ORIGINAL ARTICLE

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

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Our aim was to evaluate the onset threshold of plasma levels of the soluble fms-like tyrosine kinase 1 (sFlt-1)/placental growth factor (PIGF) ratio for predicting the imminent onset of preeclampsia (PE) within 4 weeks after blood sampling. We prospectively measured the plasma levels of sFlt-1 and PIGF by an automated electrochemiluminescence immunoassay at 19–25 weeks of gestation in 1199 women and at 26–31 weeks of gestation in 798 women. The onset threshold of the sFlt-1/PIGF ratio was determined as the 2.5th percentile of the 95th confidence interval (CI) of a regression line between the onset gestational weeks of PE and the standard deviation score of $\log_{10}(\text{sFlt-1/PIGF})$, using 25 samples taken within 1 week after the onset of PE. The imminent onset of PE was identified in 2 (0.2%) women recruited at 19–25 weeks and in 6 (0.8%) women recruited at 26–31 weeks. The onset threshold of plasma levels of the sFlt-1/PIGF ratio at 19–25 weeks showed a sensitivity (SE) of 1.00, a specificity (SP) of 1.00, a positive likelihood ratio (LR+) of ∞ and a positive predictive value (PPV) of 1.00; the onset threshold of plasma levels of the sFlt-1/PIGF ratio at 26–31 weeks showed a SE of 0.83, a SP of 0.994, a LR+ of 132 (95% CI: 51–339) and a PPV of 0.50. In conclusion, the onset threshold of plasma levels of the sFlt-1/PIGF ratio was shown to be a highly sensitive and a highly specific screening method for detecting the imminent onset of PE within 4 weeks after blood sampling at 19–31 weeks.

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INTRODUCTION

Preeclampsia (PE) is a major complication of pregnancy associated with significant morbidity and mortality of both the fetus and the mother.^{1,2} Almost 3.5% of primiparous women and 2.0% of multiparous women in Japan develop PE.³ Recent years have seen considerable advances in the identification of biomarkers related to the development of PE, especially anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) and the circulating targets of these anti-angiogenic factors such as vascular endothelial growth factor, placental growth factor (PIGF) and transforming growth factor beta.^{4–9} Basic research using a pregnant rat/mouse model of PE using adenoviral vectors containing sFlt-1 and/or sEng has clearly substantiated the mechanism underlying the

role of anti-angiogenic factors in the pathophysiology of PE.^{10–13} Despite the accumulation of evidence of the important roles of anti-angiogenic factors in the genesis of PE, the translation of these findings into clinically useful paradigms has moved at a slow pace.⁸ Indeed, no single biomarker except for the onset threshold of the sFlt-1/PIGF ratio has fulfilled the criteria of clinically useful screening of PE.^{9,14–16}

In our previous report, we proposed the onset threshold of the sFlt-1/PIGF ratio using a manual enzyme immunoassay (ELISA) for serum levels of sFlt-1 and PIGF and showed that women with an sFlt-1/PIGF ratio higher than the onset threshold at 26–31 weeks of gestation have a high risk of imminent onset of PE, although the detection rate of PE at 19–25 weeks was very poor.⁹ In the current

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study, we hypothesized that screening using the automated electrochemiluminescence immunoassay (ECLIA) for the plasma levels of sFlt-1 and PIGF would demonstrate similar performance to that of screening using ELISA, because the correlation determinations between the values determined by ECLIA and ELISA for serum/plasma sFlt-1 and PIGF were shown to be sufficiently high (all >0.7 ; Ohkuchi *et al.*¹⁷) and because the sensitivity (SE) and specificity (SP) for diagnosing early-onset PE using the plasma levels of sFlt-1 and PIGF determined by ECLIA were shown to be 100%.¹⁷ In addition, we thought that a rapid and fully automated method of measuring the circulating levels of sFlt-1 and PIGF using ECLIA would contribute to the early detection of PE.

In the current study, our first aim was to evaluate the onset threshold of the sFlt-1/PIGF ratio as determined by ECLIA for predicting the imminent onset of PE within 4 weeks after blood sampling at 19–25 and 26–31 weeks. Our second aim was to evaluate the use of plasma levels of sFlt-1 and PIGF as determined by ECLIA for predicting all PE; PE with onset at <36 , <34 and <32 weeks of gestation; gestational hypertension (GH); and small-for-gestational-age (SGA) infants.

METHODS

Subjects

We obtained written informed consent from all the women included in this study as well as approval from the Ethics Committee of our institute. Almost all study subjects were Japanese women with singleton pregnancies, and the subjects were divided into two groups. The first group consisted of 1724 unselected pregnant women who were recruited into a prospective cohort study in our tertiary center between April 2004 and September 2008. One hundred and twenty-three women were excluded, because their outcomes of PE or infant data could not be obtained. In the remaining 1601 women, PE occurred in 48 women (3.0%), early-onset PE with onset at <32 weeks of gestation occurred in 16 women (1.0%), GH occurred in 24 women (1.5%) and an SGA infant occurred in 192 women (12.0%). One thousand one hundred ninety-nine blood samples were collected at 19–25 weeks of gestation, and 798 blood samples were collected at 26–31 weeks of gestation. The second group consisted of 33 preeclamptic women from whom blood samples were drawn as soon as possible after admission to our hospital due to the clinical manifestation of PE between April 2004 and April 2007. Blood samples from some of the subjects in the first group and all of the subjects in the second group had been previously used to establish a gestational age-specific reference range of plasma levels of sFlt-1, PIGF and the sFlt-1/PIGF ratio and to evaluate the screening and diagnostic performances of those indices for the clinical manifestation of early- or late-onset PE.^{17,18}

Blood pressure in pregnancy and puerperal period

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an Omron HEM-906 automated digital oscillometric sphygmomanometer (OMRON Healthcare, Kyoto, Japan) according to the standard procedures. The mean value of the two SBP and DBP readings at 16–19 and 20–23 weeks of gestation was documented for each participant.

Measurement of uterine artery indices

Color-pulsed Doppler ultrasound examination was performed using an SSD-2200 (Aloka, Tokyo, Japan), an SSD-3500 (Aloka) or an SSD-5000 (Aloka) using a 3.5-MHz transducer and a 50-Hz high-pass filter. Details of the measurement of the uterine artery Doppler are included in the previous report.¹⁹ In brief, at least three waveforms from each side were viewed on the monitor. The peak systolic (a), end-diastolic (b), early diastolic (c) and maximum diastolic (d) Doppler velocities were measured using a built-in caliper, whereas the mean (m) Doppler velocity was automatically measured using a built-in automatic tracing system in two consecutive waveforms in each uterine artery. The pulsatility indices (PI) was defined as $(a-b)/m$ and the notch depth index (NDI) was defined as $(d-c)/d$ in the presence of notching

or the NDI was equal to zero in the absence of notching. We defined notching as positive when a notch was observed in all the three consecutive waveforms. The average PI and NDI in two consecutive waveforms were calculated, and then the mPI and mNDI of both uterine arteries were calculated and used for analysis. When there were two recordings on different examination days, we used the earlier data. The inter-observer coefficient of variability for NDI was $6.9 \pm 3.9\%$.²⁰ The normal reference values at 16–23 weeks were determined in our previous study,¹⁹ and the cutoff values of mNDI and mPI for predicting PE were set at the 90th percentile in the distributions of mNDI and mPI at 16–23 weeks in normal pregnant women.¹⁹

Measurement of plasma levels of sFlt-1 and PIGF

Blood samples were collected in tubes containing EDTA, centrifuged at 4°C at $1170 \times g$ for 15 min, and stored at -20°C until use. The ECLIA of sFlt-1 and PIGF using Elecsys sFlt-1 (Roche Diagnostics, Penzberg, Germany) and Elecsys PIGF (Roche Diagnostics) were performed using Modular Analytics E 170 (Roche Diagnostics) according to the manufacturer's instructions. The minimal detectable doses in the assays for sFlt-1 and PIGF are 15 pg ml^{-1} and 10 pg ml^{-1} , respectively. The intra-assay and inter-assay coefficients of variation for sFlt-1 are 0.5–6.8% and 0.7–11%, respectively, and the intra-assay and inter-assay coefficients of variation for PIGF are 0.6–2.6% and 0.6–5.9%, respectively (manufacturer's information). The plasma recovery rates of sFlt-1 and PIGF were 0.89 and 0.85, respectively.¹⁷

Definition of cutoff points of PIGF, sFlt-1 and the sFlt-1/PIGF ratio

Although we had determined the normal reference ranges of plasma levels of sFlt-1, PIGF and the sFlt-1/PIGF ratio during 20–38 weeks of gestation in our previous study,¹⁷ in the current study we decided to establish the 95th percentile of the plasma level of sFlt-1 (pg ml^{-1}) and the 5th percentile of the plasma level of PIGF (pg ml^{-1}) at 19–25 and 26–31 weeks of gestation. We chose these percentiles, because we thought that simple cutoff values during 19–31 weeks, but not time-dependent cutoff values determined by quadric curves in our previous study,¹⁷ might facilitate the introduction of our method into clinical practice and because the number of normal pregnant women in the current study was almost 10-fold greater than that in the previous study.¹⁷ The data of the logarithmic translations of sFlt-1 and PIGF at 19–25 weeks were almost normally distributed; therefore, we calculated the means and s.d.s. of $\log_{10}\text{sFlt-1}$ and $\log_{10}\text{PIGF}$ in 1155 normal pregnant women without GH or PE. Because the mean and s.d. of $\log_{10}\text{sFlt-1}$ at 19–25 weeks were 3.035 and 0.224, respectively, the 95th percentile of $\log_{10}\text{sFlt-1}$ was calculated as 3.404 using the equation $\text{mean} + 1.645 \times \text{s.d.}$. Then, an abnormal sFlt-1 ratio at 19–25 weeks was defined as ≥ 2540 . Because the mean and s.d. of $\log_{10}\text{PIGF}$ at 19–25 weeks were 2.480 and 0.227, respectively, the 5th percentile of $\log_{10}\text{PIGF}$ was calculated as 2.106 using the equation $\text{mean} - 1.645 \times \text{s.d.}$. Then, an abnormal PIGF at 19–25 weeks was defined as <128 . Similarly, the data of the logarithmic translation of sFlt-1 and PIGF at 26–31 weeks were almost normally distributed; therefore, we calculated the means and s.d.s of $\log_{10}\text{sFlt-1}$ and $\log_{10}\text{PIGF}$ in 769 normal pregnant women without GH or PE. Because the mean and s.d. of $\log_{10}\text{sFlt-1}$ at 26–31 weeks were 3.045 and 0.221, respectively, the 95th percentile of $\log_{10}\text{sFlt-1}$ was calculated as 3.408; thus an abnormal sFlt-1 at 26–31 weeks was defined as ≥ 2560 . Because the mean and s.d. of $\log_{10}\text{PIGF}$ at 26–31 weeks were 2.706 and 0.277, respectively, the 5th percentile of $\log_{10}\text{PIGF}$ was calculated as 2.251; thus an abnormal PIGF at 26–31 weeks was defined as <177 .

Likewise, an abnormal sFlt-1/PIGF ratio at 19–25 weeks was defined as ≥ 10.5 and that at 26–31 weeks was defined as ≥ 8.5 .

Establishment of gestational age-specific onset thresholds of the plasma levels of the sFlt-1/PIGF ratio

In our previous study, we reported that the standard deviation score (SDS) of $\log_{10}(\text{sFlt-1/PIGF})$ determined by ELISA (R&D Systems, Minneapolis, MN, USA) showed the highest correlation coefficient of -0.869 to the onset gestational weeks of PE, and thus we created the onset threshold of the sFlt-1/PIGF ratio for predicting PE with onset at <36 weeks.⁹ Because we had already determined the levels of sFlt-1 and PIGF and the sFlt-1/PIGF ratio in referred women with PE who had blood samples collected as soon as possible after

admission to our hospital due to the clinical manifestation of PE between April 2004 and April 2007,¹⁷ we also established a gestational age-specific normal reference range curve for the plasma level of the sFlt-1/PlGF ratio in normal pregnant women at 19–38 weeks of gestation.¹⁷ We analyzed the correlation coefficients between the onset gestational weeks of PE and the logarithmic-transformed raw values/SDSs of sFlt-1 and PlGF and the sFlt-1/PlGF ratio using 25 samples, which were taken within 1 week after the onset of PE. The SDS of $\log_{10}(\text{sFlt-1/PlGF})$ showed the highest correlation (-0.930), followed by the SDS of $\log_{10}\text{PlGF}$ and the raw value of $\log_{10}\text{PlGF}$ (0.922 and 0.751 , respectively), whereas the SDS of $\log_{10}\text{sFlt-1}$, the raw values of $\log_{10}(\text{sFlt-1/PlGF})$ and $\log_{10}\text{sFlt-1}$ were all <0.7 (-0.539 , -0.196 and -0.680 , respectively). Therefore, we judged that the onset threshold of the SDS of $\log_{10}(\text{sFlt-1/PlGF})$ was the most predictive, using the biomarkers determined by ECLIA. We constructed a regression line between the onset gestational weeks of PE and the SDS of $\log_{10}(\text{sFlt-1/PlGF})$ soon after onset and the lines for ± 2.0 s.d., that is, the 2.5th and 97.5th percentile confidence lines (Figure 1a). We considered the 2.5th percentile line of $\log_{10}(\text{sFlt-1/PlGF})$ as the onset threshold of PE, according to our previous method.⁹ The equation for the line representing the onset threshold of the sFlt-1/PlGF ratio was as follows:

$$\begin{aligned}\text{Onset threshold of } \log_{10}(\text{sFlt-1/PlGF}) \text{ (SDS)} &= \\ &= -0.478 \times w + 17.787 \text{ (where } w = \text{gestational weeks of the onset of PE)}.\end{aligned}$$

Next, we transformed the equation using the gestational age-specific normal reference ranges of $\log_{10}(\text{sFlt-1/PlGF})$ and the definition of SDS.¹⁷ Accordingly,

the SDS was transformed to raw values:

$$\begin{aligned}\text{Mean of the normal reference curve of } \log_{10}\text{sFlt-1/PlGF} [m] &= \\ &= 0.00668 \times w^2 - 0.363 \times w + 5.255 \text{ (where } w = \text{gestational weeks at blood sampling)}.\end{aligned}$$

$$\begin{aligned}\text{S.d. of the normal reference curve of } \log_{10}\text{sFlt-1/PlGF} [\text{SD}] &= \\ &= 0.0116 \times w - 0.00642.\end{aligned}$$

$$\begin{aligned}\text{Raw value of onset threshold of } \log_{10}(\text{sFlt-1/PlGF}) &= \\ &= \text{SDS} \times \text{SD} + m \\ &= (-0.487 \times w + 17.787) \times (0.0116 \times w - 0.00642) + 0.00668 \times w^2 \\ &\quad - 0.363 \times w + 5.255 \\ &= 0.00103 \times w^2 - 0.154 \times w + 5.141.\end{aligned}$$

Hence, the onset threshold of $\log_{10}(\text{sFlt-1/PlGF})$ was converted from a linear function to a quadratic curve (Figure 1b).

Establishment of gestational age-specific onset thresholds of the plasma levels of PlGF

The details of the methods are included in the Supplementary Information.

Definitions of PE, GH and an SGA Infant

We defined PE and GH according to the definition and classification of pregnancy-induced hypertension (2004) of the Japan Society for the Study of Hypertension in Pregnancy.²¹ In brief, PE was defined as hypertension with

Figure 1 Prediction of the imminent onset of preeclampsia (PE) using the onset threshold of the soluble fms-like tyrosine kinase 1/placental growth factor (sFlt-1/PlGF) ratio. (a) We constructed regression lines between the onset gestational weeks of PE and the standard deviation scores (SDSs) of $\log_{10}(\text{sFlt-1/PlGF})$ soon after onset and their 2.5th and 97.5th percentile confidence lines. The regression lines are represented by dotted lines, and the 2.5th and 97.5th percentile confidence lines are represented by straight lines. Thick straight lines represent the onset threshold. Open circles represent the SDSs at sampling <7 days after the onset of PE ($n=25$), whereas closed circles represent the SDSs at sampling ≥ 7 days after the onset of PE ($n=8$). (b) The thin straight curves represent the 5th and 95th percentiles of the normal reference range of the sFlt-1/PlGF ratio, and the dotted curve represents the mean of the normal reference range of the sFlt-1/PlGF ratio. We drew the onset threshold of the plasma levels of the sFlt-1/PlGF ratio (dashed and dotted curve), which was the 2.5th percentile confidence line of the regression line between the onset gestational weeks of PE and the SDSs of $\log_{10}(\text{sFlt-1/PlGF})$ soon after onset. If the plasma levels of the sFlt-1/PlGF ratio were equal to or greater than the onset threshold of the sFlt-1/PlGF ratio, the sFlt-1/PlGF ratio was abnormal. The raw values of the sFlt-1/PlGF ratio after the onset of PE are shown. Large closed circles represent the values at sampling in preeclamptic women with onset at <32 weeks of gestation, medium closed circles represent those in preeclamptic women with onset between 32 and 35 weeks of gestation and small closed circles represent those in preeclamptic women with onset at ≥ 36 weeks of gestation. (c, d) Thick straight lines represent the 5th and 95th percentiles of the distribution of $\log_{10}(\text{sFlt-1/PlGF})$ in 1155 normal pregnant women at 19–25 weeks of gestation and 769 normal pregnant women at 26–31 weeks of gestation in a prospective cohort study. The dashed and dotted curve represents the onset threshold of the plasma levels of the sFlt-1/PlGF ratio. Large, medium and small circles (c) represent the values at sampling in preeclamptic women with onset at <32 , 32–35 and ≥ 36 weeks of gestation, respectively; closed circles (c) represent cases of the imminent onset of PE within 4 weeks after blood sampling; and very small open circles (d) represent the values in normal pregnant women, whereas small open squares (d) represent the values in women with gestational hypertension.

proteinuria occurring after 20 weeks of gestation. Superimposed PE was defined as chronic hypertension diagnosed before pregnancy or before 20 weeks of gestation, with proteinuria emerging afterward. Superimposed PE was included in the category of PE in this study. GH was defined as hypertension without proteinuria occurring after 20 weeks of gestation. Hypertension was defined as SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg on two occasions ≥ 4 h apart. Proteinuria was defined as 300 mg day^{-1} from a 24-h urine collection. If only test tape was available, repeated semi-quantitative test results of 1+, which represented 30 mg dl^{-1} protein or more, were considered to constitute a positive result. An SGA infant was defined as having a birth weight below the 10th percentile for the Japanese population.²²

Statistics

We calculated the SDS as $(A - B)/C$ (where A is the raw value, B is the mean and C is the s.d.). The results are presented as the mean $\pm$ s.d. For plasma sFlt-1, PIGF and the sFlt-1/PIGF ratio, statistical testing was conducted after logarithmic transformation. An unpaired t -test was used to compare two continuous variables. The chi-square test or Fisher's exact test were used to compare categorical data. The fitted curve/line estimation was performed using software included in the IBM SPSS software package (version 21; IBM, Armonk, NY, USA). The positive likelihood ratio (LR $+$) and the 95% confidence interval were calculated using the Confidence Interval Calculator (created by Rob Herbert at the University of Sydney).²³ A LR $+$ ≥ 10 ,^{24,25} a SE ≥ 0.8 and a positive predictive value (PPV) ≥ 0.20 were considered clinically useful for screening. $P < 0.05$ was considered statistically significant.

RESULTS

Maternal and neonatal characteristics

In women with PE in our cohorts, the frequency of women with a past history of PE/GH, a family history of hypertension, obesity, chronic hypertension, SBP at 16–23 weeks ≥ 120 mm Hg, DBP at 16–23 weeks ≥ 80 mm Hg, mPI at 16–23 weeks ≥ 90 th percentile or mNDI at 16–23 weeks ≥ 90 th percentile was significantly higher than in controls, and the pre-pregnancy body mass index, SBP and DBP at 16–23 weeks were also significantly higher than in controls (Table 1). Women with PE delivered earlier and had smaller infants than controls.

Risk factors for predicting the imminent onset of PE within 4 weeks after blood sampling

The imminent onset of PE occurred within 4 weeks after blood sampling in 2 of 1199 (0.2%) women recruited at 19–25 weeks and in 6 of 798 (0.8%) women recruited at 26–31 weeks. When we evaluated the screening performance for predicting the imminent onset of PE at 19–25 weeks using a single marker alone, the LR $+$ s of a past history of PE/GH, a family history of hypertension, obesity, SBP, DBP, mPI and mNDI were all < 10 ; the LR $+$ s of the plasma levels of PIGF and sFlt-1 and of the sFlt-1/PIGF ratio were all ≥ 10 . The corresponding SEs were all 1.00, but the PPVs were very low (Table 2). When we evaluated the screening performance for predicting the imminent onset of PE at 26–31 weeks using a single marker alone, the LR $+$ s of the plasma levels of PIGF and sFlt-1 and of the sFlt-1/PIGF ratio were all ≥ 10 ; the SEs were all ≥ 0.80 , but the PPVs were < 0.20 (Table 3).

Onset threshold of sFlt-1/PIGF ratio for predicting the imminent onset of PE within 4 weeks after blood sampling

We evaluated the screening performance for predicting the imminent onset of PE within 4 weeks after blood sampling at 19–25 and 26–31 weeks using the onset threshold of plasma levels of the sFlt-1/PIGF ratio.

The onset threshold of plasma levels of the sFlt-1/PIGF ratio at 19–25 weeks showed an SE of 1.00, a LR $+$ of ∞ and a PPV of 1.00 (Table 2). Thus, the onset threshold of plasma levels of the sFlt-1/PIGF ratio at 19–25 weeks as determined by ECLIA was clinically

Table 1 Maternal and neonatal characteristics in normal pregnant women and preeclamptic women

			Number of missing values (normal/ PE)		P-value
Characteristics	Normal pregnant women ^a (n = 1529)	Women with PE (n = 48)			
Maternal					
Age (mean ± s.d., years)	32.7 ± 5.0	33.7 ± 5.5	0		0.176
Age ≥40 years (%)	104 (6.8)	4 (8.3)	0		0.566
Race: Japanese (%)	1514 (99)	48 (100)	0		1.000
Nulliparous women (%)	714 (47)	28 (58)	0		0.141
Women with past history of PE/GH (%)	57 (3.7)	9 (19)	2/1		<0.001
Women with family history of hypertension (%)	382 (25)	22 (47)	25/1		0.002
Current smoker (%)	80 (5.6)	4 (8.7)	99/2		0.328
Pre-pregnancy BMI (mean ± s.d., kg m ⁻²) ^b	22.2 ± 4.1	24.8 ± 5.7	5/0		0.003
Obesity (%)	276 (18)	20 (42)	5/0		<0.001
Chronic hypertension (%)	22 (1.4)	13 (27)	0		<0.001
SBP at 16–23 wk (mean ± s.d., mm Hg)	116 ± 14	134 ± 18	5/1		<0.001
DBP at 16–23 wk (mean ± s.d., mm Hg)	67 ± 9	81 ± 12	5/1		<0.001
SBP at 16–23 wk ≥ 120 mm Hg (%)	538 (35)	38 (81)	5/1		<0.001
DBP at 16–23 wk ≥ 80 mm Hg (%)	143 (9.4)	23 (49)	5/1		<0.001
mPI at 16–23 wk ≥ 90th percentile (%) ^b	185 (13)	21 (45)	63/1		<0.001
mNDI at 16–23 wk ≥ 90th percentile (%) ^b	162 (11)	19 (40)	63/1		<0.001
Neonatal					
Gestational age at delivery (mean ± s.d., wk)	38.8 ± 2.1	35.8 ± 4.5	0		<0.001
Preterm delivery (%)	151 (9.9)	22 (46)	0		<0.001
Birthweight (mean ± s.d., g)	2929 ± 486	2257 ± 895	0		<0.001
Small-for-gestational-age infant (%) ^c	168 (11)	18 (38)	0		<0.001

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GH, gestational hypertension; mNDI, mean notch depth index; mPI, mean pulsatility index; PE, preeclampsia; SBP, systolic blood pressure; wk, weeks of gestation.

^aNormal pregnant women were defined as women not complicated with PE or GH.

^bThe cutoff values of 90th percentiles of mPI and mNDI were selected according to the respective normal reference value in our previous paper.

^cA small-for-gestational-age infant was defined as an infant having a birth weight below the 10th percentile for the Japanese population.

useful for predicting the imminent onset of PE at 19–25 weeks. Figure 1c shows that the sFlt-1/PIGF ratio in two women with an imminent onset of PE markedly deviated from the normal reference range of the sFlt-1/PIGF ratio at 19–25 weeks, and Figure 1d shows that all of the sFlt-1/PIGF ratios at 19–25 weeks in normal pregnant women or those with GH were located much lower than the onset threshold line of the sFlt-1/PIGF ratio.

The onset threshold of plasma levels of the sFlt-1/PIGF ratio at 26–31 weeks fulfilled three criteria of clinically useful screening: a LR $+$ ≥ 10 , an SE ≥ 0.80 , and a PPV ≥ 0.20 (Table 3). Figure 1c shows that the sFlt-1/PIGF level in 83% of women with an imminent onset of PE markedly deviated from the normal reference range of the

Table 2 Screening performances for predicting imminent onset of PE within 4 wk after blood sampling, by single marker alone, and the onset thresholds of sFlt-1/PlGF ratio and PlGF, in 1199 women at 19–25 wk

	Abnormal					SE	SP	LR+ (95% CI)	LR− (95% CI)	PPV	NPV
Risk factors	value	TP	FN	FP	TN						
Single marker alone											
Past history of PE/GH	(+)	0	2	54	1142	0.00	0.95	0	1.05 (1.03–1.06)	0.000	0.998
Family history of hypertension	(+)	1	1	292	890	0.50	0.75	2.0 (0.5–8.1)	0.66 (0.17–2.7)	0.003	0.999
Obesity	(+)	1	1	225	968	0.50	0.81	2.7 (0.7–11)	0.62 (0.15–2.5)	0.004	0.999
SBP at 16–23 wk	≥120	2	0	430	761	1.00	0.64	2.8 (2.6–3.0)	0	0.005	1.000
DBP at 16–23 wk	≥80	1	1	128	1063	0.50	0.89	4.7 (1.2–19)	0.56 (0.14–2.2)	0.008	0.999
mPI at 16–23 wk	≥90th	2	0	163	993	1.00	0.86	6.7 (5.8–7.7)	0	0.012	1.000
mNDI at 16–23 wk	≥90th	1	1	143	1013	0.50	0.88	4.0 (1.00–16)	0.57 (0.14–2.3)	0.007	0.999
Plasma level of PlGF at 19–25 wk	<5.0th	2	0	67	1130	1.00	0.94	18 (14–23)	0	0.029	1.000
Plasma level of sFlt-1 at 19–25 wk	≥95th	2	0	66	1131	1.00	0.95	18 (14–23)	0	0.029	1.000
Plasma level of sFlt-1/PlGF ratio at 19–25 wk	≥95th	2	0	70	1127	1.00	0.94	17 (14–21)	0	0.028	1.000
Onset threshold of angiogenesis-related factor											
Onset threshold of sFlt-1/PlGF ratio		2	0	0	1197	1.00	1.000	∞	0	1.000	1.000
Onset threshold of PlGF		2	0	1	1196	1.00	0.999	1197 (169–8491)	0	0.667	1.000

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; FN, false negative; FP, false positive; GH, gestational hypertension; LR –, negative likelihood ratio; LR +, positive likelihood ratio; mNDI, mean notch depth index; mPI, mean pulsatility index; NPV, negative predictive value; PE, preeclampsia; PlGF, placental growth factor; PPV, positive predictive value; SBP, systolic blood pressure; sFlt-1, soluble fms-like tyrosine kinase 1; SE, sensitivity; SP, specificity; TN, true negative; TP, true positive; wk, weeks of gestation.

Bold font of SE indicates that SE was ≥0.8.

Bold font of LR+ indicates that LR+ was ≥10.0.

Bold font of PPV indicates that PPV was ≥0.20.

Table 3 Screening performances for imminent onset of PE within 4 wk after blood sampling, by single marker alone, and the onset thresholds of sFlt-1/PlGF ratio and PlGF, in 798 women at 26–31 wk

	Abnormal										
Risk factors	value	TP	FN	FP	TN	SE	SP	LR+ (95% CI)	LR– (95% CI)	PPV	NPV
Single marker alone											
Women with past history of PE/GH	(+)	3	3	31	761	0.50	0.96	13 (5.3–31)	0.52 (0.23–1.2)	0.088	0.996
Family history of hypertension	(+)	3	3	188	591	0.50	0.76	2.1 (0.9–4.7)	0.66 (0.30–1.5)	0.016	0.995
Obesity	(+)	3	3	160	630	0.50	0.80	2.5 (1.1–5.6)	0.63 (0.28–1.4)	0.018	0.995
SBP at 16–23 wk	≥120	4	2	284	504	0.67	0.64	1.9 (1.04–3.3)	0.52 (0.17–1.6)	0.014	0.996
DBP at 16–23 wk	≥80	2	4	80	708	0.33	0.90	3.3 (1.04–10)	0.74 (0.42–1.3)	0.024	0.994
mPI at 16–23 wk	≥90th	4	2	103	655	0.67	0.86	4.9 (2.7–8.9)	0.39 (0.12–1.2)	0.037	0.997
mNDI at 16–23 wk	≥90th	5	1	81	677	0.83	0.89	7.8 (5.2–12)	0.19 (0.03–1.1)	0.058	0.999
Plasma level of PlGF at 26–31 wk	<5th	5	1	48	744	0.83	0.94	14 (8.8–22)	0.18 (0.03–1.06)	0.094	0.999
Plasma level of sFlt-1 at 26–31 wk	≥95th	5	1	39	753	0.83	0.95	17 (11–27)	0.18 (0.03–1.05)	0.114	0.999
Plasma level of sFlt-1/PlGF ratio at 26–31 wk	≥95th	5	1	45	747	0.83	0.94	15 (9.3–23)	0.18 (0.03–1.06)	0.100	0.999
Onset threshold of angiogenesis-related factor											
Onset threshold of sFlt-1/PlGF ratio		5	1	5	787	0.83	0.994	132 (51–339)	0.17 (0.03–1.00)	0.500	0.999
Onset thershold of PlGF		5	1	28	764	0.83	0.965	24 (14–39)	0.17 (0.03–1.03)	0.152	0.999

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; FN, false negative; FP, false positive; GH, gestational hypertension; LR –, negative likelihood ratio; LR +, positive likelihood ratio; mNDI, mean notch depth index; mPI, mean pulsatility index; NPV, negative predictive value; PE, preeclampsia; PlGF, placental growth factor; PPV, positive predictive value; SBP, systolic blood pressure; sFlt-1, soluble fms-like tyrosine kinase 1; SE, sensitivity; SP, specificity; TN, true negative; TP, true positive; wk, weeks of gestation.

Bold font of SE indicates that SE was ≥0.8.

Bold font of LR+ indicates that LR+ was ≥10.0.

Bold font of PPV indicates that PPV was ≥0.20.

sFlt-1/PlGF ratio at 26–31 weeks, and Figure 1d shows that only 0.6% of women without an imminent onset of PE (four normal pregnant women and one woman with GH with onset 4 weeks after sampling) had an abnormal sFlt-1/PlGF ratio.

Onset threshold of PlGF for predicting the imminent onset of PE within 4 weeks after blood sampling

The details of the results are included in the Supplementary Information.

Comparison of screening properties of angiogenesis-related factors at 19–25 and 26–31 weeks for predicting all PE; PE with onset at <36, <34 and <32 weeks; GH; and an SGA infant

To predict all PE and PE with onset at <36, <34 and <32 weeks using the plasma levels of PlGF of <5th percentile at 19–25 weeks, PE with onset at <36, <34 and <32 weeks showed a LR+ of ≥10, PE with onset at <32 weeks showed an SE of ≥0.80 and no outcomes showed PPV of ≥0.20 (Table 4). As for the plasma levels of sFlt-1 of

Table 4 Screening performances for predicting imminent onset of PE within 4 wk after blood sampling in all PE; PE with onset at <36, <34 and <32 wk; GH; and SGA in 1199 women at 19–25 wk

Risk factors outcomes	Incidence rates	TP	FN	FP	TN	SE	SP	LR+ (95% CI)	LR- (95% CI)	PPV	NPV
<i>PIGF < 5th</i>											
all PE	0.028	13	21	56	1109	0.38	0.95	8.0 (4.8–13)	0.65 (0.50–0.85)	0.188	0.981
PE with onset at <36 wk	0.015	10	8	59	1122	0.56	0.95	11 (6.9–18)	0.47 (0.28–0.78)	0.145	0.993
PE with onset at <34 wk	0.012	10	4	59	1126	0.71	0.95	14 (9.5–22)	0.30 (0.13–0.69)	0.145	0.996
PE with onset at <32 wk	0.009	9	2	60	1128	0.82	0.95	16 (11–24)	0.19 (0.06–0.67)	0.130	0.998
GH	0.017	3	17	66	1113	0.15	0.94	2.7 (0.92–7.8)	0.90 (0.75–1.08)	0.043	0.985
SGA	0.116	18	121	51	1009	0.13	0.95	2.7 (1.6–4.5)	0.92 (0.86–0.98)	0.261	0.893
<i>sFlt-1 ≥ 95th</i>											
all PE	0.028	5	29	63	1102	0.15	0.95	2.7 (1.2–6.3)	0.90 (0.78–1.04)	0.074	0.974
PE with onset at <36 wk	0.015	4	14	64	1117	0.22	0.95	4.1 (1.7–10)	0.82 (0.64–1.05)	0.059	0.988
PE with onset at <34 wk	0.012	3	11	65	1120	0.21	0.95	3.9 (1.4–11)	0.83 (0.63–1.09)	0.044	0.990
PE with onset at <32 wk	0.009	3	8	65	1123	0.27	0.95	5.0 (1.8–13)	0.77 (0.54–1.11)	0.044	0.993
GH	0.017	2	18	66	1113	0.10	0.94	1.8 (0.47–6.8)	0.95 (0.82–1.10)	0.029	0.984
SGA	0.116	7	132	61	999	0.05	0.94	0.88 (0.41–1.9)	1.01 (0.97–1.05)	0.103	0.883
<i>sFlt-1/PIGF ratio ≥ 95th</i>											
all PE	0.028	13	21	59	1106	0.38	0.95	7.6 (4.6–12)	0.65 (0.50–0.85)	0.181	0.981
PE with onset at <36 wk	0.015	11	7	61	1120	0.61	0.95	12 (7.6–18)	0.41 (0.23–0.73)	0.153	0.994
PE with onset at <34 wk	0.012	10	4	62	1123	0.71	0.95	14 (9.1–21)	0.30 (0.13–0.69)	0.139	0.996
PE with onset at <32 wk	0.009	9	2	63	1125	0.82	0.95	15 (11–22)	0.19 (0.06–0.67)	0.125	0.998
GH	0.017	2	18	70	1109	0.10	0.94	1.7 (0.44–6.4)	0.96 (0.83–1.11)	0.028	0.984
SGA	0.116	11	128	61	999	0.08	0.94	1.4 (0.74–2.5)	0.98 (0.93–0.95)	0.153	0.886

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; FN, false negative; FP, false positive; GH, gestational hypertension; LR-, negative likelihood ratio; LR+, positive likelihood ratio; mNDI, mean notch depth index; mPI, mean pulsatility index; NPV, negative predictive value; PE, preeclampsia; PIGF, placental growth factor; PPV, positive predictive value; SBP, systolic blood pressure; SE, sensitivity; sFlt-1, soluble fms-like tyrosine kinase 1; SGA, small-for-gestational age; SP, specificity; TN, true negative; TP, true positive; wk, weeks of gestation.

Bold font of SE indicates that SE was ≥0.8.

Bold font of LR+ indicates that LR+ was ≥10.0.

Bold font of PPV indicates that PPV was ≥0.20.

≥95th percentile at 19–25 weeks, no outcomes fulfilled the criteria for clinically useful screening performance. As for the plasma levels of sFlt-1/PIGF ratio of ≥95th percentile at 19–25 weeks, PE with onset at <36, <34 and <32 weeks showed a LR+ of ≥10, PE with onset at <32 weeks showed an SE of ≥0.80 and no outcomes showed a PPV of ≥0.20.

To predict all PE and PE with onset at <36, <34 and <32 weeks using the plasma levels of PIGF of <5th percentile at 26–31 weeks, PE with onset at <34 and <32 weeks showed a LR+ of ≥10 and an SE of ≥0.80 but no outcomes showed a PPV of ≥0.20 (Table 5). As for the plasma levels of sFlt-1 of ≥95th percentile at 26–31 weeks, PE with onset at <36, <34 and <32 weeks showed a LR+ of ≥10, PE with onset at <34 and <32 weeks showed an SE of ≥0.80 and no outcomes showed a PPV of ≥0.20. As for the plasma levels of sFlt-1/PIGF ratio of ≥95th percentile at 26–31 weeks, PE with onset at <34 and <32 weeks showed a LR+ of ≥10 and an SE of ≥0.80 but no outcomes showed a PPV of ≥0.20.

In predicting GH and SGA infants, none of the angiogenesis-related markers at 19–25 or 26–31 weeks showed an SE ≥0.80 or a LR+ ≥10 (Tables 4 and 5).

Comparison of receiver operating characteristics (ROC) curves of angiogenesis-related factors at 19–25 and 26–31 weeks for predicting all PE; PE with onset at <36, <34 and <32 weeks; GH; and an SGA infant

The details of the results are included in the Supplementary Information.

DISCUSSION

In this study, we disclosed two new findings. First, the onset threshold of the sFlt-1/PIGF ratio at 19–25 and 26–31 weeks as determined by ECLIA showed not only a high SE and a high LR+ but also high PPV for predicting the imminent onset of PE within 4 weeks after blood sampling, showing an SE of ≥0.83, an LR+ of ≥132 and a PPV of ≥0.50. Thus the onset threshold of the sFlt-1/PIGF ratio at 19–31 weeks may be clinically useful for detecting imminent risk for the onset of PE within 4 weeks after blood sampling. Second, the plasma levels of PIGF of <5th percentile and an sFlt-1/PIGF ratio ≥95th percentile at 19–25 weeks showed a high SE and a high LR+ but low PPV for predicting the imminent onset of PE and PE with onset at <32 weeks. Plasma levels of PIGF of <5th percentile, plasma levels of sFlt-1 of ≥95th percentile and an sFlt-1/PIGF ratio of ≥95th percentile at 26–31 weeks also showed a high SE and a high LR+ but low PPV for predicting the imminent onset of PE and PE with onset at <34 and <32 weeks. Thus, the cutoff levels of 5th percentile of PIGF and 95th percentile of sFlt-1 at 19–31 weeks may be useful for screening for high risk for PE with onset at <34 weeks.

The onset threshold of the sFlt-1/PIGF ratio at 19–31 weeks as determined by ECLIA may be clinically useful for detecting imminent risk for the onset of PE within 4 weeks after blood sampling. In our previous report, we reported the onset threshold of the sFlt-1/PIGF ratio and showed that women with an sFlt-1/PIGF ratio higher than the onset threshold at 26–31 weeks have a high risk of imminent onset of PE with onset at <36 weeks: at 2.2 ± 0.6 weeks after blood sampling, the ratio showed an SE of 0.36; however, the detection rate of PE at 19–25 weeks was very poor, with an SE = 0.⁹ In the current

Table 5 Screening performances for predicting imminent onset of PE within 4 wk after blood sampling in all PE; PE with onset at <36, <34 and <32 wk; GH; and SGA in 798 women at 26–31 wk

Risk factors outcomes	Incidence rates	TP	FN	FP	TN	SE	SP	LR+ (95% CI)	LR– (95% CI)	PPV	NPV
<i>PlGF < 5th</i>											
All PE	0.028	7	15	46	730	0.32	0.94	5.4 (2.7–11)	0.73 (0.55–0.97)	0.132	0.980
PE with onset at <36 wk	0.014	6	5	47	740	0.55	0.94	9.1 (5.0–17)	0.48 (0.25–0.92)	0.113	0.993
PE with onset at <34 wk	0.008	5	1	48	744	0.83	0.94	14 (8.8–22)	0.18 (0.03–1.06)	0.094	0.999
PE with onset at <32 wk	0.004	3	0	50	745	1.00	0.94	16 (12–21)	0	0.057	1.000
GH	0.016	2	11	51	734	0.15	0.94	2.4 (0.64–8.7)	0.91 (0.72–1.14)	0.038	0.985
SGA	0.096	14	63	39	682	0.18	0.95	3.4 (1.9–5.9)	0.87 (2.0–7.5)	0.264	0.915
<i>sFlt-1 ≥ 95th</i>											
All PE	0.028	7	15	37	739	0.32	0.95	6.7 (3.4–13)	0.72 (0.54–0.95)	0.159	0.980
PE with onset at <36 wk	0.015	6	5	38	749	0.55	0.95	11 (6.1–21)	0.48 (0.25–0.91)	0.136	0.993
PE with onset at <34 wk	0.012	5	1	39	753	0.83	0.95	17 (11–27)	0.18 (0.03–846)	0.114	0.999
PE with onset at <32 wk	0.009	3	0	41	754	1.00	0.95	19 (14–26)	0	0.068	1.000
GH	0.016	2	11	42	743	0.15	0.95	2.9 (0.78–11)	0.89 (0.71–1.13)	0.045	0.985
SGA	0.096	8	69	36	685	0.10	0.95	2.1 (1.00–4.3)	0.94 (0.87–1.02)	0.182	0.908
<i>sFlt-1/PlGF ratio ≥ 95th</i>											
All PE	0.028	8	14	42	734	0.36	0.95	6.7 (3.6–13)	0.67 (0.49–0.92)	0.160	0.981
PE with onset at <36 wk	0.015	6	5	44	743	0.55	0.94	9.8 (5.3–18)	0.48 (0.25–0.92)	0.120	0.993
PE with onset at <34 wk	0.012	5	1	45	747	0.83	0.94	15 (9.3–23)	0.18 (0.03–1.06)	0.100	0.999
PE with onset at <32 wk	0.009	3	0	47	748	1.00	0.94	17 (13–22)	0	0.060	1.000
GH	0.016	3	10	47	738	0.23	0.94	3.9 (1.4–11)	0.82 (0.61–1.10)	0.060	0.987
SGA	0.096	14	63	36	685	0.18	0.95	3.6 (2.1–6.4)	0.86 (2.2–8.3)	0.280	0.916

Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; FN, false negative; FP, false positive; GH, gestational hypertension; LR–, negative likelihood ratio; LR+, positive likelihood ratio; mNDI, mean notch depth index; mPI, mean pulsatility index; NPV, negative predictive value; PE, preeclampsia; PlGF, placental growth factor; PPV, positive predictive value; SBP, systolic blood pressure; SE, sensitivity; sFlt-1, soluble fms-like tyrosine kinase 1; SGA, small-for-gestational age; SP, specificity; TN, true negative; TP, true positive; wk, weeks of gestation.

Bold font of SE indicates that SE was ≥0.8.
Bold font of LR+ indicates that LR+ was ≥10.0.
Bold font of PPV indicates that PPV was ≥0.20.

study, the SEs of the onset threshold of the sFlt-1/PlGF ratio at 19–25 and 26–31 weeks were sufficiently high (1.00 and 0.83, respectively), showing an LR+ of >100 and a PPV of ≥0.50, indicating that the onset threshold of sFlt-1/PlGF as determined by ECLIA at 19–31 weeks may be a good biomarker for predicting the imminent onset of PE. Improvement of the SE using the onset threshold of the sFlt-1/PlGF ratio in our current study appeared to be the result of the improved SE of PE by PlGF using ECLIA.

PlGF <5th percentile and an sFlt-1/PlGF ratio ≥95th percentile at 19–25 weeks showed a high SE and a high LR+ but low PPV for predicting the imminent onset of PE and PE with onset at <32 weeks. These results suggested that plasma levels of PlGF and the sFlt-1/PlGF ratio at 19–25 weeks may be useful in the detection of women at high risk for not only the imminent onset of PE within 4 weeks but also a later onset of PE (after 4 weeks). The imminent onset of PE occurred in only two pregnant women with blood sampling at 19–25 weeks, but PE with onset at <32 weeks occurred in 11 women with blood sampling at 19–25 weeks. Therefore, we should consider that a decrease of PlGF plasma levels or an increase of the sFlt-1/PlGF ratio at 19–25 weeks indicates a high risk for the occurrence of early-onset PE with onset at <32 weeks. In our previous study, based on PlGF plasma levels <2.5th percentile and an sFlt-1/PlGF ratio ≥97.5th percentile as determined by ELISA, the use of PlGF levels at 19–25 weeks for predicting PE with onset at <32 weeks showed an SE, SP and LR+ of 44%, 96% and 12, respectively. Furthermore, using the sFlt-1/PlGF ratio at 19–25 weeks for predicting PE with onset at <32 weeks showed an SE, SP and LR+ of 78%, 92% and 47, respectively.⁹ Kleinrouweler *et al.*¹⁶ reviewed the accuracy of PlGF and sFlt-1

detection in the serum or plasma of pregnant women with blood sampling before 30 weeks of gestation and before the clinical onset of PE; of the 15 studies on PlGF, one study performed at 24 weeks reported an outcome of early-onset PE with onset at <32 weeks, with an SE of 84.4% and an SP of 95%.²⁶ Taken together, low PlGF levels and a high sFlt-1/PlGF ratio in the second trimester appear to be high-risk markers for early-onset PE.

Plasma levels of PlGF of <5th percentile, plasma levels of sFlt-1 of ≥95th percentile and an sFlt-1/PlGF ratio of ≥95th percentile at 26–31 weeks also showed a high SE and a high LR+ but low PPV for predicting the imminent onset of PE and PE with onset at <34 and <32 weeks. However, when we checked the predicted women in a database, the five women detected with imminent onset within 4 weeks after blood sampling using the onset threshold of the sFlt-1/PlGF ratio at 26–31 weeks were identical to the five women detected with PE with onset at <34 weeks using the plasma levels of PlGF of <5th percentile, the plasma levels of sFlt-1 of ≥95th percentile and an sFlt-1/PlGF ratio of ≥95th percentile at 26–31 weeks. Although the sFlt-1/PlGF ratio of ≥95th percentile at 26–31 weeks detected three other women with a later onset of PE after 34 weeks, the LR+ decreased from 15 for the prediction of PE with onset at <34 weeks to 6.7 for the prediction of all PE, suggesting that the screening of PE using PlGF and sFlt-1 at 26–31 weeks may be useful for detecting PE with onset at <34 weeks, but not for detecting PE with onset at ≥34 weeks.

We also evaluated the screening performance for predicting the imminent onset of PE within 4 weeks after blood sampling at 19–25 and 26–31 weeks using the onset threshold of plasma levels of PlGF.

The onset threshold of PIGF at 19–25 weeks was clinically useful but that at 26–31 weeks showed low PPV, although the $LR \pm$ was ≥ 10 and the SE was ≥ 0.8 . Therefore, if we select only one biomarker for predicting the imminent onset of PE at 19–31 weeks, we should select the sFlt-1/PIGF ratio rather than PIGF.

When we evaluated the ROC curves of the plasma levels of sFlt-1 and of PIGF and the sFlt-1/PIGF ratio at 19–25 and 26–31 weeks for predicting all PE and PE with onset at <36 , <34 and <32 weeks, the areas under the curve (AUCs) of the sFlt-1/PIGF ratio and PIGF at 19–25 weeks of gestation were more accurate for predicting PE with earlier onset; however, the AUC of sFlt-1 at 19–25 weeks of gestation was very weak, even for predicting PE with onset at <32 weeks of gestation. In contrast, the AUCs of the three indices at 26–31 weeks of gestation were more accurate for predicting PE with earlier onset, and the AUCs of the three indices for earlier onset PE were identical. Thus, the results using ROC curves were almost consistent with those using the arbitrary cutoff values of PIGF plasma levels <5 th percentile, sFlt-1 plasma levels ≥ 95 th percentile and an sFlt-1/PIGF ratio ≥ 95 th percentile.

In this study, the onset threshold of plasma levels of the sFlt-1/PIGF ratio was a highly sensitive and a highly specific screening method for detecting the imminent onset of PE within 4 weeks after blood sampling at 19–31 weeks; however, the imminent onset of PE occurred in only 2 of the 1199 (0.2%) women recruited at 19–25 weeks and in only 6 of the 798 (0.8%) women recruited at 26–31 weeks. Because we planned to draw blood samples at 20–23 and 28–29 weeks in our study design, the time between the two samplings was relatively long, almost 8 weeks, resulting in several missed outcomes of PE for predicting the imminent onset of PE within 4 weeks after blood sampling. We are now planning a new study protocol with blood sampling every 4 weeks from 20 to 35 weeks. In addition, because our sample numbers for the target outcomes were relatively small, our findings should be confirmed in a larger multicenter cohort study. In this study, we did not evaluate the effects of sEng on predicting the imminent onset of PE within 4 weeks after blood sampling. We are now preparing to measure the levels of sEng in all the subjects in our current study and to analyze the onset threshold of sEng for predicting the imminent onset of PE.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Hypertension Research website (<http://www.nature.com/hr>)