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Longitudinal changes in maternal serum placental growth factor and soluble fms-like tyrosine kinase-1 in women at increased risk of pre-eclampsia

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KEYWORDS: blood pressure; gestational hypertension; placental growth factor; pre-eclampsia; pregnancy screening; soluble fms-like tyrosine kinase-1

ABSTRACT

Objectives *To investigate longitudinal changes in maternal serum levels of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) in pregnant women who develop pre-eclampsia (PE) or gestational hypertension (GH).*

Methods *This was a prospective longitudinal study in women with singleton pregnancies identified by screening at 11 + 0 to 13 + 6 weeks' gestation as being at high-risk of PE. Blood samples were taken every 4 weeks until delivery. Values were compared in women who developed preterm PE (requiring delivery before 37 weeks' gestation), term PE or GH and those who remained normotensive.*

Results *A total of 1069 samples were analyzed in 234 women, including 172 who remained normotensive, 18 who developed GH, 22 who developed preterm PE and 22 who developed term PE. In the preterm PE group, compared to the normotensive group, sFlt-1 levels were significantly higher from 15 weeks' gestation onward and the difference increased with gestational age (*$P < 0.001$*). In the preterm PE group, compared to the normotensive group, PlGF levels were significantly lower from 11 weeks' gestation onward and the difference increased significantly with gestational age (*$P < 0.001$*). Similarly, in the term PE and GH groups, PlGF levels were lower from 13 and 27 weeks onward, respectively, and the differences increased significantly with gestational age (*$P < 0.001$* for both groups). In the preterm PE group, compared to the normotensive group, the sFlt-1/PlGF ratio was significantly higher from 11 weeks onward and the difference increased significantly with gestational age (*$P < 0.001$*). A random slope model provided a significantly better fit to the data than did a single-level model for sFlt-1 (likelihood ratio (LR) = 516; degrees of freedom (df) = 3;* $P < 0.001$*), PlGF (LR = 542; df = 3;* $P < 0.001$*) and the sFlt-1/PlGF ratio (LR = 468; df = 3;* $P < 0.001$*).*

Conclusion *Repeat measurements of the biochemical markers used in this study are likely to be better predictors of PE than are measurements at a single time point during pregnancy, as the differences between normotensive and hypertensive pregnancies increase with gestational age. In screening for preterm PE, maternal serum level of PlGF is a useful marker from the first trimester onward, while the level of sFlt-1 is likely to have a predictive value from the second trimester onward.* *Copyright © 2015 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Pre-eclampsia (PE) remains a leading cause of maternal and perinatal mortality and morbidity in both developed and developing countries[1–3]. PE is thought to result from an imbalance between angiogenic factors, such as placental growth factor (PlGF), and antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1)[4]. Several studies have reported that the maternal serum concentration of PlGF is reduced, while that of sFlt-1 and the sFlt-1/PlGF ratio are increased, both at the time of and prior to, the clinical diagnosis of PE[5–13]. Maternal serum concentrations of PlGF, but not those of sFlt-1, are altered from as early as 11 weeks' gestation in women who eventually develop PE[13,14]. Moreover, maternal serum levels of PlGF, sFlt-1 and the sFlt-1/PlGF ratio could also have prognostic value[7,9–11]. When the results of these studies were combined in a meta-analysis,

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the predictive accuracy of maternal serum PlGF and sFlt-1 levels measured before 30 weeks' gestation was too poor to be useful in clinical practice[15]. However, in a recent screening study, maternal serum PlGF and sFlt-1 levels measured at 30–33 weeks' gestation allowed detection of 100%, 76% and 62% of pregnancies with PE requiring delivery within 4, 6 and 8 weeks of the assessment, respectively, for a fixed false-positive rate of 5%[16].

Despite the fact that maternal serum levels of PlGF and sFlt-1 are affected by maternal characteristics, many screening studies have failed to take this into account[7–12]. Furthermore, most studies were cross-sectional and focused on specific gestational age ranges, commonly 11–13, 20–24 or 30–33 weeks' gestation[16–19]. Few studies, most of which were nested case–control studies, have investigated longitudinal changes in sFlt-1 and PlGF levels in women who eventually developed PE[20–24]. The results of these studies are variable. Nevertheless, some have demonstrated that longitudinal changes are better predictors of PE than is measurement at a single time point during pregnancy and they appear to be more strongly associated with early-onset disease[21,25]. Importantly, most of these longitudinal studies did not involve adjustment for maternal characteristics.

The aim of this study was to investigate longitudinal changes in maternal serum concentrations of sFlt-1 and PlGF, from the first trimester onward, in women identified as being at high risk following first-trimester screening and who subsequently developed PE or gestational hypertension (GH) or remained normotensive.

METHODS

At University College London Hospital, the risk of developing PE is assessed routinely at 11 + 0 to 13 + 6 weeks' gestation using a combination of maternal history, uterine artery Doppler mean pulsatility index, mean arterial pressure and serum pregnancy-associated plasma protein-A[26]. The present study took place between December 2009 and May 2012 and those women considered to be at high risk of early-onset PE were followed in a specialist hypertension clinic in which blood samples were collected every 4 weeks until delivery. Written informed consent was obtained from all women participating in the study which was approved by the London-Surrey Borders Research Ethics Committee. None of these pregnancies was complicated by aneuploidy or major structural abnormalities.

Maternal serum PlGF and sFlt-1 levels were measured in 234 women, with blood samples taken every 4 weeks until delivery. None of the samples was previously thawed and refrozen. Recorded patient characteristics included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian or mixed), method of conception (spontaneous or assisted, requiring the use of ovulation-inducing drugs), cigarette smoking during pregnancy, history of chronic hypertension or pre-existing diabetes mellitus, history of PE in the mother of the patient, obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks) and previous pregnancy with PE. Maternal weight and height were also measured, and body mass index was calculated.

The scientist performing the assays for sample analysis was not aware of clinical data concerning the patient and was blinded to pregnancy outcome. Serum PlGF and sFlt-1 levels were measured in parallel, using an automated electrochemiluminescence immunoassay system (Cobas e411, Roche Diagnostics, Penzberg, Germany). The interassay coefficients of variation for low and high concentrations were 5.4% and 3.0% for PlGF and 3.0% and 3.2% for sFlt-1, respectively. The Cobas e411 analyzer PlGF assay has a detection range of 3–10 000 pg/mL and the sFlt-1 assay of 10–85 000 pg/mL.

Data on pregnancy outcomes were collected from hospital records. The obstetric records of women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was chronic hypertension, PE or GH. The diagnoses of PE and GH were made according to guidelines of the International Society for the Study of Hypertension in Pregnancy[27]. GH was defined as systolic blood pressure (BP) of $\geq$ 140 mmHg and/or diastolic BP of $\geq$ 90 mmHg on at least two occasions 4 h apart, developing after 20 weeks' gestation in a previously normotensive woman, in the absence of significant proteinuria. PE was defined as GH with proteinuria of $\geq$ 300 mg in 24 h or two readings of at least ++ on dipstick analysis of a midstream or catheter urine specimen, if 24-h collection was not available. PE superimposed on chronic hypertension was defined as significant proteinuria (as defined above) developing after 20 weeks' gestation in a woman with known chronic hypertension (history of hypertension before conception or presence of hypertension at the booking visit before 20 weeks' gestation in the absence of trophoblastic disease).

Statistical analysis

Maternal baseline characteristics were compared using the chi-square test or Fisher's exact test for categorical variables and the Kruskal–Wallis test for continuous variables; comparisons among different outcome groups were made using the Mann–Whitney *U*-test with *post-hoc* Bonferroni correction for multiple comparisons. Data are presented as median (interquartile range) for continuous variables and as *n* (%) for categorical variables.

The distribution of maternal plasma sFlt-1 and PlGF values were $\log_{10}$ transformed. Analysis of repeat measurements with multilevel mixed-effects linear models (fixed effects and random effects) was performed. The fixed-effect component included up to third-order polynomial terms of gestational age, hypertensive disorders (PE or GH) and first-order interaction between gestational age and each hypertensive disorder. The random-effect component included the intercept and linear effects of gestational age. Repeat measurements at different weeks' gestation in the same woman constituted Level 1 and each individual constituted Level 2. The multilevel model was

Table 1 Maternal characteristics, according to outcome group, in 234 pregnant women assessed for development of pre-eclampsia (PE) and gestational hypertension (GH)

Characteristic	*Normal* (n = *172*)	*GH* (n = *18*)	*Term PE* (n = *22*)	*Preterm PE* (n = *22*)
Age (years)	32.5 (29.0–36.0)	34.0 (30.8–37.3)	29.6 (25.8–32.3)	31.0 (26.8–35.3)
Weight (kg)	69.7 (59.1–79.0)	69.5 (63.5–78.2)	78.0 (64.5–92.8)	66.5 (58.7–79.5)
Height (cm)	164.5 (160.0–168.0)	165.0 (162.9–170.5)	161.0 (155.8–167.6)*	161.5 (152.8–167.3)
Racial origin				
Caucasian	122 (70.9)	11 (61.1)	13 (59.1)	10 (45.5)
Afro-Caribbean	22 (12.8)	5 (27.8)	4 (18.2)	6 (27.3)
South Asian	16 (9.3)	2 (11.1)	3 (13.6)	5 (22.7)
East Asian	9 (5.2)	0 (0)	2 (9.1)	0 (0)
Mixed	3 (1.7)	0 (0)	0 (0)	1 (4.5)
Parous	51 (29.7)	5 (27.8)	6 (27.3)	8 (36.4)
Cigarette smoker	8 (4.7)	1 (5.6)	0 (0)	0 (0)
Mode of conception				
Spontaneous	162 (94.2)	16 (88.9)	20 (90.9)	21 (95.5)
Ovulation induction	3 (1.7)	0 (0)	1 (4.5)	0 (0)
IUI	4 (2.3)	2 (11.1)	0 (0)	0 (0)
IVF	3 (1.7)	0 (0)	1 (4.5)	1 (4.5)
Chronic hypertension	4 (2.3)	2 (11.1)	2 (9.1)	2 (9.1)

Data are given as median (interquartile range) or *n* (%). Comparisons between outcome groups by chi-square test for categorical variables and Kruskal–Wallis test for continuous variables, and comparison of each outcome group with normal outcome by Mann–Whitney *U*-test with *post-hoc* Bonferroni correction: $^*P < 0.05$. IUI, intrauterine insemination; IVF, *in-vitro* fertilization.

compared to the one-level model by the likelihood ratio (LR) test. Prior to performing regression analysis, continuous variables were centered by subtracting the mean from each measured value (70 from maternal weight in kg, 164 from maternal height in cm and 32 from maternal age in years).

The predictive accuracy of preterm PE using PlGF and sFlt-1 levels as well as the sFlt-1/PlGF ratio by a single measurement at 11–13 and 19–23 weeks' gestation, and by repeat measurements was determined by receiver–operating characteristics (ROC) curve analysis. The areas under the ROC curves (AUC) of sFlt-1, PlGF and sFlt-1/PlGF values were compared to assess their performance in the prediction of preterm PE.

The software programs MLwiN 2.28 (Centre for Multilevel Modelling, University of Bristol, Bristol, UK)[28] and IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA) were used for statistical analysis.

RESULTS

Both PlGF and sFlt-1 values were analyzed in a total of 1069 samples from 234 women, with a median of three (range, 1–8) samples per woman. Of the 234 pregnant women included, 172 remained normotensive, 18 developed GH, 22 developed PE requiring delivery after 37 weeks (term PE) and 22 developed PE requiring delivery at or before 37 weeks (preterm PE). Maternal characteristics of each outcome group are summarized in Table 1. Median maternal height was significantly lower in women who developed term PE ($P = 0.017$). There were no significant differences in maternal age ($P = 0.051$), weight ($P = 0.232$), ethnicity ($P = 0.260$), parity ($P = 0.907$), smoking status ($P = 0.531$), chronic hypertension ($P = 0.105$) or mode of conception ($P = 0.368$) among the different outcome groups.

Maternal serum sFlt-1 levels decreased significantly with maternal weight (Table 2). Levels were significantly higher in women of Afro-Caribbean and East Asian racial origin and in those who underwent ovulation induction, but was not significantly affected by maternal age ($P = 0.589$), height ($P = 0.596$), smoking status ($P = 0.864$), history of PE ($P = 0.196$) or chronic hypertension ($P = 0.830$). A random-slope model provided a significantly better fit to the data than did a single-level model (LR = 516; degrees of freedom (df) = 3; $P < 0.001$) or a random-intercept model (LR = 122; df = 2; $P < 0.001$). In the normotensive group, there was a quadratic increase of $\log_{10}$sFlt-1 with gestational age (Table 2 and Figure 1). Maternal serum sFlt-1 levels decreased until 19 weeks' gestation and increased thereafter. In the preterm PE group, compared to the normotensive group, sFlt-1 levels were significantly increased from 15 weeks' gestation onward and the difference increased with gestational age (Table 2 and Figure 1b). In the term PE and GH groups, maternal serum sFlt-1 levels did not differ significantly from those in the normotensive group ($P = 0.044$ and $P = 0.999$, respectively; Figures 1c and d).

Maternal serum PlGF levels were significantly higher in women of Afro-Caribbean and South Asian racial origin but were not significantly affected by maternal age ($P = 0.366$), weight ($P = 0.107$), height ($P = 0.317$), smoking status ($P = 0.261$), mode of conception ($P = 0.555$), history of PE ($P = 0.208$) or chronic hypertension ($P = 0.718$). A random-slope model provided a significantly better fit to the data than did a single-level model (LR = 542; df = 3; $P < 0.001$) or a random-intercept model (LR = 152; df = 2; $P < 0.001$) (Table 2). In the

Table 2 Application of multilevel linear mixed-effects model on $\log_{10}$ soluble fms-like tyrosine kinase-1 (sFlt-1), $\log_{10}$ placental growth factor (PlGF) and $\log_{10}$sFlt/PlGF ratio in 234 pregnant women assessed for development of pre-eclampsia (PE) and gestational hypertension (GH)

	sFlt-1			*PlGF*			*sFlt-1/PlGF ratio*		
Parameter	*Estimate*	*SE*	P	*Estimate*	*SE*	P	*Estimate*	*SE*	P
Fixed part									
Intercept	3.493954	0.052847	< 0.001	−0.828887	0.065869	< 0.001	4.352011	0.090010	< 0.001
GH	0.000125	0.093181	0.999	0.130851	0.111781	0.243	−0.172696	0.153676	0.262
Term PE	−0.164263	0.080796	0.044	−0.070197	0.096876	0.470	−0.120514	0.133512	0.368
Preterm PE	−0.399078	0.090865	< 0.001	0.242647	0.109999	0.028	−0.694381	0.151147	< 0.001
GA (weeks)	−0.045029	0.00403	< 0.001	0.241150	0.005111	< 0.001	−0.286617	0.007023	< 0.001
GA (weeks)^2	0.001201	0.000077	< 0.001	−0.004246	0.000098	< 0.001	0.005455	0.000134	< 0.001
Interaction									
GH with GA (weeks)	0.003478	0.003736	0.353	−0.009774	0.004737	0.040	0.013794	0.006893	0.047
Term PE with GA (weeks)	0.007455	0.003255	0.023	−0.003279	0.004120	0.427	0.010408	0.006005	0.085
Preterm PE with GA (weeks)	0.033273	0.003822	< 0.001	−0.036469	0.004842	< 0.001	0.071051	0.006977	< 0.001
Weight (−70)*	−0.003564	0.000762	< 0.001						
Racial origin									
Caucasian (reference)									
Afro-Caribbean	0.077127	0.033911	0.024	0.172856	0.041402	< 0.001			
South Asian	−0.006728	0.039316	0.864	0.138745	0.048325	0.004			
East Asian	0.153643	0.056153	0.007	0.067048	0.068460	0.329			
Mixed	0.082440	0.097163	0.397	−0.018237	0.119767	0.879			
Mode of conception									
Spontaneous (reference)									
Ovulation induction	0.255146	0.090781	0.005						
Intrauterine insemination	0.054834	0.076294	0.473						
In-vitro fertilization	0.049281	0.081410	0.546						
Previous PE									
Nulliparous (reference)									
Parous without history of PE							−0.141036	0.048670	0.004
Parous with history of PE							−0.138011	0.064212	0.033
Random part									
Level 2									
Variance (constant)	0.084682	0.013954		0.114565	0.020003		0.219234	0.035342	
Variance (GA)	0.000135	0.000022		0.000215	0.000034		0.000485	0.000070	
Covariance (constant, GA)	−0.002779	0.000523		−0.004007	0.000784		−0.008684	0.001477	
Level 1									
Residual	0.015445	0.000899		0.025009	0.001427		0.046666	0.002646	

*Subtracted from maternal weight in kg. GA, gestational age; SE, standard error.

normotensive group, there was a quadratic association of $\log_{10}$PlGF with gestational age (Table 2 and Figure 2a). PlGF levels increased until 28 weeks' gestation and decreased thereafter. In the preterm PE group, compared to the normotensive group, PlGF levels were significantly lower from 11 weeks' gestation onward and the difference increased significantly with gestational age (Table 2 and Figure 2b). Similarly, in the term PE and GH groups, maternal serum PlGF levels were lower from 13 weeks' gestation and from 27 weeks' gestation onward, respectively, than in women who remained normotensive and the difference increased significantly with gestational age (Figures 2c and d).

The maternal serum sFlt-1/PlGF ratio was significantly lower in parous women, with or without previous PE, but was not significantly affected by maternal age ($P = 0.799$), weight ($P = 0.215$), height ($P = 0.729$), racial origin ($P = 0.196$), smoking status ($P = 0.480$), mode of conception ($P = 0.083$) or chronic hypertension ($P = 0.832$). A random-slope model provided a significantly better fit to the data than did a single-level model (LR = 468; df = 3; $P < 0.001$) or a random-intercept model (LR = 183; df = 2; $P < 0.05$) (Table 2). In the normotensive group, there was a quadratic association of $\log_{10}$sFlt-1/PlGF with gestational age (Table 2 and Figure 3a). The maternal serum sFlt-1/PlGF ratio decreased until 26 weeks' gestation but increased thereafter. In the preterm PE group, compared to the normotensive group, the maternal serum sFlt-1/PlGF ratio was significantly higher from 11 weeks' gestation onward and the difference increased significantly with gestational age (Table 2 and Figure 3b). Similarly, in the term PE and GH groups, the sFlt-1/PlGF ratio was significantly higher from 21 weeks' and 23 weeks' gestation onward, respectively, and the difference increased significantly with gestational age (Figures 3c and d).

The performance of screening for preterm PE was not significantly improved by repeat measurements of PlGF levels, when compared to a single measurement obtained at 11–13 weeks ($P = 0.348$) or a single measurement at 19–22 weeks ($P = 0.781$), by repeat measurements of sFlt-1 levels, when compared to a single measurement at 11–13 weeks ($P = 0.871$) or a single measurement

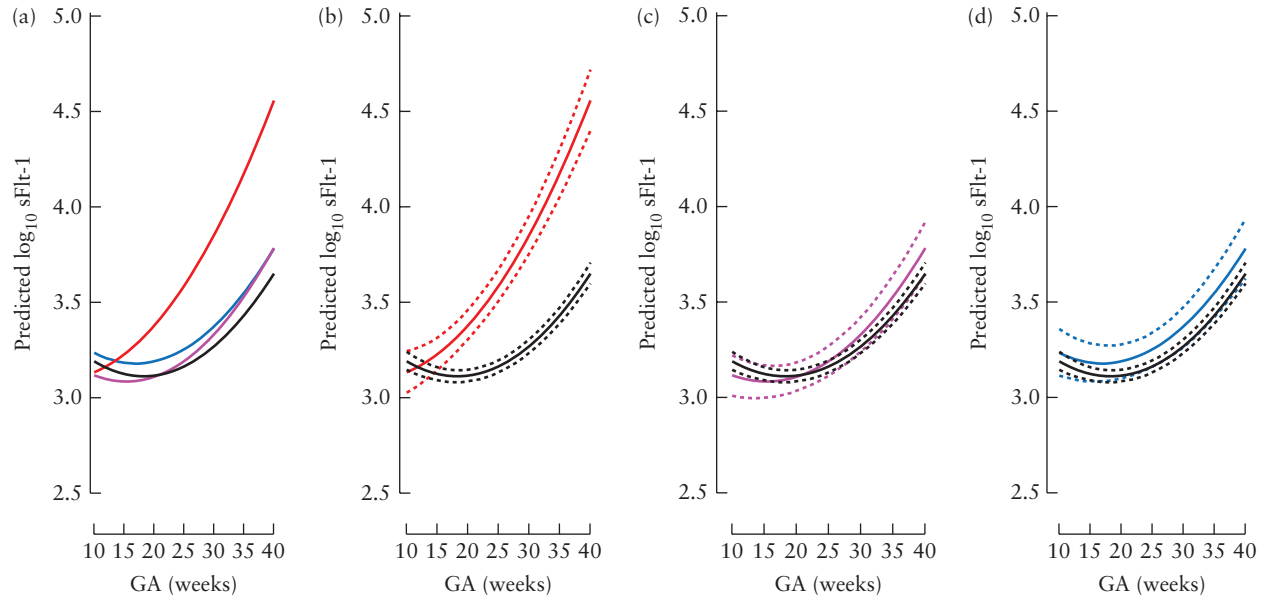

Figure 1 Maternal serum soluble fms-like tyrosine kinase-1 (sFlt-1) in 234 pregnancies with normal outcome (——) (a) and those complicated by preterm pre-eclampsia (PE) (——) (b), term PE (——) (c) or gestational hypertension (——) (d). Mean values with 95% CIs in each outcome group are shown (b–d). GA, gestational age.

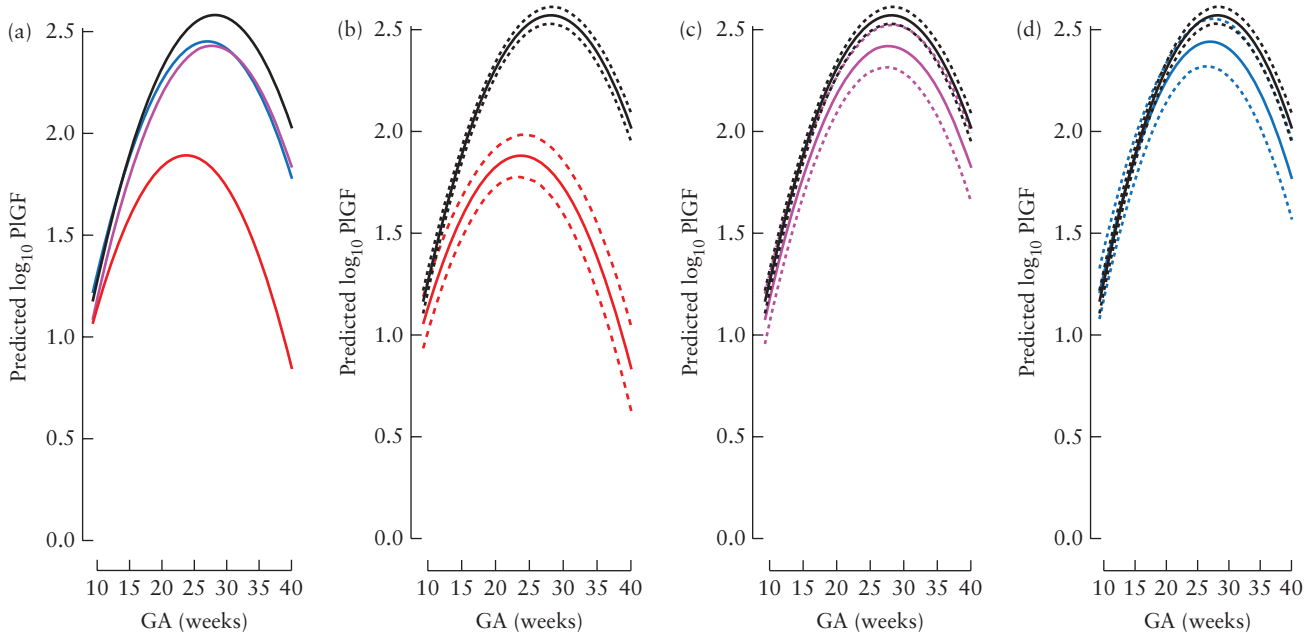

Figure 2 Maternal serum placental growth factor (PlGF) in 234 pregnancies with normal outcome (——) (a) and those complicated by preterm pre-eclampsia (PE) (——) (b), term PE (——) (c) or gestational hypertension (——) (d). Mean values with 95% CIs in each outcome group are shown (b–d). GA, gestational age.

at 19–22 weeks ($P = 0.093$) or by repeat measurements of the sFlt-1/PlGF ratio, when compared to a single measurement at 11–13 weeks ($P = 0.376$) or a single measurement at 19–22 weeks ($P = 0.084$) (Table 3). However, the study is likely to be underpowered to determine whether repeat measurements of sFlt-1 or PlGF levels are superior to a single measurement. A random-slope model provided a significantly better fit to the data than did a single-level model for sFlt-1 (LR = 516; df = 3; $P < 0.001$), PlGF (LR = 542; df = 3; $P < 0.001$) and sFlt-1/PlGF ratio (LR = 468; df = 3; $P < 0.001$).

DISCUSSION

This study demonstrates that, in normotensive pregnancies, there is a quadratic association between gestational age and maternal serum concentrations of sFlt-1 and PlGF and their ratio. Both sFlt-1 levels and the sFlt-1/PlGF ratio increase with gestational age, with a second-trimester trough (at 19 weeks for sFlt-1 and 26 weeks for the sFlt-1/PlGF ratio), whereas PlGF levels increase with advancing gestation, with a peak at 28 weeks and a decrease thereafter.

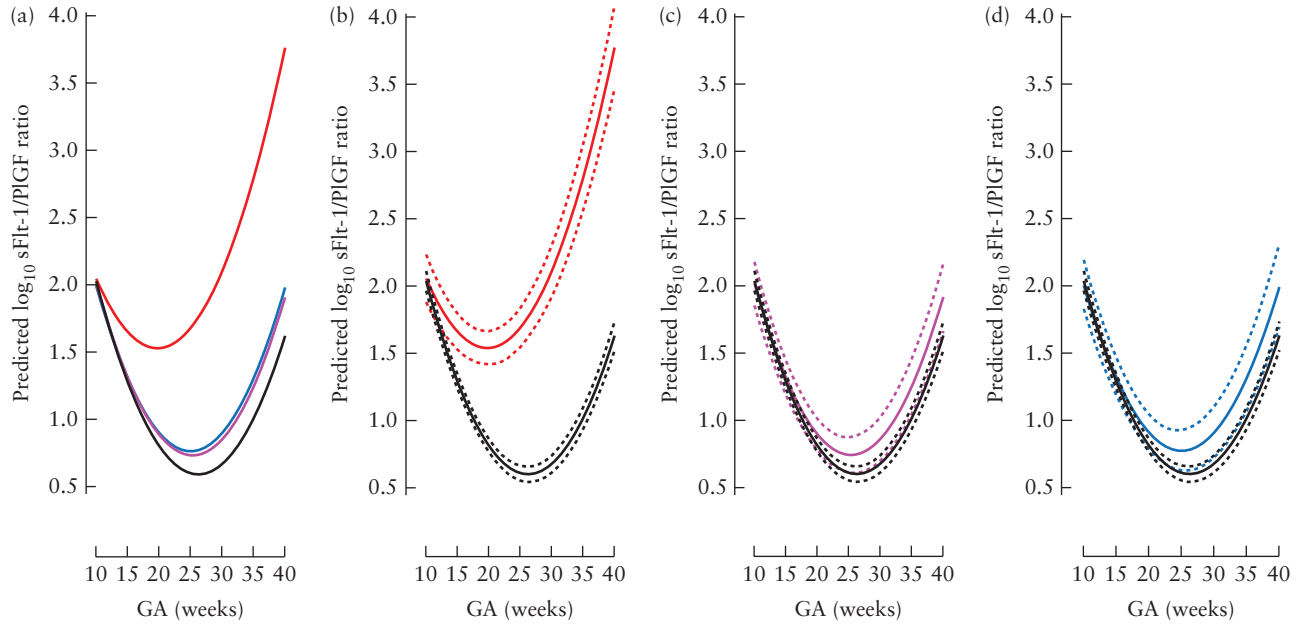

Figure 3 Maternal serum soluble fms-like tyrosine kinase-1/placental growth factor (sFlt-1/PlGF) ratio in 234 pregnancies with normal outcome (——) (a) and those complicated by preterm pre-eclampsia (PE) (——) (b), term PE (——) (c) or gestational hypertension (——) (d). Mean values with 95% CIs in each outcome group are shown. GA, gestational age.

Table 3 Performance of soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF) and sFlt-1/PlGF ratio measured either at single time points or repeatedly throughout pregnancy for prediction of pre-eclampsia

Screening measurement	*AUC (95% CI)*
PlGF	
11–13 weeks	0.70 (0.54–0.87)
19–22 weeks	0.77 (0.63–0.91)
Longitudinal	0.79 (0.74–0.84)
sFlt-1	
11–13 weeks	0.58 (0.44–0.73)
19–22 weeks	0.73 (0.61–0.85)
Longitudinal	0.74 (0.69–0.80)
sFlt-1/PlGF ratio	
11–13 weeks	0.73 (0.60–0.86)
19–22 weeks	0.79 (0.67–0.91)
Longitudinal	0.85 (0.80–0.89)

AUC, area under the receiver–operating characteristics curve.

In women with preterm PE, compared to normotensive controls, maternal serum levels of PlGF are significantly lower and the sFlt-1/PlGF ratio is significantly higher from 11 weeks' gestation onward, while sFlt-1 levels are significantly higher from 15 weeks' gestation onward. The difference in maternal serum levels of these biochemical markers between women who develop preterm PE, compared to those who remain normotensive, increases with gestation.

In women with term PE, compared to normotensive controls, maternal serum levels of PlGF are significantly lower and the sFlt-1/PlGF ratio is significantly higher from 13 weeks' and 21 weeks' gestation, respectively, and the difference increases with gestation. These changes are detected from 27 weeks' and 23 weeks' gestation, respectively, in women who develop GH. Again, these differences increase with gestation. Maternal serum levels of sFlt-1 were not significantly different from those in the normotensive group in women who developed term PE or GH.

This study has several strengths, including its prospective longitudinal design, the use of a well-defined methodology and the application of a robust statistical approach that takes into account not only the difference in marker levels in outcome groups but also the change throughout gestation. This approach is different from the calculation of trends with gestational age that are based on large numbers of cross-sectional and unrelated measurements. A limitation of the study is the relatively small number of cases.

PE is characterized by impairment in the physiological process of trophoblastic invasion of the maternal spiral arteries, resulting in persistence of high impedance in the uterine vessels[29–31]. It is likely that the resulting hypoxia suppresses PlGF and upregulates sFlt-1 expression in trophoblast cells, leading to an exaggerated angiogenic/antiangiogenic imbalance[5,32,33]. Soluble sFlt-1 is a splice variant of vascular endothelial growth factor (VEGF) receptor-1 (Flt-1), while both VEGF and PlGF are key vascular endothelial growth factors in angiogenesis and vasculogenesis[34]. Circulating sFlt-1 can bind to PlGF and VEGF, effectively inhibiting their actions, hence its role as an antiangiogenic factor[35,36].

Soluble sFlt-1 was found to be downregulated by the heme oxygenase (Hmox1)/carbon monoxide (CO) pathway[37]. This phenomenon might account for the protective effect of smoking against development of PE[37]. Moreover, statins induce heme oxygenase, and therefore suppress the release of sFlt-1 and induce PlGF[38,39]. Ongoing studies are investigating the role of statins and Hmox1 activators as potential novel therapeutic agents

for treating PE[37–40]. Promising results were reported in a pilot study in which Thadhani *et al.* performed extracorporeal apheresis that resulted in lowering of sFlt-1 levels, reducing proteinuria, stabilizing BP and prolonging pregnancy without apparent adverse effects for mother and fetus[41].

Our finding that in normotensive pregnancies maternal serum levels of PlGF decrease with increasing gestational age and are higher in women of Afro-Caribbean and South Asian origin than in Caucasian women is compatible with results of previous screening studies[16]. Similarly, consistent with published evidence, maternal serum sFlt-1 levels increased with gestational age and were higher in women of Afro-Caribbean origin and women who conceived by assisted conception[16]. The incidence of PE in the study population was 18.8%, and that of GH was 7.7%. This is consistent with the fact that our study involved a population identified as being at high risk of PE following first-trimester screening.

Our findings in normotensive pregnancies, of a non-linear relationship between gestational age and maternal serum levels of PlGF and sFlt-1, as well as the sFlt1/PlGF ratio, are compatible with results of previous longitudinal studies[20,23]. Similarly, our finding of reduced maternal serum PlGF levels in preterm PE before the clinical onset of PE, as early as 11 weeks' gestation, is consistent with both cross-sectional and longitudinal studies[13,17,20,21,23,42,43]. In term PE and GH, we found significantly lower maternal serum PlGF levels, from as early as 13 weeks' and 27 weeks' gestation, respectively[43]. In our study cohort, during the first trimester, maternal serum sFlt-1 levels were not significantly different in women who developed PE and those who remained normotensive. The levels were significantly different from 15 weeks' gestation onward in those who developed preterm PE and the difference increased with gestation. These findings are consistent with earlier studies[14,17,20,22,23,42,43]. Maternal sFlt-1 levels were not significantly different in women who later developed GH, an observation consistent with previous studies[43]. Finally, the maternal serum sFlt-1/PlGF ratio was significantly higher in women who later developed PE or GH, compared to women with normotensive pregnancies, and the difference increased with gestational age. It seems that using the ratio, as compared to the use of sFlt-1 levels alone, allows detection of the risk of preterm PE at an earlier gestational age. Furthermore, the ratio has useful predictive value for pregnancies destined to be complicated by term PE or GH, in which maternal serum sFlt-1 levels were not significantly different from those in normotensive pregnancies. These findings are also consistent with those of previous studies[17,23,42–44]. It remains to be determined whether it is cost-effective to use the maternal serum sFlt-1/PlGF ratio rather than PlGF levels alone in the prediction of PE.

The difference in sFlt-1 and PlGF levels as well as the sFlt-1/PlGF ratio in pregnancies complicated by preterm PE and those that remained normotensive increased with gestation. This finding could have implications for the prediction of PE; measuring maternal serum sFlt-1 and PlGF levels during the second and third trimesters, as well as the first trimester, could potentially improve their predictive accuracy in diagnosing PE. Erez *et al.* reported an odds ratio (OR) of 3.9 for the increase in sFlt-1levels alone, and an OR of 4.3 for an increase in PlGF concentration less than the median[21]. Our results are in agreement with previous findings that changes in biochemical markers, when measured longitudinally, are better predictors of PE than measurement at a single time point during pregnancy[25].

In conclusion, this study described longitudinal changes in maternal serum levels of sFlt-1 and PlGF as well as the sFlt-1/PlGF ratio in women with high-risk pregnancies who developed PE or GH and those who remained normotensive. Repeat measurements of these biochemical markers are likely to be better predictors of PE than measurement at a single time point during pregnancy, as the differences between normotensive and complicated pregnancies increase with gestation. In screening for preterm PE, the maternal serum level of PlGF is a useful marker from the first trimester onward, while the sFlt-1 level has a predictive value from the second trimester onward. In term PE, maternal serum PlGF levels are reduced from the first trimester onward, while sFlt-1 levels are unlikely to be useful for prediction. The sFlt-1/PlGF ratio might provide added predictive value, compared to the use of a single marker. These results are likely to be useful in continuing efforts to improve the accuracy of predicting PE.

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REFERENCES

1. Cantwell R, Clutton-Brock T, Cooper G, Dawson A, Drife J, Garrod D, Harper A, Hulbert D, Lucas S, McClure J, Millward-Sadler H, Neilson J, Nelson-Piercy C, Norman J, O'Herlihy C, Oates M, Shakespeare J, de Swiet M, Williamson C, Beale V, Knight M, Lennox C, Miller A, Parmar D, Rogers J, Springett A. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom. *BJOG* 2011; **118** (Suppl 1)1–203.
2. Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol* 2009; **33**: 130–137.
3. World Health Organization. Make Every Mother and Child Count. World Health Report, 2005. Geneva, Switzerland: World Health Organization; 2005.
4. Bdolah Y, Sukhatme VP, Karumanchi SA. Angiogenic imbalance in the pathophysiology of preeclampsia: newer insights. *Semin Nephrol* 2004; **24**: 548–556.
5. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003; **111**: 649–658.
6. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; **350**: 672–683.
7. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Moertl M, Zeisler H, Calda P, Holzgreve W, Galindo A, Engels T, Denk B, Stepan H. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am J Obstet Gynecol* 2012; **206**: 58. e1–e8.
8. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, Lim KH, Wenger JB, Thadhani R, Karumanchi SA. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation* 2012; **125**: 911–919.

9. Chaiworapongsa T, Romero R, Savasan ZA, Kusanovic JP, Ogge G, Soto E, Dong Z, Tarca A, Gaurav B, Hassan SS. Maternal plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in patients presenting to the obstetrical triage area with the suspicion of preeclampsia. *J Matern Fetal Neonatal Med* 2011; **24**: 1187–1207.
10. Sibiude J, Guibourdenche J, Dionne MD, Le Ray C, Anselem O, Serreau R, Goffinet F, Tsatsaris V. Placental growth factor for the prediction of adverse outcomes in patients with suspected preeclampsia or intrauterine growth restriction. *PLoS One* 2012; **7**: e50208.
11. Chappell LC, Duckworth S, Seed PT, Griffin M, Myers J, Mackillop L, Simpson N, Waugh J, Anumba D, Kenny LC, Redman CW, Shennan AH. Diagnostic accuracy of placental growth factor in women with suspected preeclampsia: a prospective multicenter study. *Circulation* 2013; **128**: 2121–2131.
12. Ohkuchi A, Hirashima C, Takahashi K, Suzuki H, Matsubara S, Suzuki M. Onset threshold of the plasma levels of soluble fms-like tyrosine kinase 1/placental growth factor ratio for predicting the imminent onset of preeclampsia within 4 weeks after blood sampling at 19–31 weeks of gestation. *Hypertens Res* 2013; **36**: 1073–1080.
13. Akolekar R, Zaragoza E, Poon LC, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11 + 0 to 13 + 6 weeks of gestation in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2008; **32**: 732–739.
14. Akolekar R, de Cruz J, Foidart JM, Munaut C, Nicolaides KH. Maternal plasma soluble fms-like tyrosine kinase-1 and free vascular endothelial growth factor at 11 to 13 weeks of gestation in preeclampsia. *Prenat Diagn* 2010; **30**: 191–197.
15. Kleinrouweler C, Wiegerinck M, Ris-Stalpers C, Bossuyt P, van der Post J, von Dadelszen P, Mol B, Pajkrt E, for the EBM CONNECT Collaboration. Accuracy of circulating placental growth factor, vascular endothelial growth factor, soluble fms-like tyrosine kinase 1 and soluble endoglin in the prediction of pre-eclampsia: a systematic review and meta-analysis. *BJOG* 2012; **119**: 778–787.
16. Lai J, Garcia-Tizon Larroca S, Peeva G, Poon LC, Wright D, Nicolaides KH. Competing risks model in screening for preeclampsia by serum placental growth factor and soluble fms-like tyrosine kinase-1 at 30-33 weeks' gestation. *Fetal Diagn Ther* 2014; **35**: 240–248.
17. De Vivo A, Baviera G, Giordano D, Todarello G, Corrado F, D'Anna R. Endoglin, PlGF and sflt-1 as markers for predicting pre-eclampsia. *Acta Obstet Gynecol Scand* 2008; **87**: 837–842.
18. Baumann MU, Bersinger NA, Mohaupt MG, Raio L, Gerber S, Surbek DV. First-trimester serum levels of soluble endoglin and soluble fms-like tyrosine kinase-1 as first-trimester markers for late-onset preeclampsia. *Am J Obstet Gynecol* 2008; **199**: 266.e1–e6.
19. Savvidou MD, Noori M, Anderson JM, Hingorani AD, Nicolaides KH. Maternal endothelial function and serum concentrations of placental growth factor and soluble endoglin in women with abnormal placentation. *Ultrasound Obstet Gynecol* 2008; **32**: 871–876.
20. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Mazaki-Tovi S, Gomez R, Edwin S, Chaiworapongsa T, Levine RJ, Karumanchi SA. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *J Matern Fetal Neonatal Med* 2008; **21**: 9–23.
21. Erez O, Romero R, Espinoza J, Fu W, Todem D, Kusanovic JP, Gotsch F, Edwin S, Nien JK, Chaiworapongsa T, Mittal P, Mazaki-Tovi S, Than NG, Gomez R, Hassan SS. The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. *J Matern Fetal Neonatal Med* 2008; **21**: 279–287.
22. Rana S, Karumanchi SA, Levine RJ, Venkatesha S, Rauh-Hain JA, Tamez H, Thadhani R. Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia. *Hypertension* 2007; **50**: 137–142.
23. Perni U, Sison C, Sharma V, Helseth G, Hawfield A, Suthanthiran M, August P. Angiogenic factors in superimposed preeclampsia: a longitudinal study of women with chronic hypertension during pregnancy. *Hypertension* 2012; **59**: 740–746.
24. Myatt L, Clifton RG, Roberts JM, Spong CY, Wapner RJ, Thorp JM Jr, Mercer BM, Peaceman AM, Ramin SM, Carpenter MW, Sciscione A, Tolosa JE, Saade G, Sorokin Y, Anderson GD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Can changes in angiogenic biomarkers between the first and second trimesters of pregnancy predict development of pre-eclampsia in a low-risk nulliparous patient population? *BJOG* 2013; **120**: 1183–1191.
25. Smith GC, Wear H. The perinatal implications of angiogenic factors. *Curr Opin Obstet Gynecol* 2009; **21**: 111–116.
26. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. *Prenat Diagn* 2011; **31**: 66–74.
27. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20**: IX–XIV.
28. Rasbash J, Charlton C, Browne WJ, Healy M, Cameron B. (2009) MLwiN Version 2.1. Centre for Multilevel Modelling, University of Bristol.
29. Redman CWG. Pre-eclampsia and the placenta. *Placenta* 1991; **12**: 301–308.
30. Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet* 1993; **341**: 1447–1451.
31. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of hypertension during pre-eclampsia linking placental ischemia with endothelial dysfunction. *Hypertension* 2001; **38**: 718–722.
32. Torry DS, Mukherjea D, Arroyo J, Torry RJ. Expression and function of placenta growth factor: implications for abnormal placentation. *J Soc Gynecol Investig* 2003; **10**: 178–188.
33. Munaut C, Lorquet S, Pequeux C, Blacher S, Berndt S, Frankenne F, Foidart JM. Hypoxia is responsible for soluble vascular endothelial growth factor receptor-1 (VEGFR-1) but not for soluble endoglin induction in villous trophoblast. *Hum Reprod* 2008; **23**: 1407–1415.
34. Roy H, Bhardwaj S, Yla-Herttuala S. Biology of vascular endothelial growth factors. *FEBS Lett* 2006; **580**: 2879–2887.
35. Kendall RL, Wang G, Thomas KA. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. *Biochem Biophys Res Commun* 1996; **226**: 324–328.
36. He Y, Smith SK, Day KA, Clark DE, Licence DR, Charnock-Jones DS. Alternative splicing of vascular endothelial growth factor (VEGF)-R1 (FLT-1) pre-mRNA is important for the regulation of VEGF activity. *Mol Endocrinol* 1999; **13**: 537–545.
37. Cudmore M, Ahmad S, Al-Ani B, Fujisawa T, Coxall H, Chudasama K, Devey LR, Wigmore SJ, Abbas A, Hewett PW, Ahmed A. Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation* 2007; **115**: 1789–1797.
38. Saad AF, Kechichian T, Yin H, Sbrana E, Longo M, Wen M, Tamayo E, Hankins GD, Saade GR, Costantine MM. Effects of pravastatin on angiogenic and placental hypoxic imbalance in a mouse model of preeclampsia. *Reprod Sci* 2014; **21**: 138–145.
39. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. *Proc Natl Acad Sci USA* 2011; **108**: 1451–1455.
40. Ramma W, Ahmed A. Therapeutic potential of statins and the induction of heme oxygenase-1 in preeclampsia. *J Reprod Immunol* 2014; **101–102**: 153–160.
41. Thadhani R, Kisner T, Hagmann H, Bossung V, Noack S, Schaarschmidt W, Jank A, Kribs A, Cornely OA, Kreyssig C, Hemphill L, Rigby AC, Khedkar S, Lindner TH, Mallmann P, Stepan H, Karumanchi SA, Benzing T. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. *Circulation* 2011; **124**: 940–950.
42. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. *Circulation* 2010; **122**: 478–487.
43. McElrath TF, Lim KH, Pare E, Rich-Edwards J, Pucci D, Troisi R, Parry S. Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. *Am J Obstet Gynecol* 2012; **207**: 407.e1–7.
44. Rizos D, Eleftheriades M, Karampas G, Rizou M, Haliassos A, Hassiakos D, Vitoratos N. Placental growth factor and soluble fms-like tyrosine kinase–1 are useful markers for the prediction of preeclampsia but not for small for gestational age neonates: a longitudinal study. *Eur J Obstet Gynecol Reprod Biol* 2013; **171**: 225–230.

