations in the sFlt-1 and PlGF levels were also more pronounced before the onset of preeclampsia in women who had preeclampsia before term (<37 weeks of gestation) than in women who had an onset of preeclampsia at term (≥37 weeks) (at 21 to 32

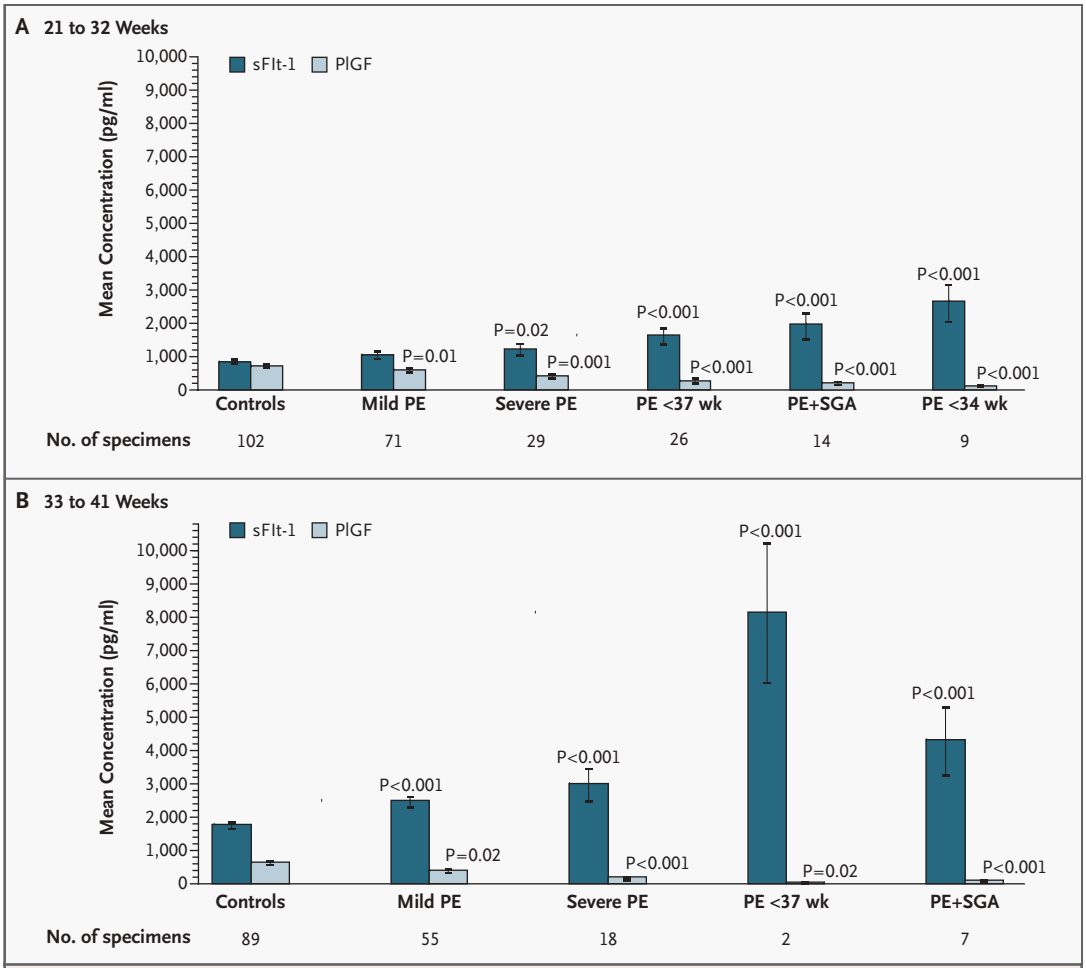



**Figure 3. Mean Concentrations of Soluble fms-like Tyrosine Kinase 1 (sFlt-1) and Placental Growth Factor (PlGF) According to Preeclampsia Status and Severity.**

Panel A shows the concentrations at 21 to 32 weeks of gestation, and Panel B the concentrations at 33 to 41 weeks of gestation, in controls and in women who later had clinical preeclampsia (PE) according to whether they had mild preeclampsia, severe preeclampsia, preeclampsia with an onset at less than 37 weeks of gestation, preeclampsia and a small-for-gestational-age infant (SGA), or preeclampsia with an onset at less than 34 weeks of gestation. Specimens from women in whom preeclampsia developed were obtained before the onset of clinical disease. The P values given are for the comparisons with the specimens from the controls. I bars represent SEs.

weeks: sFlt-1 level, 1672 pg per milliliter in women with preeclampsia before term vs. 935 pg per milliliter in women with preeclampsia at term; P<0.001; PlGF level, 297 vs. 676 pg per milliliter; P<0.001; at 33 to 41 weeks: sFlt-1 level, 8150 vs. 2467 pg per milliliter; P<0.001; PlGF level, 73 vs. 370 pg per milliliter; P=0.05). Furthermore, the sFlt-1 and PlGF levels in specimens obtained before the onset of preeclampsia from women who later had preeclampsia and a small-for-gestational-age infant were significantly different from the concentrations in the women who later had preeclampsia but whose infants were not small for gestational age (at 21 to 32 weeks: sFlt-1 level, 1993 vs. 985 pg per milliliter; P<0.001; PlGF level, 229 vs. 634 pg per milliliter; P<0.001; at 33 to 41 weeks: sFlt-1 level, 4329 vs. 2442 pg per milliliter; P=0.003; PlGF level, 120 vs. 388 pg per milliliter; P=0.009).

### ODDS RATIOS FOR PREECLAMPSIA ASSOCIATED WITH ANGIOGENIC FACTORS

To determine whether the concentrations of sFlt-1 or PlGF in specimens obtained before the onset of clinical signs of preeclampsia were associated with the risk of this condition, we calculated odds ratios for preeclampsia in each quartile of values for sFlt-1

and PlGF in the controls, as compared with the lowest or highest quartile, respectively (Table 2). We also examined the risk of preeclampsia in the extreme quartiles with respect to all the other quartiles, as follows. For specimens obtained during the second trimester or the early part of the third trimester, the lowest quartile of PlGF was associated with an increased risk of preterm preeclampsia (at <37 weeks' gestation; odds ratio for specimens from weeks 13 to 20, 7.4; 95 percent confidence interval, 1.8 to 30.2; odds ratio for specimens from weeks 21 to 32, 7.9; 95 percent confidence interval, 2.9 to 21.5). A PlGF level in the lowest quartile, however, was not a significant predictor of preeclampsia with an onset at term (≥37 weeks). Associations between the sFlt-1 level and preeclampsia were observed only closer to the onset of disease. An sFlt-1 level in the highest quartile from 21 to 32 weeks of gestation (but not earlier) predicted preterm preeclampsia (odds ratio, 5.1; 95 percent confidence interval, 2.0 to 13.0), and a level in the highest quartile between 33 and 41 weeks (but not earlier) predicted preeclampsia at term (odds ratio, 6.0; 95 percent confidence interval, 2.9 to 12.5). These findings are consistent with the results presented in Figure 1B, which shows that the elevation in the sFlt-1 level occurs largely within the five weeks before the onset of clinical disease. The lowest quartile of VEGF was not predictive of preeclampsia.

## DISCUSSION

The experimental production of a preeclampsia-like phenotype in rats with the induction of high circulating levels of sFlt-1 raised the possibility that this antiangiogenic factor might have a pathogenic role in preeclampsia.[5] Our findings suggest that concentrations of sFlt-1, which were previously reported to be increased in women with established preeclampsia,[5,8] begin to increase steeply about five weeks before the onset of clinical disease. Parallel with the increase in the sFlt-1 level, there are decreases in the free PlGF and free VEGF levels, suggesting that the decrease in these factors may be attributable in part to binding by sFlt-1. Women with preterm preeclampsia or preeclampsia and a small-for-gestational-age infant had higher sFlt-1 levels and lower PlGF levels at 21 to 32 weeks and at 33 to 41 weeks than those with an onset of preeclampsia at term or preeclampsia without a small-for-gestational-age infant, respectively.

We have also demonstrated that there is a moderate but significant decrease in the PlGF level beginning early in the second trimester among women in whom preeclampsia later develops, which is consistent with previous observations.[11,18-21] Finally, we have shown that women with low concentrations of PlGF during early gestation have a much greater risk of early-onset preeclampsia.

In normotensive pregnancy, the sFlt-1 levels are stable during the early and middle stages of gestation, and there is a steady increase beginning at 33 to 36 weeks. This increase corresponds to the late-gestational decrease in the free PlGF level in normal pregnancy that is reported here and has been observed by others.[11,22] During the second trimester, the PlGF concentrations are high and the sFlt-1 concentrations low, creating a proangiogenic state. We speculate that in later gestation, placental vascular growth may be tempered by the increase in the levels of antiangiogenic sFlt-1 and the decrease in the levels of proangiogenic VEGF and PlGF. In women with preeclampsia, sFlt-1 appears to increase earlier in gestation and to reach a higher concentration than it does in controls. Thus, in preeclampsia, the antiangiogenic "brakes" may be applied too soon and too hard — an exaggeration of a normal process governing placental growth and function. A study involving renal biopsies that demonstrated mild glomerular endotheliosis in normal term pregnancy and severe lesions in preeclampsia[23] is consistent with the hypothesis that preeclampsia is an exacerbation of the antiangiogenic state of normotensive pregnancy at term.

Since they are not accompanied by reciprocal increases in systemic sFlt-1 levels, diminished PlGF levels early in the pregnancies of women in whom preeclampsia later develops might reflect either less placental production or increased binding to local circulating and membrane-bound receptors. Diminished levels of placental PlGF could play a role in the aberrant cytotrophoblast invasion ("pseudovasculogenesis") that is characteristic of preeclampsia.[24,25] Alterations of the sFlt-1 and PlGF levels appeared to be greater in women who had early-onset preeclampsia and in women with preeclampsia who delivered a small-for-gestational-age infant, suggesting that defective angiogenesis may be especially important in these cases. The VEGF levels were decreased during and within five weeks before established preeclampsia, but low levels were not a significant predictor of future preeclampsia.

Our study has limitations. The data are primarily cross-sectional and do not permit the distinction

**Table 2. Odds Ratios for Preeclampsia at Less Than 37 Weeks of Gestation and at 37 Weeks or More of Gestation According to Quartile of Total sFlt-1 and Quartile of Free PlGF.***

| Quartile | No. of Controls | Onset of Preeclampsia <37 Wk | | Onset of Preeclampsia ≥37 Wk | |
|---|---|---|---|---|---|
| | | No. of Women | Odds Ratio (95% CI) | No. of Women | Odds Ratio (95% CI) |
| **sFlt-1 concentration** | | | | | |
| 13–20 Wk of gestation | | | | | |
| Q4: >1047 pg/ml | 25 | 6 | 1.3 (0.4–5.0) | 20 | 1.5 (0.6–3.7) |
| Q3: 699–1047 pg/ml | 25 | 8 | 2.2 (0.6–7.8) | 23 | 1.9 (0.8–4.5) |
| Q2: 532–698 pg/ml | 25 | 4 | 0.5 (0.1–2.3) | 16 | 1.1 (0.4–2.7) |
| Q1: ≤531 pg/ml | 25 | 6 | 1.0 | 16 | 1.0 |
| 21–32 Wk of gestation | | | | | |
| Q4: >1131 pg/ml | 25 | 16 | 4.7 (1.3–16.6) | 18 | 1.7 (0.7–4.4) |
| Q3: 744–1131 pg/ml | 26 | 5 | 1.4 (0.3–6.0) | 21 | 1.7 (0.7–4.2) |
| Q2: 513–743 pg/ml | 25 | 1 | 0.3 (0.0–2.8) | 21 | 1.9 (0.8–4.7) |
| Q1: ≤512 pg/ml | 26 | 4 | 1.0 | 14 | 1.0 |
| 33–41 Wk of gestation | | | | | |
| Q4: >2191 pg/ml | 22 | | | 44 | 7.5 (2.6–21.8) |
| Q3: 1634–2191 pg/ml | 22 | | | 12 | 1.7 (0.5–5.5) |
| Q2: 1288–1633 pg/ml | 22 | | | 7 | 1.0 (0.3–3.3) |
| Q1: ≤1287 pg/ml | 23 | | | 8 | 1.0 |
| **PlGF concentration** | | | | | |
| 13–20 Wk of gestation | | | | | |
| Q4: >307 pg/ml | 25 | 4 | 1.0 | 4 | 1.0 |
| Q3: 161–307 pg/ml | 25 | 2 | 0.6 (0.1–3.5) | 22 | 5.6 (1.7–19.0) |
| Q2: 88–160 pg/ml | 25 | 6 | 1.9 (0.4–8.2) | 26 | 6.4 (1.9–22.1) |
| Q1: ≤87 pg/ml | 25 | 12 | 9.6 (1.6–57.6) | 23 | 6.7 (1.6–27.5) |
| 21–32 Wk of gestation | | | | | |
| Q4: >1021 pg/ml | 25 | 1 | 1.0 | 14 | 1.0 |
| Q3: 678–1021 pg/ml | 26 | 1 | 1.1 (0.1–18.2) | 19 | 1.2 (0.5–3.1) |
| Q2: 364–677 pg/ml | 25 | 5 | 5.3 (0.6–49.3) | 20 | 1.3 (0.5–3.2) |
| Q1: ≤363 pg/ml | 26 | 19 | 19.6 (2.3–163.8) | 21 | 1.2 (0.5–3.1) |
| 33–41 Wk of gestation | | | | | |
| Q4: >948 pg/ml | 22 | | | 6 | 1.0 |
| Q3: 378–948 pg/ml | 22 | | | 18 | 2.7 (0.9–8.3) |
| Q2: 176–377 pg/ml | 22 | | | 19 | 2.8 (0.9–8.5) |
| Q1: ≤175 pg/ml | 23 | | | 28 | 4.1 (1.4–12.2) |

* Odds ratios were adjusted for gestational age and body-mass index (with 95 percent confidence intervals [CIs]). Quartiles (Q) were determined on the basis of specimens from the controls. Specimens were obtained from all women before the onset of clinical signs of preeclampsia. For the odds ratios shown, the reference category was the lowest quartile of soluble fms-like tyrosine kinase 1 (sFlt-1) or the highest quartile of placental growth factor (PlGF).

of subgroups in which the sFlt-1 and PlGF concentrations might be altered even more or not at all. Nevertheless, longitudinal plots of sFlt-1 concentrations in individual women according to the gestational age of the fetus have confirmed the third-trimester increase in the sFlt-1 level in controls and the greater increase in women with preeclampsia. We found the sFlt-1 levels in women with established preeclampsia to be lower than others have reported them to be.[5,8] Longer freezer-storage times

may have resulted in decreased sFlt-1 levels in our study. A more likely explanation is that the women in our study may have had milder preeclampsia. The mean gestational age of the fetus at the time when specimens were obtained from women with clinical preeclampsia was 38 weeks in this study, as compared with 34 weeks or less in other studies. Finally, we did not examine other complications of pregnancy, such as intrauterine growth restriction in the absence of hypertension or gestational hypertension without proteinuria.

In summary, we have demonstrated a marked increase in the circulating sFlt-1 concentration beginning five weeks before the onset of preeclampsia, accompanied by decreases in the circulating free PlGF and VEGF levels. These findings lend support to the hypothesis that circulating angiogenic proteins may have an important biologic role in preeclampsia. Data from prospective, longitudinal studies in which serial concentrations of sFlt-1 and free PlGF are measured throughout pregnancy are needed to better assess the relevance of these markers to the early identification of preeclampsia and the prediction of its severity.

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Drs. Maynard, Sukhatme, and Karumanchi report being named as coinventors on a pending patent filed by Beth Israel Deaconess Medical Center for the use of angiogenesis-related proteins for the diagnosis and treatment of preeclampsia.

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