Circulating Angiogenic Factors and the Risk of Preeclampsia in Systemic Lupus Erythematosus Pregnancies

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ABSTRACT. ***Objective.*** To investigate whether angiogenic factors are associated with risk of developing preeclampsia in pregnant women with systemic lupus erythematosus (SLE).

Methods. We performed a nested case–control study within a cohort of SLE women with singleton pregnancies. The study included 42 patients with SLE who eventually developed preeclampsia and 75 normal SLE pregnancies. Serum samples were collected at 4-week intervals (from weeks 12 to 36). Serum samples were analyzed for soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), and soluble endoglin (sEng).

Results. Women destined to develop preeclampsia had lower PlGF levels and higher sFlt-1 and sEng levels, and a higher sFlt-1/PlGF ratio than normal pregnancies. These changes became significant at 12 weeks in patients destined to develop either early onset (< 34 weeks, $p \leq 0.003$) or late-onset preeclampsia (≥ 34 weeks, $p \leq 0.02$). The risk to develop preeclampsia was higher among patients with PlGF concentration values in the lowest quartile or with sFlt-1 and sEng levels, and sFlt-1/PlGF ratio, in the highest quartile of the normal SLE pregnancies distribution. The OR were higher and appeared earlier in patients destined to develop early onset preeclampsia (OR ≥ 16.2, from Week 12 onward) than in patients who presented preeclampsia later (OR ≥ 8.9, from Week 24 onward).

Conclusion. Changes in circulating concentrations of sFlt-1, PlGF, sEng, and the sFlt-1/PlGF ratio precede the onset of preeclampsia in SLE pregnancies. The risk profile of circulating angiogenic factors for developing preeclampsia distinctly evolves depending on whether this condition is manifested earlier or later. (J Rheumatol First Release May 15 2015; doi:10.3899/jrheum.141571)

Key Indexing Terms:
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Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that commonly affects women of childbearing age. Women with SLE usually have normal fertility; therefore pregnancy is common[1]. Although obstetrical management in patients with SLE has improved, and the rate of fetal loss has decreased in recent decades[2], they still have an increased frequency of adverse pregnancy outcomes including pregnancy loss, intrauterine growth restriction, fetal loss, prematurity, and preeclampsia[1,3,4,5,6,7,8]. Preeclampsia occurs in 7.6–35% of SLE pregnancies[1,3,6,7,8,9,10]. Several factors have been associated to preeclampsia including preeclampsia in a previous pregnancy, SLE activity, preexisting or active lupus nephritis, hypertension (HTN), thrombocytopenia, and the presence of antiphospholipid antibodies[1,10,11,12].

Although the cause of this pregnancy-specific syndrome is unclear, accumulating evidence suggests that preeclampsia results from an imbalance between placental proangiogenic and antiangiogenic factors that damage maternal vascular

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endothelium, leading to the clinical manifestations of this condition[13,14,15,16,17,18,19,20].

Higher circulating concentrations of both soluble vascular endothelial growth factor receptor-1 [also referred to as soluble fms-like tyrosine kinase-1 (sFlt-1)] and soluble endoglin (sEng), and lower concentrations of placental growth factor (PlGF) are present at the time of diagnosis of preeclampsia, and have also been associated with increased risk to develop this condition[13,14,16,17,18,19,20]. Circulating concentrations of these factors are significantly different in both low-risk and high-risk patients who later develop preeclampsia several weeks to months before the onset of clinical manifestations of preeclampsia when compared with similar women who had an uneventful pregnancy[14,16,18,19,20]. Nevertheless, there is no available information on the changes in circulating levels of these proangiogenic and antiangiogenic factors throughout gestation in SLE patients with normal pregnancies and in those who later developed preeclampsia or on the accuracy of these factors to predict this condition in patients with SLE.

The goal of our present study was to investigate whether differences in circulating levels of sFlt-1, PlGF, sEng, and the sFlt-1/PlGF ratio in patients with SLE may identify those patients at high risk to develop early-onset or late-onset preeclampsia, as reported in women with or without high-risk pregnancies.

MATERIAL AND METHODS

The protocol study was approved by our institute's review board. Written informed consent was obtained from all participants. Between March 2009 and May 2013, 131 Mexican women with singleton pregnancies and who fulfilled 4 or more of the American College of Rheumatology revised criteria for SLE[21,22] were recruited from the Maternal Fetal Medicine Outpatients Clinic of a tertiary care level hospital. For the purpose of this study, samples from all 42 women who developed preeclampsia as well as from all 75 women who did not develop preeclampsia at any time during their pregnancies, and who delivered a healthy full-term infant (≥ 38 weeks) were included. The patients with preeclampsia were divided into those who developed late-onset (≥ 34 weeks, n = 22), and early-onset (less than 34 weeks, n = 20) preeclampsia.

Routine visits to the clinic were scheduled at 4-week intervals starting from Week 12 of gestational age and ending at Week 36. Blood was drawn from an antecubital vein during each visit and the serum obtained after centrifugation was aliquoted and stored at –80°C until assayed. At each visit, disease activity was also scored according to a published index (SLE Disease Activity Index, SLEDAI)[23]. For this study, any value above 4 was considered active disease. All women with antiphospholipid syndrome (APS) were taking low-dose aspirin plus low molecular weight heparin. The diagnosis of APS was made according to the updated APS criteria[24].

Hypertensive disorder of pregnancy was defined according to the American College of Obstetricians and Gynecology criteria[25]. HTN was defined as systolic blood pressure (SBP) at least 140 mmHg and/or diastolic blood pressure (DBP) at least 90 mmHg, measured twice at least 6 h apart, and that returned to normal values within 3 months after delivery. Mild preeclampsia was defined as HTN and significant proteinuria (≥ 300 mg of protein in a 24-h urine specimen or a protein:creatinine ratio ≥ 0.30 mg/mg in a random urine sample)[26]. Severe preeclampsia was considered when either HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count), eclampsia, or preeclampsia with severe HTN (SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg) or massive proteinuria (≥ 5 g/day or protein:creatinine ratio ≥ 3.87)[26] was present. Other variables included were an infant small for gestational age (SGA; defined as an infant whose birth weight was below the 10th percentile), persistent headache or visual disturbances, upper right quadrant or epigastric pain, abnormal liver enzyme levels, thrombocytopenia (< 100,000/μl), serum creatinine above 1.2 mg/dl, pulmonary edema, oliguria (< 500 ml per day), or oligohydramnios. Superimposed preeclampsia was defined as new onset of significant proteinuria in women with HTN diagnosed before 20 weeks of gestation. In women with HTN and proteinuria before 20 weeks of gestation, examination was done for any of the following: sudden increase in proteinuria, sudden increase in blood pressure, the development of HELLP syndrome, eclampsia, abnormal liver enzyme levels, or thrombocytopenia.

Serum analysis. Measurements of sFlt-1, PlGF, and sEng were performed in duplicate in serum samples obtained at each gestational age using ELISA commercial kits (R&D Systems), following the manufacturer's instructions. The minimal detectable quantities for sFlt-1, PlGF, and sEng were 3.5, 7, and 7 pg/ml, respectively, and the intraassay and interassay coefficients ranged from 3.8 to 5.9% and 4.9 to 7.8%, respectively. The sFlt-1/PlGF ratio was calculated from the corresponding sFlt-1 and PlGF values.

Statistical analysis. Differences between continuous variables were determined by the unpaired Student's t-test (or the Mann-Whitney U test for non-normally distributed variables). Differences between categorical variables were determined by the chi-square test with Yates continuity correction or Fisher's exact test for small samples (or the Mantel-Haenszel test with linear tendency for variables with > 2 categories). Differences among ≥ 3 continuous variables were determined by 1-way ANOVA followed by posthoc procedures (Scheffe's F test) or by the Kruskal-Wallis 1-way test, then the Mann–Whitney U test for non-normally distributed variables.

Association among circulating angiogenic factors and the subsequent risk of preeclampsia was analyzed in a cross-sectional manner using all samples within intervals of gestational age studied and according to the time before the onset of preeclampsia. On the basis of the distribution exhibited by the samples from normal SLE pregnancies, the serum concentrations of sFlt-1, PlGF, sEng, and the resulting sFlt-1/PlGF ratios at each gestational age were divided into quartiles, and the OR was calculated and used to assess the association between quartiles and the risk of early-onset or late-onset preeclampsia. The 3 lowest quartiles were used as reference categories for sFlt-1, sEng, and sFlt-1/PlGF ratio, whereas for PlGF, the 3 highest quartiles were taken as reference. Logistic regression analysis was used to adjust the OR for maternal age, median prednisone dosage at followup, prior hematological manifestations to pregnancy, APS, SLE flare during pregnancy, quiescent renal SLE, and creatinine clearance and 24-h proteinuria at enrollment. This analysis was necessary considering that these variables may significantly affect circulating levels of angiogenic factors and thereby explain the risk of subsequent preeclampsia. A 2-tailed p value less than 0.05 was considered statistically significant.

RESULTS

General description of the population. Of the 131 SLE pregnancies in 131 women enrolled, 14 (10.7%) were excluded for the following reasons: 5 patients for miscarriage between 13 and 17 weeks of gestation (3 with renal activity and 2 with APS); 5 patients for premature rupture of membranes between 25 and 34 weeks of gestation (including 1 patient with mucocutaneous and articular activity); 2 for renal activity and nonreassuring fetal status at weeks 33 and 34 of gestational age, respectively; 1 for renal activity and impaired renal function at Week 31 of gestational age; and 1 lost to followup at Week 28. Therefore, a total of 117 SLE pregnancies were available for final analysis. Among 42

patients (35.9%) who developed preeclampsia, 2 had mild preeclampsia, 11 had superimposed preeclampsia, and 29 had severe preeclampsia (including 7 patients who developed HELLP syndrome, 1 eclampsia, and 2 both conditions). The demographic and clinical characteristics of the participants are shown in Table 1. The late-onset preeclampsia group was younger and was taking higher prednisone doses, and had lower baseline creatinine clearance and higher baseline proteinuria levels compared with normal SLE pregnancies ($p \leq 0.02$). Patients who developed early-onset preeclampsia had a significantly higher proportion of APS than those with normal SLE pregnancies ($p < 0.001$). Compared with normal SLE pregnancies, patients with preeclampsia had lower gestational age at delivery, delivered infants with lower birth weights, and had a greater proportion of SGA infants regardless of the time of onset of preeclampsia ($p < 0.001$). These outcomes were more pronounced in patients with early-onset preeclampsia than in those with late-onset preeclampsia ($p < 0.001$). Other adverse perinatal and maternal outcomes that occurred only in patients with early-onset preeclampsia were stillbirths or neonatal deaths (n = 12, 60%) and 1 maternal death (5.0%) due to cerebral hemorrhage (Table 1).

Gestational changes in circulating levels of sFlt-1, PlGF, sEng, and sFlt-1/PlGF ratio. Figure 1 shows the serum sFlt-1, PlGF, and sEng levels, and the sFlt-1/PlGF ratio throughout gestation in normal SLE pregnancies and in those destined to develop preeclampsia. In normal SLE pregnancies, serum sFlt-1 and sEng concentrations progressively increased throughout gestation; meanwhile, serum PlGF concentrations rose from Week 12 to Week 28, decreasing thereafter until the end of sampling (at Week 36). The sFlt-1/PlGF ratio decreased from Week 12 to Week 28 and then progressively increased until the end of sampling.

At each gestational age studied, patients destined to develop preeclampsia exhibited higher serum sFlt-1 and sEng concentrations from Week 12 onward than did women with normal SLE pregnancies ($p \leq 0.005$). Moreover, concentrations of sFlt-1 and sEng were higher from Week 12 to Week 28 of gestation in patients destined to develop early-onset

Table 1. Clinical and demographic characteristics of women with systemic lupus erythematosus (SLE) who have normal pregnancies and women with SLE who eventually developed preeclampsia.

Variable	Normal Pregnancies, n = 75	Late-onset Preeclampsia, n = 22	p	Early-onset Preeclampsia, n = 20	p
Maternal age, yrs	29.8 ± 4.9	26.1 ± 5.5	0.02[a]	29.3 ± 5.8	
Body mass index, kg/m^2	26.4 ± 4.4	26.0 ± 3.6		28.4 ± 5.1	
Gravidity	2 (1–6)	2 (1–6)		2 (1–6)	
Miscarriage	0 (0–4)	0 (0–2)		0 (0–4)	
Nulliparity, n (%)	32 (42.7)	5 (22.7)		5 (25.0)	
Prior preeclampsia, n (%)	9 (12.0)	3 (13.6)		5 (25.0)	
Smoker, n (%)	18 (24.0)	3 (13.6)		2 (10.0)	
SLE disease duration, yrs	5.8 ± 2.9	5.2 ± 3.8		4.3 ± 2.8	
PDN dosage at followup, mg/day	5 (0–60)	10 (0–50)	0.01[a]	5 (0–50)	
Treatment with chloroquine, n (%)	31 (41.3)	5 (22.7)		9 (45.0)	
Treatment with azathioprine, n (%)	10 (13.3)	5 (22.7)		2 (10.0)	
Antiphospholipid syndrome, n (%)	8 (10.7)	5 (22.7)		10 (50.0)	< 0.001[a]
Previous SLE manifestations to pregnancy, n (%)					
Renal involvement	27 (36.0)	13 (59.1)		5 (25.0)	
Arthritis	64 (85.3)	19 (86.4)		20 (100.0)	
Mucocutaneous	54 (72.0)	15 (68.2)		12 (60.0)	
Hematological	6 (8.0)	6 (27.3)	0.04[a]	5 (25.0)	
Neurological	2 (2.7)	1 (4.5)		1 (5.0)	
Serositis	3 (4.0)	0 (0.0)		3 (15.0)	
Chronic hypertension	4 (5.3)	0 (0.0)		3 (15.0)	
Serum creatinine*, mg/dl	0.6 (0.32–0.85)	0.6 (0.48–1.17)		0.6 (0.42–1.94)	
Creatinine clearance*, ml/min/1.73 m^2	135.4 ± 24.2	115.8 ± 33.4	0.02[a]	133.5 ± 35.8	
24-h proteinuria*, mg	102.4 (25.8–3129.2)	159.2 (54.2–6424.7)	0.01[a]	110.8 (48.9–3362.1)	
Quiescent renal lupus, n (%)	8 (10.7)	8 (36.4)	0.008[a]	4 (20.0)	
SLE flares during pregnancy, n (%)	6 (8.0)	5 (22.7)		2 (10.0)	
Gestational age at delivery, weeks	38.5 ± 0.6	36.0 ± 1.7	< 0.001[a]	27.2 ± 4.5	< 0.001[a,b]
Infant birth weight, g	3019 ± 243	2103 ± 350	< 0.001[a]	786 ± 405	< 0.001[a,b]
Small for gestational age infant, n (%)	0	16 (72.7)	< 0.001[a]	15 (75.0)	< 0.001[a]
Stillbirths or neonatal deaths, n (%)	0	0		12 (60.0)	< 0.001[a,b]
Maternal death, n (%)	0	0		1 (5.0)	

Plus-minus values are means ± SD; other values are medians (range), except where indicated. * at enrollment. P value is given only for significant differences. [a]versus normal pregnancies. [b]versus late-onset preeclampsia after application of appropriate statistical tests. PDN: prednisone.

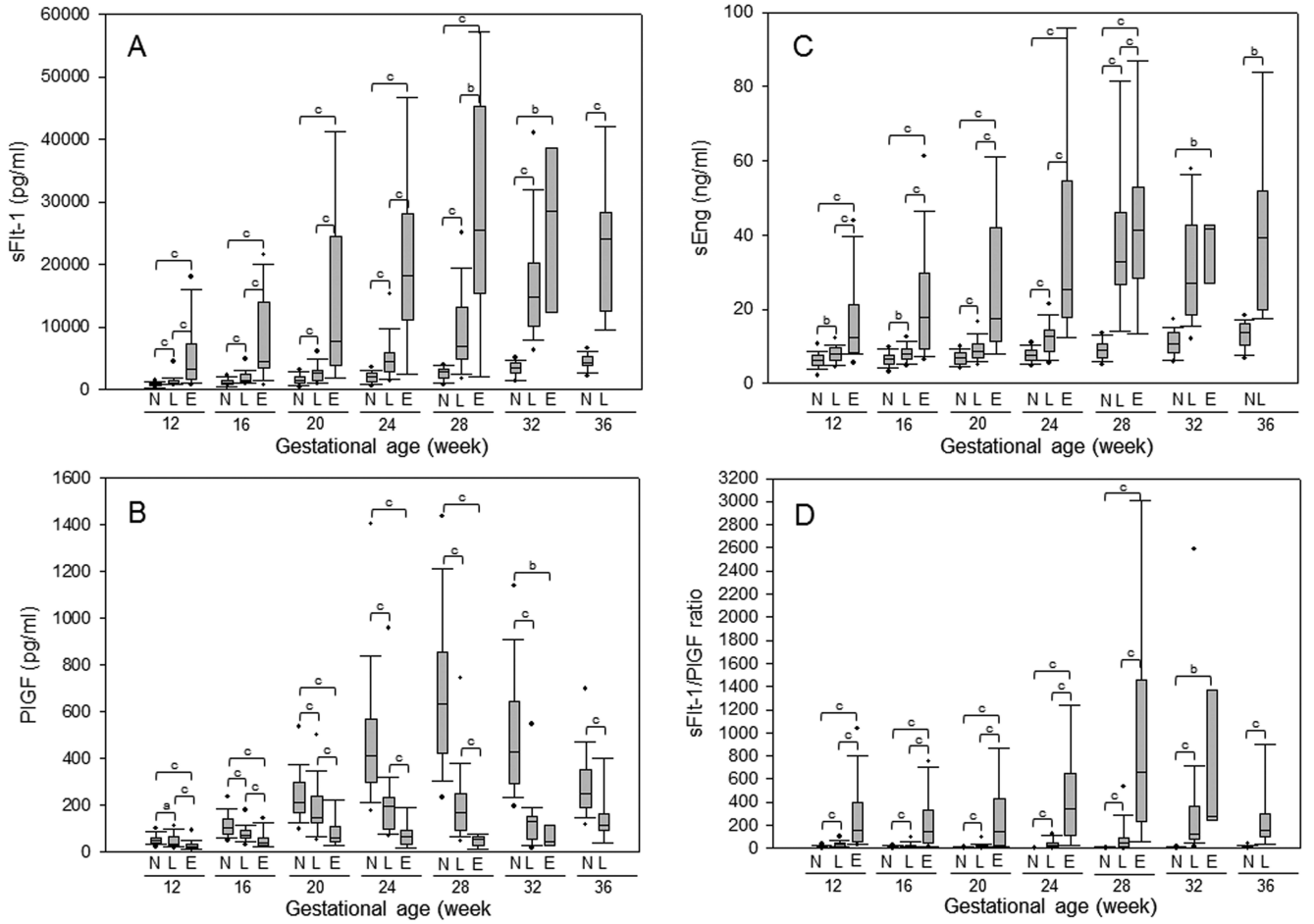

Figure 1. Box and whisker plot comparing the dynamics of changes in soluble fms-like tyrosine kinase-1 (sFlt-1; A), placental growth factor (PlGF; B), soluble endoglin (sEng; C), and sFlt-1/PlGF ratio (D) in maternal serum from women with SLE who had normal pregnancies (N) or who developed later-onset preeclampsia (L) or early-onset preeclampsia (E). Boxes represent interquartile range in which the horizontal line represents the median; the top and bottom horizontal lines of the box are the 75th and 25th percentiles of the data for each group, and whiskers at top and bottom are the 90th and 10th percentiles. Closed dots represent extreme values. Significant differences at various gestational timepoints between normal SLE pregnancies and patients who eventually developed preeclampsia at different times are marked with letters (a, $p \leq 0.05$; b, $p \leq 0.01$; c, $p \leq 0.001$). SLE: systemic lupus erythematosus.

preeclampsia than in those who presented late-onset preeclampsia ($p \leq 0.003$).

As a group, patients destined to develop preeclampsia had lower serum PlGF concentrations throughout gestation than those with normal SLE pregnancies; these differences became significant at 12 weeks of gestation for both early-onset and late-onset preeclampsia ($p \leq 0.005$). Further, in patients who developed early-onset preeclampsia, the decrement in serum PlGF levels was more pronounced during weeks 12–28 than in patients who presented preeclampsia later ($p \leq 0.001$).

At each gestational age studied, patients destined to develop preeclampsia exhibited higher serum sFlt-1/PlGF ratios than did patients with normal SLE pregnancies. This ratio was significantly higher from Week 12 onward in women who developed early-onset or late-onset preeclampsia ($p < 0.001$ vs normal SLE pregnancies). The increment in serum sFlt-1/PlGF ratio exhibited by patients who developed early-onset preeclampsia surpassed during weeks 12–28 of gestation ($p < 0.001$) that observed in patients who later presented preeclampsia.

Angiogenic factors and the risk of preeclampsia. The effect of changes in serum concentrations of sFlt-1, PlGF, and sEng on the association with the development of preeclampsia was investigated by grouping the levels into quartiles based on the distribution of these factors among normal SLE pregnancies. Table 2 shows that the OR for developing preeclampsia progressively increased among patients in the higher (for serum sFlt-1 and sEng levels, and sFlt-1/PlGF ratio) or lower (for serum PlGF levels) quartiles of the control distribution (normal SLE pregnancies). At each gestational age studied, there was a clear association between sFlt-1 and

Table 2. The association (OR) among quartiles of serum soluble fms-like tyrosine kinase-1, placental growth factor, and soluble endoglin concentrations, and soluble fms-like tyrosine kinase-1/placental growth factor ratio and subsequent preeclampsia (late onset and early onset).

	Normal Pregnancies	Late-onset Preeclampsia		Early-onset Preeclampsia	
	Controls	Cases	OR (95% CI)	Cases	OR (95% CI)
sFlt-1, pg/ml					
12 weeks of gestation					
Q4: ≥ 980.0	18	17	9.9 (2.4–40.9)	19	199.9 (10.2–3919)
Q1-3: < 980.0	57	5	1.0	1	1.0
20 weeks of gestation					
Q4: ≥ 1523.1	19	10	2.7 (0.7–9.6)	18	39.0 (5.6–271.6)
Q1-3: < 1523.1	56	11	1.0	2	1.0
20 weeks of gestation					
Q4: ≥ 2130.5	18	13	4.3 (1.2–15.0)	16	26.0 (4.4–154.9)
Q1-3: < 2130.5	57	9	1.0	2	1.0
24 weeks of gestation					
Q4: ≥ 2716.6	19	17	15.0 (3.2–69.5)	15	24.0 (4.0–144.3)
Q1-3: < 2716.6	56	5	1.0	2	1.0
28 weeks of gestation					
Q4: ≥ 3234.0	19	19	30.4 (4.7–194.9)	8	37.3 (3.3–426.4)
Q1-3: < 3234.0	56	3	1.0	1	1.0
32 weeks of gestation					
Q4: ≥ 4253.8	19	22	ND	3	ND
Q1-3: < 4253.8	56	0	ND	0	ND
36 weeks of gestation					
Q4: ≥ 5235.2	19	11	ND		
Q1-3: < 5235.2	56	0	ND		
PlGF, pg/ml					
12 weeks of gestation					
Q2-4: > 39.4	56	8	1.0	3	1.0
Q1: ≤ 39.4	19	14	10.6 (2.5–45.6)	17	27.0 (4.1–178.6)
16 weeks of gestation					
Q2-4: > 78.5	56	9	1.0	3	1.0
Q1: ≤ 78.5	19	13	5.0 (1.4–18.3)	17	28.5 (4.3–188.5)
20 weeks of gestation					
Q2-4: > 166.3	56	8	1.0	3	1.0
Q1: ≤ 166.3	19	14	7.7 (1.9–31.9)	15	54.2 (4.8–610.9)
24 weeks of gestation					
Q2-4: > 296.9	56	2	1.0	1	1.0
Q1: ≤ 296.9	19	20	59.6 (5.0–707.1)	16	47.2 (5.9–379.8)
28 weeks of gestation					
Q2-4: > 423.9	56	1	1.0	0	ND
Q1: ≤ 423.9	19	21	516.2 (10.9–24,392)	9	ND
32 weeks of gestation					
Q2-4: > 291.4	56	1	1.0	0	ND
Q1: ≤ 291.4	19	21	516.2 (10.9–24,392)	3	ND
36 weeks of gestation					
Q2-4: > 291.4	56	1	1.0		
Q1: ≤ 291.4	19	10	64.1 (2.2–1838)		
sFlt-1/PlGF ratio					
12 weeks of gestation					
Q4: ≥ 21.9	19	14	8.8 (2.1–36.9)	20	ND
Q1-3: < 21.9	56	8	1.0	0	ND
16 weeks of gestation					
Q4: ≥ 14.0	19	19	27.1 (4.4–168.5)	20	ND
Q1-3: < 14.0	56	3	1.0	0	ND
20 weeks of gestation					
Q4: ≥ 10.4	19	16	7.3 (1.9–28.4)	18	ND
Q1-3: < 10.4	56	6	1.0	0	ND
24 weeks of gestation					
Q4: ≥ 7.5	19	19	17.2 (3.4–86.1)	17	ND
Q1-3: < 7.5	56	3	1.0	0	ND

Table 2. Continued

	Normal Pregnancies	Late-onset Preeclampsia		Early-onset Preeclampsia	
	Controls	Cases	OR (95% CI)	Cases	OR (95% CI)
28 weeks of gestation					
Q4: ≥ 6.3	19	20	46.9 (5.2–426.6)	8	ND
Q1-3: < 6.3	56	2	1.0	0	ND
32 weeks of gestation					
Q4: ≥ 12.2	19	21	1334 (9.7–1834)	3	ND
Q1-3: < 12.2	56	1	1.0	0	ND
36 weeks of gestation					
Q4: ≥ 25.0	19	10	67.0 (2.2–2045)		
Q1-3: < 25.0	56	1	1.0		
sEng, ng/ml					
12 weeks of gestation					
Q4: ≥ 7.6	21	12	4.5 (1.1–18.4)	19	70.1 (6.2–797.7)
Q1-3: < 7.6	54	10	1.0	1	1.0
16 weeks of gestation					
Q4: ≥ 7.7	19	11	4.5 (1.1–20.2)	18	38.1 (5.5–263.1)
Q1-3: < 7.7	56	11	1.0	2	1.0
20 weeks of gestation					
Q4: ≥ 8.2	21	13	4.5 (1.2–16.8)	16	16.2 (2.9–88.8)
Q1-3: < 8.2	54	9	1.0	2	1.0
24 weeks of gestation					
Q4: ≥ 8.96	20	17	8.9 (2.2–36.6)	17	ND
Q1-3: < 8.9	55	5	1.0	0	ND
28 weeks of gestation					
Q4: ≥ 10.6	20	17	11.9 (2.7–53.0)	9	ND
Q1-3: < 10.6	55	5	1.0	0	ND
32 weeks of gestation					
Q4: ≥ 13.8	19	21	137.2 (9.8–1913)	3	ND
Q1-3: < 13.8	56	1	1.0	0	ND
36 weeks of gestation					
Q4: ≥ 16.2	19	11	ND		
Q1-3: < 16.2	56	0	ND		

Quartiles (Q) were determined on the basis of the distribution among women with SLE who had normal pregnancies (controls). For the OR shown in the highest quartile (sFlt-1/PlGF ratio and sEng), the reference categories were the lower 3 quartiles, whereas for the OR shown in the lowest quartile (PlGF), the reference categories were the higher 3 quartiles. OR undefined because all measurements from SLE women destined to develop preeclampsia were located in the highest or lowest quartile. OR were adjusted for maternal age, median prednisone dosage at followup, prior hematological manifestations to pregnancy, antiphospholipid syndrome, SLE flare during pregnancy; and creatinine clearance and 24-h proteinuria at enrollment. SLE: systemic lupus erythematosus; ND: not determined; sFlt-1: soluble fms-like tyrosine kinase-1; PlGF: placental growth factor; sEng: soluble endoglin.

sEng concentrations and sFlt-1/PlGF ratio in the highest quartiles, and an increased risk to develop preeclampsia. Patients in the highest quartile for either sFlt-1 or sEng at 12 to 32 weeks and at 20–24 to 36 weeks of gestation exhibited higher risk for early-onset and late-onset preeclampsia, respectively. On the other hand, patients in the highest quartile for sFlt-1/PlGF ratio had an increased risk for early-onset or late-onset preeclampsia at Week 12 onward.

For serum PlGF concentrations, there was an increase in the risk of preeclampsia when values fell in the lowest quartile at 12 to 32 weeks for early-onset preeclampsia, and at 20–24 to 36 weeks of gestation for late-onset preeclampsia.

DISCUSSION

To our knowledge, our present report represents the first that has prospectively examined the relationship between circulating sFlt-1, PlGF, and sEng concentrations and the risk to subsequently develop preeclampsia in SLE pregnancies. In the present nested case–control study, we found that serum sFlt-1, PlGF, and sEng levels in patients with SLE who eventually developed either early-onset or late-onset preeclampsia were distinctly different during pregnancy from levels in normal SLE pregnancies (controls). The levels of sFlt-1 and sEng rose and those of PlGF concomitantly declined during weeks 12–36 and 28–36 of gestation, respectively. Even more important was the observation that the dynamics of changes in concentration values of these particular angiogenic factors preceded the appearance of either early-onset or late-onset preeclampsia. In fact, some differences in the temporal changes of sFlt-1, PlGF, and sEng levels between patients who developed early-onset or late-onset preeclampsia were detected: patients who

developed early-onset preeclampsia exhibited significantly higher sFlt-1 and sEng levels and lower PlGF concentrations as early as at Week 12 of gestation, whereas in those who developed later-onset preeclampsia, these factors rose or declined at Week 20 to 24 of gestation. Collectively, these data provide further support for the notion that alterations in circulating sFlt-1, PlGF, and sEng concentrations are present in women destined to develop preeclampsia and that these changes are apparently more pronounced in women who will develop early-onset preeclampsia[14,16,19,20].

We also analyzed the dynamics of changes in the sFlt-1/PlGF ratio as an index to determine the angiogenic balance during pregnancy. The results showed that the dynamics of the sFlt-1/PlGF ratio exhibited a trend similar to that shown by sFlt-1 and sEng in patients who subsequently developed early-onset or late preeclampsia. In line with the present study, similar results were also noted by Salmon, *et al*[27], who demonstrated that levels of sFlt-1 and sFlt-1/PlGF ratios were significantly higher from weeks 12–15 onward in pregnant patients with SLE destined to develop preeclampsia than in those who had an uneventful pregnancy.

Our results confirm and extend previous observations reported among low-risk and high-risk women, including those with SLE[14,16,18,19,20,27], that indicated that the imbalance of circulating angiogenic factors (i.e., high concentrations of sFlt-1 and sEng, and low concentrations of PlGF) is associated with preeclampsia and that these changes in circulating levels of sFlt-1, PlGF, and sEng precede the onset of clinical disease.

In contrast to low-risk or other high-risk pregnant populations, there has been limited investigation of the differences in these angiogenic factors throughout pregnancy among patients with SLE. Qazi, *et al* evaluated the concentrations of maternal sFlt-1 and PlGF in only 1 serum sample taken between 22–32 weeks of gestation in 52 patients with SLE[3]. This study reported that mean serum sFlt-1 was higher in patients who developed preeclampsia compared with uncomplicated pregnancies. The authors conclude that sFlt-1 is associated with preeclampsia in SLE pregnancies. However, and in contrast to the data presented herein, that study was unable to detect differences in PlGF or sFlt-1/PlGF ratio values between normal SLE pregnancies and those complicated with preeclampsia. This discrepancy may be due to the small number of samples (18 preeclamptic patients total); also, the study did not take into account the differences in the gestational age intervals studied, the severity of preeclampsia, and the time of onset of preeclampsia. In this vein, several studies in patients without SLE have shown that circulating concentrations of sFlt-1, PlGF, sEng, and sFlt-1/PlGF ratio are markedly different in women who have severe preeclampsia[14] or in those women who will develop early-onset preeclampsia[14,16,18,19,20], which underlines the need to measure these particular factors early in pregnancy, particularly in those women with 1 or more clinical risk factors for preeclampsia, such as SLE. Moreover, because preeclampsia can appear anytime during the last half of pregnancy, no single sampling point during pregnancy will be sufficient to rule out preeclampsia.

Patients with SLE whose values for sFlt-1, sEng, or sFlt-1/PlGF ratio fall within the highest or the lowest quartile for PlGF showed an increased risk for preeclampsia. When the same analysis based on gestational age was performed, it was found that the risk for developing preeclampsia progressively increased throughout pregnancy. Further, the risk to develop early-onset preeclampsia exceeded and appeared earlier than in patients who subsequently developed late-onset preeclampsia. These data are similar to those previously reported in women without SLE[14,16,18,19,20].

We showed that the sFlt-1/PlGF ratio markedly improved the sensitivity to predict either early-onset or late-onset preeclampsia risk, more than any of the individual factors. These data are consistent with previous studies that showed that measurement of this ratio is a better predictor of preeclampsia than either measure alone[16,20].

Consistent with the idea that SLE women are at high risk for developing preeclampsia, we found a high frequency of preeclampsia in our cohort (35.9%), but it was similar to the frequency in previous studies, which ranged from 7.6% to 35%[1,3,6,7,9,10]. In our univariate analysis, we found that the following variables were significantly associated with late-onset preeclampsia: median prednisone dosage at followup, prior hematological manifestations in pregnancy, quiescent renal SLE, and creatinine clearance and 24-h proteinuria at enrollment. Only APS was strongly associated with early-onset preeclampsia. Nevertheless, the logistic regression analysis revealed that only the concentrations of all angiogenic factors studied showed an increased risk of both early-onset and late-onset preeclampsia at each gestational age studied, even after taking into account other variables. In this regard, several risk factors have been reported to be associated with preeclampsia in patients with SLE, including thrombocytopenia, preexisting renal disease, HTN, SLE activity during pregnancy, and the use of corticosteroids[1,6,8,10,11]. In our study, unfortunately we were unable to find such associations; however, they may have been missed owing to the small number of patients studied who developed preeclampsia. In addition, on logistic regression analysis, only the presence of APS remained as a risk factor for early-onset preeclampsia (OR 3.4, 95% CI 1.1–10.8). This finding is in line with previous studies[1,6,8,10,11]. Collectively, these data, coupled with the profiles of changes in levels of circulating angiogenic factors in early-onset and late-onset preeclampsia, as well as pathological studies of the placenta[28,29], suggest that early-onset preeclampsia in patients with SLE might be associated to poor placentation (which can be aggravated by placental thrombosis in those patients with APS), whereas late-onset preeclampsia is

associated to some maternal factors, such as chronic HTN or renal disease, among others, rather than to poor placentation alone[29]. Further prospective longitudinal studies are needed to assess these possible associations.

The strengths of our study are the following: it was designed to determine whether, when, and how the circulating concentrations of sFlt-1, PlGF, and sEng change in SLE pregnancies destined to develop preeclampsia; as the levels of the 3 angiogenic factors change depending on gestational age, measurements at regular (every 4 weeks) time intervals were performed in the same participants; and to rule out the effects of potential confounders, the estimated OR were adjusted for established risk factors. Nonetheless, it is important to acknowledge that our study is limited because it did not include patients with other obstetrical conditions sharing similarities with preeclampsia, such as gestational HTN or pregnancies complicated by SGA infants.

Our results demonstrate that an imbalance in circulating angiogenic factors is associated with the potential to develop preeclampsia in SLE pregnancies, and that changes in circulating levels of these particular factors precede the onset of clinical disease. In particular, measurement of the sFlt-1/PlGF ratio at 12 weeks of gestation onward has a high discrimination power to reveal the risk of developing preeclampsia in SLE pregnancies.

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