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Association between angiogenic factors and signs of arterial aging in women with pre-eclampsia

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KEYWORDS: cardiovascular disease; common carotid artery; fms-like tyrosine kinase-1; fms-like tyrosine kinase-1:placental growth factor ratio; high-frequency ultrasonography; intima:media ratio; placental growth factor; pre-eclampsia

ABSTRACT

Objective *Pre-eclampsia (PE) is associated with an increased risk of cardiovascular disease later in life. In cases with PE there is a substantial increase in levels of the antiangiogenic factor soluble fms-like tyrosine kinase-1 (sFlt-1) and decreased levels of the proangiogenic factor placental growth factor (PlGF). Elevated levels of sFlt-1 are also found in individuals with cardiovascular disease. The aims of this study were to assess levels of sFlt-1, PlGF and the sFlt-1/PlGF ratio and their correlation with signs of arterial aging by measuring the common carotid artery (CCA) intima and media thicknesses and their ratio (I/M ratio) in women with and without PE.*

Methods *Serum sFlt-1 and PlGF levels were measured using commercially available enzyme-linked immunosorbent assay kits, and CCA intima and media thicknesses were estimated using high-frequency (22-MHz) ultrasonography in 55 women at PE diagnosis and in 64 women with normal pregnancy at a similar gestational age, with reassessment at 1 year postpartum.*

Results *During pregnancy, higher levels of sFlt-1, lower levels of PlGF, a thicker intima, a thinner media and a higher I/M ratio of the CCA were found in women with PE* vs *controls (all* $P < 0.0001$*). Further, sFlt-1 and the sFlt-1/PlGF ratio were positively correlated with intima thickness and I/M ratio (all* $P < 0.0001$*). At 1 year postpartum, levels of sFlt-1 and the sFlt-1/PlGF ratio had decreased in both groups; however, their levels in the PE group were still higher than in the controls (*$P = 0.001$ *and* < 0.0001*, respectively). Levels of sFlt-1 and the sFlt-1/PlGF ratio remained positively correlated with intima thickness and I/M ratio at 1 year postpartum.*

Conclusions *Higher sFlt-1 levels and sFlt-1/PlGF ratio in women with PE were positively associated with signs of arterial aging during pregnancy. At 1 year postpartum, sFlt-1 levels and the sFlt-1/PlGF ratio were still higher in the PE group and were associated with the degree of arterial aging.* © *2016 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of the International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Pre-eclampsia (PE) is a pregnancy-related complication that affects 3–5% of all pregnancies[1] and is a leading cause of maternal and perinatal morbidity and mortality worldwide[2]. Normal pregnancy is a state of mild systemic inflammation[3,4], whereas PE is associated with exaggerated inflammation[5,6]. Soluble fms-like tyrosine kinase-1 (sFlt-1) is an antiangiogenic factor and placental growth factor (PlGF) is a proangiogenic factor produced by the placenta[7,8]. In cases of PE, serum concentrations of sFlt-1 are increased[9], whereas those of PlGF are decreased[10]. An imbalance between sFlt-1 and PlGF[11–13], together with exaggerated inflammation[6], play a major role in the development of endothelial dysfunction that leads to the development of PE.

Antiangiogenesis also contributes to the development of cardiovascular disease (CVD). In the last few years several studies have shown that sFlt-1 levels are higher in individuals with acute myocardial infarction than in those without[14–16]. Circulating sFlt-1 is an effective biomarker for predicting the progression of heart failure in subjects with CVD[14,15]. Endothelial dysfunction is the key factor

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in the pathogenesis of atherosclerosis and CVD[17], and meta-analyses have shown that PE is an independent risk factor for subsequent CVD[18,19].

According to histomorphometry[20] and intravascular high-frequency ultrasonography[14,21], aging and the development of atherosclerosis are associated with increased arterial intima thickness and decreased media thickness. However, these differential changes are not observed by means of conventional measurement of common carotid artery (CCA) intima–media thickness (IMT). Therefore, our group has used high-frequency ultrasonography to assess intima and media thicknesses separately, in order to calculate the intima to media (I/M) ratio. Using this method, we have shown that women with PE have more vascular damage (preclinical atherosclerosis) than those with normal pregnancy, at the time of PE diagnosis[22], 1 year postpartum[22] and about 10 years later[23]. In contrast, conventional CCA-IMT measurement is unable to reveal any cardiovascular risk at any of these time points[22,23].

The aims of this study were to investigate whether higher serum levels of sFlt-1 and an elevated sFlt-1/PlGF ratio in women with PE reflect the degree of preclinical atherosclerosis, as estimated by high-frequency ultrasonography, during pregnancy and at 1 year postpartum.

SUBJECTS AND METHODS

Women diagnosed with PE and women with normal pregnancy and pregnancy outcomes were recruited in 2007–2010. The method of recruitment of this population has been described extensively in our previous study[22]. The local ethics committee of the Medical Faculty of Uppsala University approved the study protocol and informed written consent was obtained from each woman included in the study.

PE was defined as new-onset hypertension (systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg observed on at least two occasions $\geq$ 6 h apart) combined with proteinuria ($\geq$ 2 on a dipstick test or a 24-h urine sample showing leakage of $\geq$ 300 mg albumin/24 h) after 20 weeks' gestation. PE was diagnosed as early onset if it occurred before 34 weeks' gestation and late onset if it occurred at or after 34 weeks' gestation. The condition was classified as severe when the increase in blood pressure was marked (SBP $\geq$ 160 mmHg and/or DBP $\geq$ 110 mmHg) and/or proteinuria was excessive ($\geq$ 5000 mg/24 h).

Among women in the normal pregnancy group, mean gestational age at inclusion was similar to that in the PE group. Normal pregnancy was defined as a normotensive pregnancy resulting in term delivery ($\geq$ 37 weeks) of an appropriate-weight infant (within $\pm$ 2 SD of the mean birth weight for gestational age)[24].

The women were examined first during pregnancy and thereafter at about 1 year after delivery (postpartum). At the postpartum examination, all but three of the women with PE had restarted menstruation and all but three had stopped breastfeeding. Among the women with normal pregnancy, all but two had restarted menstruation and all had stopped breastfeeding. The women who had not restarted menstruation were taking contraceptive medication and the women who were still breastfeeding did so partially and had restarted menstruation.

Assessment during pregnancy and postpartum

Based on routine early second-trimester ultrasonographic dating, gestational age was defined in terms of completed weeks. At inclusion, data on maternal age, reproductive history, smoking habits and height were collected. Maternal weight, enabling calculation of body mass index (BMI), and blood pressure were monitored at both prenatal and postpartum visits. Blood pressure was measured manually in women with PE and automatically in controls, in the upper right arm after about 15 min rest, with the woman in a supine position, using Umedico (Helsinborg, Sweden) blood pressure equipment (cuff size 12×35 cm or a size appropriate for the arm circumference). Mean arterial pressure (MAP), which is a better predictor of PE than are SBP and DBP, was calculated as $DBP + (SBP - DBP)/3$[25]. Data were collected from the delivery records with regard to possible pregnancy-related complications, gestational age at delivery, mode of delivery and birth weight of the infant. Small-for-gestational age (SGA) and large-for-gestational age were defined as infants with a birth weight > 2 SD below or above, respectively, the reference population's mean birth weight for gestational age[24].

A venous blood sample was collected from each woman at both examinations. The samples were kept at room temperature (22°C) for about 30 min before being centrifuged for 10 min at 2000 *g*. Serum samples were separated and stored at −70 °C until required for analysis of the levels of sFlt-1 and PlGF.

Enzyme-linked immunosorbent assays

Levels of sFlt-1 and PlGF were analyzed using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The ELISAs were performed without knowledge of the clinical diagnosis and the kits (R&D Systems, Minneapolis, MN, USA) contained microtiter plates on which specific monoclonal antibodies were coated. Standards and samples were pipetted into the wells and the peptide was bound to the immobilized antibodies. After washing, an enzyme-conjugated polyclonal antipeptide antibody was added to the wells. After incubation and washing, a substrate solution was added. Development was stopped and absorbance was measured using SpectraMax 250 equipment (Molecular Devices, Sunnyvale, CA, USA). The peptide concentrations in the samples were determined by comparing the optical density of the sample against the standard curve. The manufacturer determined the specificity of the assays, which do not exhibit any cross-reactivity with a panel of other recombinant human and mouse cytokines. The detection limit

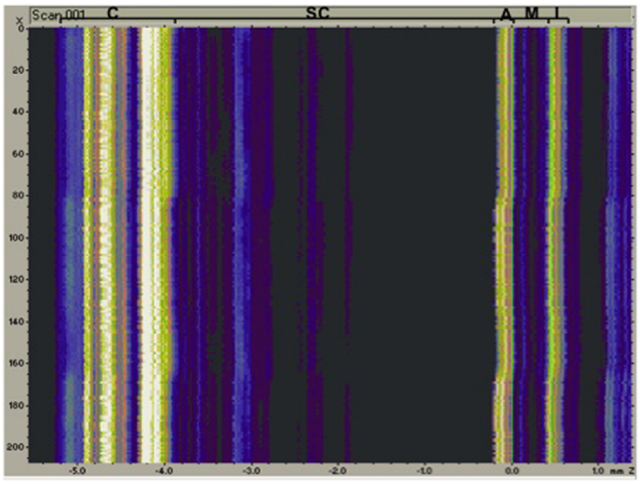

Figure 1 Layers of common carotid arterial wall, examined by non-invasive 22-MHz ultrasonography. A, adventitia; C, cutis; I, intima; M, media; SC, subcutis. Image reused with permission from the American Heart Association[22].

of the PlGF test was 10 pg/mL and PlGF levels below this limit were assigned as 10 pg/mL.

High-frequency ultrasonography of arterial wall

The left CCA wall layers were imaged (Figure 1) using high-resolution ultrasonographic equipment fitted with a broadband probe with 22-MHz center frequency (Collagenoson®, Minhorst Company, Meudt, Germany). The method has been described extensively elsewhere[26,27]. Point estimates of the arterial wall, not adjusted to the cardiac cycle, were obtained and about 20 point estimates were saved on a computer by one researcher (M.L.). Individual arterial wall layer dimensions were measured offline for all participants by another researcher (T.A.) who was blinded to the study group and time of assessment. The means of about 10 technically acceptable measurements were calculated and used in the analysis. In our laboratory, the coefficient of variation was 3.9% for intima thickness and 3.4% for media thickness[26].

Statistical analysis

Median and interquartile range were used to present the data. Differences in distributions were tested using the chi-square test. Between-group differences in continuous variables were tested by the Mann–Whitney *U*-test and within-group differences by Wilcoxon's signed-rank test. Spearman's rank correlation test was used to assess correlations between serum levels of sFlt-1 and PlGF, and the sFlt-1/PlGF ratio *vs* arterial wall layer dimensions and cardiovascular risk factors in the combined groups (PE and normal pregnancy), justified by substantial overlapping between groups with regard to sFlt-1 and PlGF levels, and sFlt-1/PlGF ratios and similar directions in the associations (Figure 2). Multivariate linear regression analysis was used to assess if the differences in angiogenic factors and arterial wall layer dimensions between the groups remained significant after adjustment for possible confounders. The level of significance was set at $P < 0.05$. Statistical analyses were performed using SPSS software for Windows (PASW statistics, version 20.0, IBM Corp., Armonk, NY, USA).

RESULTS

Fifty-five women with PE and 64 with normal pregnancy were recruited to the study. At the postpartum examination, five women in the PE group were pregnant again and two did not wish to participate. Among the women with normal pregnancy, four were pregnant again, one did not wish to participate and one had moved away from Sweden. Thus, 48 women in the PE group and 58 in the normal pregnancy group were included in the postpartum evaluation. Demographic data of the study population are shown in Table 1 and have been described in our previous publication[22]. Of the women with PE, 42% had early-onset PE, 69% had severe PE and 86% were on antihypertensive medication at the time of inclusion. Gestational age at delivery was on average 3 weeks earlier in the PE group than in the normal pregnancy group ($P < 0.001$). Infants born to mothers with PE had lower birth weights than those born to mothers with normal pregnancy, even after adjustment for gestational age.

In women with PE, BMI, SBP, DBP and MAP were all significantly higher than in women with normal pregnancy, at both inclusion and 1 year postpartum (Table 2), as described in our previous publication[22]. Of the women who started antihypertensive medication at PE diagnosis, most finished the treatment within a few days and all women were without antihypertensive medication within 6 weeks after delivery. None was receiving antihypertensive medication at the examination at 1 year postpartum.

At inclusion, women with PE had significantly higher levels of serum sFlt-1, a higher sFlt-1/PlGF ratio and significantly lower levels of serum PlGF than did women with normal pregnancy (all $P < 0.0001$) (Table 3). In 56% of women with PE and in 5% of normal pregnancies, serum PlGF levels were < 10 pg/mL, the detection limit of the ELISA. As described previously[22], women with PE had significantly thicker CCA intima ($P < 0.0001$) and thinner media ($P = 0.001$) dimensions and a higher I/M ratio ($P < 0.0001$) than did women with a normal pregnancy; however, there was no difference in the conventional IMT measurement between groups (Table 3).

We found a clear reduction in serum levels of sFlt-1 and sFlt-1/PlGF ratio values from pregnancy to the postpartum assessment, in both PE and normal pregnancies. There were still significant group differences in sFlt-1 levels and the sFlt-1/PlGF ratio ($P = 0.001$ and $P < 0.0001$, respectively) at analyses at 1 year postpartum (Table 3).

At the time of inclusion, there were strong positive correlations between both serum sFlt-1 and the sFlt-1/PlGF ratio and intima thickness ($r_s = 0.51$ and 0.63, respectively; both $P < 0.0001$) (Figure 2a) and the I/M ratio ($r_s = 0.50$ and 0.61, respectively; both

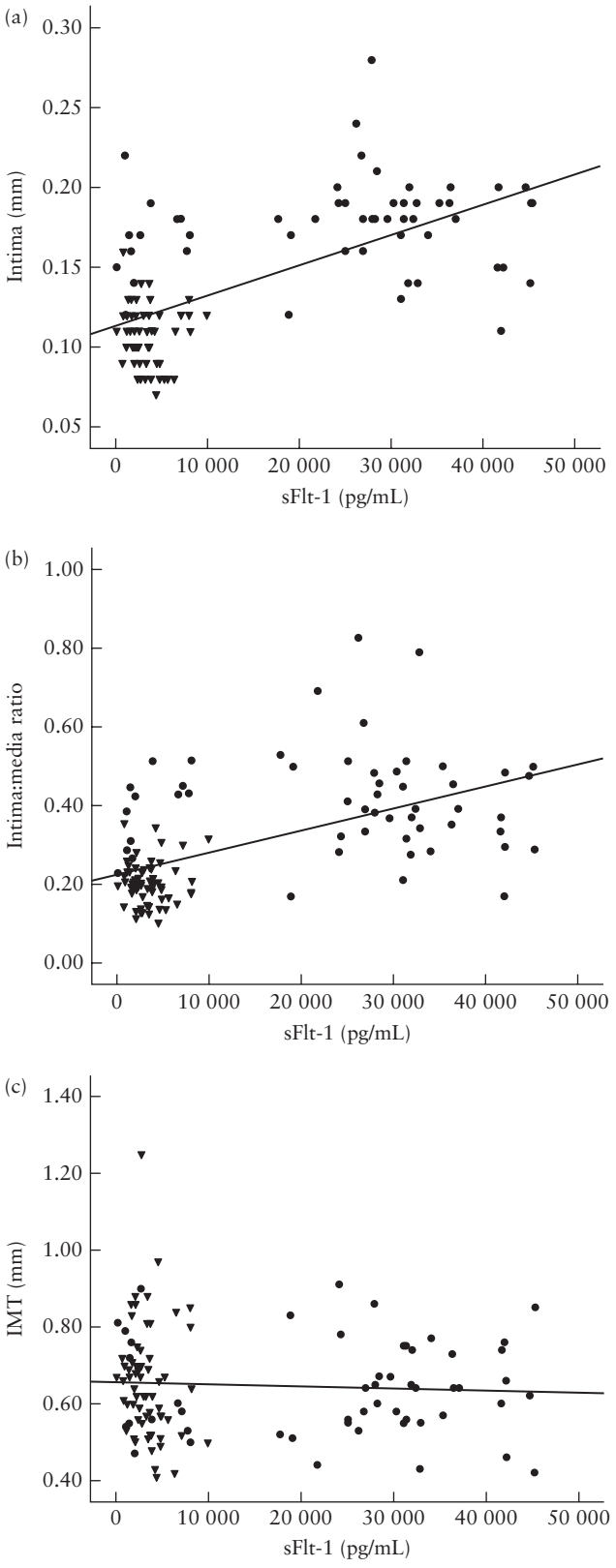

Figure 2 Correlation between soluble fms-like tyrosine kinase-1 (sFlt-1) and intima thickness (a), intima:media ratio (b) and intima–media thickness (IMT) (c) of common carotid artery in women with pre-eclampsia (●) and those with normal pregnancy (▼) at study inclusion. (a) $r_s = 0.51$, $P < 0.0001$; (b) $r_s = 0.50$, $P < 0.0001$; (c) $r_s = -0.11$, $P = 0.22$.

Table 1 Characteristics of 55 women with pre-eclampsia (PE) and 64 women with normal pregnancy

Characteristic	*PE*	*Normal*
Maternal age (years)	30 (26–34)	30 (28–33)
GA at examination (weeks)	35 (27–37)	36 (34–37)
Current smoker	0 (0)	2 (3)
Nulliparous	39 (71)*	32 (50)
Early-onset PE	23 (42)	—
Severe PE	38 (69)	—
Taking anti-hypertensive medication	47 (86)	—
GA at delivery (weeks)	37 (34–38)†	40 (39–41)
Birth weight (g)	2560 (1970–3160)‡	3645 (3363–4030)
Time of postpartum evaluation (months)	13 (11.5–13)	13 (11.5–13)

Data are given as median (interquartile range) or *n* (%). Comparison of groups: *$P < 0.05$; †$P < 0.001$; ‡$P < 0.001$, adjusted for gestational age (GA) at delivery. Data reused with permission from the American Heart Association[22].

$P < 0.0001$) (Figure 2b) for the combined group of PE and normal pregnancies. Similarly, we found inverse correlations between PlGF and intima thickness and I/M ratio ($r_s = -0.44$ and -0.47, respectively; both $P < 0.0001$). After adjusting for common confounding factors (BMI, blood pressure, smoking status and family history of CVD), angiogenic factors and arterial wall layer dimensions still differed significantly between PE and normal pregnancy (Table 3). At 1 year postpartum, there were still significant positive correlations between sFlt-1 and intima thickness ($r_s = 0.38$, $P = 0.007$) and between the sFlt-1/PlGF ratio and CCA intima thickness and I/M ratio ($r_s = 0.48$ and 0.41; $P < 0.0001$ and 0.003, respectively). Similarly, negative correlations were found between PlGF and CCA intima thickness and I/M ratio ($r_s = -0.21$ and -0.21; $P = 0.04$ and 0.03, respectively) (data not shown). When we analyzed findings of the PE and normal pregnancy groups separately, we found no correlation between levels of angiogenic factors and arterial wall layer dimensions. There were no correlations between levels of sFlt-1 and PlGF, and the sFlt-1/PlGF ratio *vs* CCA-IMT at inclusion (Figure 2c) or at 1 year postpartum.

For the combined groups at inclusion, we found that women with higher BMI, SBP, DBP and MAP often had higher sFlt-1 levels and sFlt-1/PlGF ratios and lower levels of PlGF. Similarly, we also found that these women with higher BMI, SBP, DBP and MAP often had thicker intima and thinner media dimensions and a higher I/M ratio of the CCA[22]. No correlations were found between maternal age *vs* angiogenic factors and arterial wall layer dimensions (Table 4). At 1 year postpartum, BMI and blood pressure had decreased in both groups compared with during pregnancy and no significant correlations were found between BMI and blood pressure *vs* angiogenic factors. However, there were still positive

Table 2 Measurements of modifiable cardiovascular risk factors in women with pre-eclampsia (PE) and with normal pregnancy at study inclusion and 1 year postpartum (PP)

	PE		*Normal*	
Characteristic	*At inclusion* (n = *55*)	*1 year PP* (n = *48*)	*At inclusion* (n = *64*)	*1 year PP* (n = *58*)
Body mass index (kg/m^2)	33 (27–35)*†	27 (23–32)‡	27 (25–30)†	23 (21–27)
Systolic blood pressure (mmHg)	145 (140–151)*†	120 (115–125)*	113 (110–120)§	110 (105–115)
Diastolic blood pressure (mmHg)	91 (83–100)*†	80 (71–80)*	70 (65–75)	70 (65–75)
Mean arterial pressure (mmHg)	110 (103–117)*†	93 (87–97)*	85 (80–88)	83 (78–87)

Data are given as median (interquartile range). Comparisons with corresponding assessment in normal pregnancy: *$P < 0.0001$; ‡$P < 0.001$. Comparisons with postpartum assessment in same group: †$P < 0.0001$; §$P = 0.001$. Data reused with permission from the American Heart Association[22].

Table 3 Serum levels of proangiogenic and antiangiogenic factors and dimensions of common carotid arterial wall layers in women with pre-eclampsia (PE) and women with normal pregnancy at study inclusion and 1 year postpartum (PP)

	PE		*Normal*	
Variable	*At inclusion* (n = *55*)	*1 year PP* (n = *48*)	*At inclusion* (n = *64*)	*1 year PP* (n = *58*)
sFlt-1 (pg/mL)	27 994 (12 876–33 463)*†‡	536 (367–674)¶	3011 (1962–4509)†	231 (190–427)
PlGF (pg/mL)	30 (25–48)*§	32 (19–99)	146 (70–235)†	28 (18–77)
sFlt-1/PlGF ratio	1909 (507–3139)*†‡	39 (27–55)*	24 (8–69)	10 (3–23)
Intima thickness (mm)	0.18 (0.16–0.19)*†‡	0.12 (0.11–0.13)*	0.11 (0.09–0.12)†	0.08 (0.07–0.09)
Media thickness (mm)	0.45 (0.37–0.54)¶	0.48 (0.40–0.57)**	0.54 (0.46–0.62)	0.53 (0.48–0.61)
Intima:media thickness ratio	0.39 (0.32–0.49)*†‡	0.26 (0.20–0.30)*	0.20 (0.16–0.24)†	0.15 (0.13–0.18)
Intima–media thickness (mm)	0.64 (0.55–0.75)	0.60 (0.51–0.71)	0.63 (0.55–0.72)	0.62 (0.54–0.70)

Data are given as median (interquartile range). Comparisons with corresponding assessment in normal pregnancy: *$P < 0.0001$; ¶$P = 0.001$; **$P < 0.05$. Comparisons with postpartum assessment in the same group: †$P < 0.0001$. Comparisons with corresponding assessment in normal pregnancy, after adjustment for body mass index, blood pressure, smoking status and family history of cardiovascular disease: ‡$P < 0.0001$; §$P = 0.001$. Data on arterial wall layers reused with permission from the American Heart Association[22]. PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

Table 4 Associations between cardiovascular risk factors *vs* angiogenic factors and dimensions of common carotid artery (CCA) wall layers at study inclusion in women with pre-eclampsia or normal pregnancy

Cardiovascular risk factors	*Angiogenic factors*	r_s	*CCA wall layer dimensions*	r_s
Body mass index (kg/m^2)	sFlt-1	0.30*	Intima	0.34†
	PlGF	−0.40*	Media	−0.24‡
	sFlt-1/PlGF	0.38*	Intima:media ratio	0.37*
Systolic blood pressure (mmHg)	sFlt-1	0.61†	Intima	0.70*
	PlGF	−0.66*	Media	−0.17
	sFlt-1/PlGF	0.71*	Intima:media ratio	0.60*
Diastolic blood pressure (mmHg)	sFlt-1	0.60*	Intima	0.65*
	PlGF	−0.66*	Media	−0.18
	sFlt-1/PlGF	0.70*	Intima:media ratio	0.55*
Mean arterial pressure (mmHg)	sFlt-1	0.62*	Intima	0.68*
	PlGF	−0.67*	Media	−0.17
	sFlt-1/PlGF	0.72*	Intima:media ratio	0.58*
Age (years)	sFlt-1	−0.05	Intima	0.04
	PlGF	−0.025	Media	0.03
	sFlt-1/PlGF	−0.009	Intima:media ratio	0.01

*$P < 0.0001$; †$P = 0.001$; ‡$P = 0.008$. PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

correlations between BMI and blood pressure *vs* arterial wall layer dimensions in postpartum analyses (data not shown for postpartum analyses).

At inclusion, among women with PE, we found no differences in serum levels of sFlt-1 and PlGF and the sFlt-1/PlGF ratio between early- and late-onset PE, women who were on antihypertensive medication *vs* those who were not, women with CVD heredity *vs* those without (except for PlGF, $P = 0.04$), women who delivered preterm *vs* at term or women who delivered an SGA *vs* appropriate-for-gestational age infant (data not shown).

DISCUSSION

In women with PE, we found substantially higher levels of serum sFlt-1, a higher sFlt-1/PlGF ratio and lower

levels of serum PlGF compared with those in women with normal pregnancy, at both inclusion and 1 year postpartum. We also found that serum levels of sFlt-1 and the sFlt-1/PlGF ratio were positively associated with CCA intima thickness and I/M ratio, and negatively associated with CCA media thickness, i.e. signs of arterial aging. Women with elevated BMI and blood pressure often had higher levels of antiangiogenic factors and negatively affected arterial wall layer dimensions.

sFlt-1 is a splice variant of vascular endothelial growth factor 1 (Flt-1) and is produced mainly by the placenta[7] and endothelial cells[28,29]. sFlt-1 is a strong inhibitor of angiogenic activity by binding to and inactivating the proangiogenic factor PlGF[30]. High serum levels of sFlt-1 and low levels of PlGF predict and correlate with the onset of clinical signs of PE[9,31]. We and others[9,10,31] have shown that PE is associated with higher circulating levels of sFlt-1 and lower levels of PlGF compared with women with normal pregnancy. In the present study we found that, after 1 year, women in the PE group still had higher levels of sFlt-1 compared with normal pregnancies. Two earlier studies have revealed persisting elevated sFlt-1 levels after delivery in women who had PE, but the investigators examined sFlt-1 levels at 2 days[32] and 1 week postpartum[33]. Our finding of elevated levels of sFlt-1 at 1 year postpartum could be explained by the presence of extraplacental production of sFlt-1[28,29] and persistent endothelial dysfunction in women with previous/recent PE[34–36].

Probably due to the small sample size and the risk of Type II error, we could not find any significant differences in sFlt-1 and PlGF levels between early- *vs* late-onset PE, preterm *vs* term delivery or SGA *vs* appropriate-for-gestational-age infant births. Previous studies have found pronounced alterations in sFlt-1 and PlGF levels in early-onset compared with late-onset PE[37,38], and in pregnancies with preterm delivery[39] and SGA infants[40].

PE is thought to be a 'stress test' with regard to future risk of CVD[41]. Recently, two large meta-analyses, by McDonald *et al.*[18] and Bellamy *et al.*[19], showed that women with PE have higher risks of coronary heart disease and stroke later in life. The serum concentration of sFlt-1 has been shown to be increased in individuals with acute myocardial infarction compared with those without, and it is a good predictor of development of heart failure in patients with coronary heart disease[14–16]. Further, in an earlier study, we showed that women with PE had thicker intima and thinner media dimensions and a higher I/M ratio compared with women with normal pregnancy, both during pregnancy and at 1 year postpartum[22]. Our current findings of highly significant and logical correlations of sFlt-1 and PlGF and the sFlt-1/PlGF ratio with signs of arterial aging are in line with the findings of McDonald *et al.*[18] and Bellamy *et al.*[19], and support our previous findings[22].

High blood pressure and obesity are two of the modifiable risk factors in regard to the development of CVD[8]. We found that, at inclusion, BMI and blood pressure were higher in women with PE than in those with normal pregnancy[22]. At postpartum analysis, BMI and blood pressure had decreased in both PE and normal pregnancy groups, but there was still a difference between the groups. These postpartum differences in BMI and blood pressure, together with persistent positive correlations between BMI and blood pressure *vs* arterial wall layer thicknesses indicate that the effects of PE on the cardiovascular system are longstanding. These findings are in line with our main findings of highly significant positive correlations between sFlt-1 levels and sFlt-1/PlGF ratio *vs* CCA intima thickness and I/M ratio, at both inclusion and 1 year postpartum.

Because of substantial overlap in levels of serum sFlt-1 (Figure 2) and PlGF (data not shown in figure) between women with PE and those with normal pregnancy, we tested the correlations between serum levels of sFlt-1 and PlGF and sFlt-1/PlGF ratio *vs* arterial wall layer dimensions in the groups combined. Previous studies have shown that normal pregnancy represents a state of mild systemic inflammation[3,4] and in an earlier study we showed that normal pregnancy is also associated with increased levels of sFlt-1 compared with those in non-pregnant women[37]. Further, our group has shown previously that women with normal pregnancy, who are older, with higher BMI and blood pressure, also have negatively affected arterial wall layer dimensions during pregnancy[22].

A strength of our study is that we obtained serum levels of sFlt-1 and PlGF and values of CCA wall layer measurements both during pregnancy and at about 1 year after delivery, which permitted analysis of postpartum changes. We have found repeatedly that the use of CCA intima thickness and I/M ratio is superior to that of IMT for imaging the effects of vascular aging and CVD[27] and long-term estrogen therapy[26]. In addition, our unpublished data indicate that the method correctly images the expected vascular benefits of menopausal hormone therapy[26], which was not possible when tested in very large randomized controlled trials using CCA-IMT[42,43]. A limitation of our study is the relatively small sample size with the associated potential risk of Type II error.

In conclusion, we have shown that levels of serum sFlt-1 and sFlt-1/PlGF ratio are associated with signs of arterial aging, as estimated by high-frequency ultrasonography, both during pregnancy and at 1 year postpartum. Further, we have shown that levels of sFlt-1 and the sFlt-1/PlGF ratio are associated with two of the modifiable cardiovascular risk factors during pregnancy. These parameters therefore seem to reflect the degree of vascular damage during pregnancy and also at 1 year postpartum. In addition, our data confirm previous findings of higher serum sFlt-1 and lower PlGF levels in women with PE compared with women with normal pregnancy and add new information concerning a persistent difference between the groups at 1 year postpartum. Further study is needed to investigate the long-term effects of PE-related antiangiogenic factors on arterial wall layers.

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