sFlt-1/PlGF for prediction of early-onset pre-eclampsia: STEPS (Study of Early Pre-eclampsia in Spain)

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ABSTRACT

Objective A high ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) has been linked to pre-eclampsia (PE). We evaluated the sFlt-1/PlGF ratio as a predictive marker for early-onset PE in women at risk of PE.

Methods This prospective, Spanish, multicenter study included pregnant women with a risk factor for PE, including intranatal growth restriction, PE, eclampsia or hemolysis, elevated liver enzymes and low platelet count syndrome in previous pregnancy, gestational diabetes or abnormal uterine artery Doppler. The primary objective was to show that the sFlt-1/PlGF ratio at 20, 24 and 28 weeks' gestation was predictive of early-onset PE (< 34 + 0 weeks). Serum sFlt-1 and PlGF were measured at 20, 24 and 28 weeks. Multivariate logistic regression was used to develop a predictive model.

Results A total of 819 women were enrolled, of which 729 were suitable for analysis. Of these, 78 (10.7%) women developed PE (24 early onset and 54 late onset). Median sFlt-1/PlGF ratio at 20, 24 and 28 weeks was 6.3 (interquartile range (IQR), 4.1–9.3), 4.0 (IQR, 2.6–6.3) and 3.3 (IQR, 2.0–5.9), respectively, for women who did not develop PE (controls); 14.3 (IQR, 5.5–43.7), 18.4 (IQR, 8.2–57.9) and 51.9 (IQR, 11.5–145.6) for women with early-onset PE; and 6.7 (IQR, 4.6–9.9), 4.7 (IQR, 2.8–7.2) and 6.0 (IQR, 3.8–10.5) for women with late-onset PE. Compared with early-onset PE, the sFlt-1/PlGF ratio was significantly lower in controls (P < 0.001 at each timepoint) and in women with chronic hypertension (P < 0.001 at each timepoint), gestational hypertension (P < 0.001 at each timepoint) and late-onset PE (P < 0.001 at each timepoint). A prediction model for early-onset PE was developed, which included the sFlt-1/PlGF ratio plus mean arterial pressure, being parous and previous PE, with areas under the receiver–operating characteristics curves of 0.86 (95% CI, 0.77–0.95), 0.91 (95% CI, 0.85–0.97) and 0.93 (95% CI, 0.86–0.99) at 20, 24 and 28 weeks, respectively, and was superior to models using the sFlt-1/PlGF ratio alone or uterine artery mean pulsatility index.

Conclusions The sFlt-1/PlGF ratio can improve prediction of early-onset PE for women at risk of this condition. © 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) affects 2–5% of pregnancies and can result in intrauterine growth restriction (IUGR), renal or hepatic impairment, HELLP syndrome (hemolysis, elevated liver enzyme levels and low platelet count), eclampsia, and maternal and fetal mortality. Early and late manifestations of PE differ in time of onset of symptoms, relative frequency, placental morphology, genetic risk and risk of adverse outcomes. Early-onset PE is associated with a higher incidence of adverse perinatal outcomes, including oligohydramnios, Apgar score < 7, stillbirth and early neonatal death, compared with late-onset PE. As early intervention is important to improve maternal and fetal outcomes and the classical...
clinical markers of PE (hypertension and proteinuria) are poorly predictive of those who will develop the condition, markers of angiogenesis have been examined as aids to PE prediction. A key feature of PE is placental insufficiency. Dysregulation of pro- and antiangiogenic factors is thought to be causally linked to the condition\textsuperscript{7,17,18}; before and during PE, maternal serum concentrations of antiangiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) are increased and levels of proangiogenic placental growth factor (PIGF) are decreased\textsuperscript{19,20}. A high sFlt-1/PIGF ratio has been linked with PE and demonstrated before clinical onset of the condition, and differences in sFlt-1 and PIGF have been observed between early- and late-onset PE\textsuperscript{21–29}. The Elecsys\textsuperscript{®} immunoassay sFlt-1/PIGF ratio is CE-IVD (Conformité Européenne–In Vitro Diagnostics) approved as a diagnostic aid for PE with gestational age-specific cut-off values, and as an aid in short-term prediction of PE in women with suspected PE\textsuperscript{26,30,31}. The Prediction of Short-Term Outcome in Pregnant Women with Suspected PE Study (PROGNOSIS) developed a cut-off-based PE prediction model. Optimum sFlt-1/PIGF ratio cut-off levels of ≤ 38 and > 38 were identified to rule out and rule in, respectively, PE, in women with singleton pregnancy at 24 + 0 to 36 + 6 weeks’ gestation\textsuperscript{32}. However, the predictive value of the sFlt-1/PIGF ratio has not been examined specifically for early-onset PE. This study, the Study of Early Pre-eclampsia in Spain (STEPS), aimed to evaluate the sFlt-1/PIGF ratio at 20, 24 and 28 weeks as a predictive marker for early-onset PE in women at risk of PE.

METHODS

Study design and participants

STEPS was a prospective, double-blind, multicenter (10 study sites in Spain) study, performed between October 2010 and March 2013, and enrolled pregnant women at risk of PE. To be considered at risk of PE, women had to meet one of the following inclusion criteria: PE, eclampsia, HELLP syndrome or IUGR in a previous pregnancy; pre-existing chronic hypertension without proteinuria; gestational hypertension (new-onset hypertension in pregnancy); pre-existing renal disease (kidney transplantation or creatinine clearance < 60 mL/min); pre-existing diabetes mellitus Type I (insulin dependent); mean uterine artery Doppler pulsatility index (UaA-PI) > 1.45 (at 19–20 weeks); thrombophilia (antiphospholipid syndrome, protein C deficiency, protein S deficiency, antithrombin deficiency, factor V Leiden mutation); multiple pregnancy; age ≥ 40 years and conceived with assisted reproductive technologies (ART). Women with two or more of the following risk factors were also included: nulliparity; body mass index ≥ 35 kg/m\(^2\); diastolic blood pressure > 80 mmHg at study inclusion; age ≥ 40 years; and family history (mother or sister) of PE, eclampsia or HELLP syndrome. Women were excluded if they were both hypertensive and had proteinuria or if major fetal malformations/germ cell disorders were observed. Women provided informed, signed consent. The protocol was approved by applicable national/regional independent ethics committees and institutional review boards (Table S1). The study adhered to the Guidelines for Good Clinical Practice.

The primary objective of the study was to demonstrate that the sFlt-1/PIGF ratio was a predictive marker for early-onset PE. Secondary objectives included evaluation of the sFlt-1/PIGF ratio as a predictor of late-onset PE and the use of the sFlt-1/PIGF ratio for differentiation of hypertension from PE.

Diagnostic criteria

For consistency, investigators used predefined diagnostic criteria (Table 1) based on the Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy\textsuperscript{33}. PE was defined as newly occurring hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) with newly occurring proteinuria after 20 weeks. To be considered early onset, PE had to occur before 34 + 0 weeks.

Data collection and visits

At gestational weeks 19–20 (Visit 1), 23–24 (Visit 2) and 27–28 (Visit 3), participants underwent a blood test to determine the sFlt-1/PIGF ratio, Doppler examination of the uterine arteries and assessment of blood pressure (measured by validated automated devices), proteinuria, PE status, hemoglobin, platelets and uric acid levels. Postpartum, additional data were collected, including blood pressure, type of delivery, Apgar score, weight of placenta, neonatal outcomes (perinatal/fetal death, delivery < 34 weeks, IUGR, placental abruption, respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage) and maternal outcomes (maternal death, pulmonary edema, acute renal failure, cerebral hemorrhage, cerebral thrombosis, disseminated intravascular coagulation). Unplanned visits could be carried out in the event of complications.

Serum samples (≥ 2 mL) were collected according to a standard operating procedure and were analyzed at the individual study sites. Results were checked for consistency between study sites by central analysis at Hospital Universitario Central de Asturias.

Maternal serum levels of sFlt-1 and PIGF were determined using the fully automated Elecsys sFlt-1 and Elecsys PIGF assays on the cobas® e electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany) and the sFlt-1/PIGF ratio was calculated\textsuperscript{27,30,31}. The sFlt-1/PIGF ratio results were concealed from both patients and carers to ensure that they did not affect the clinical monitoring of patients.

Adverse events were recorded, although the study was non-interventional.
Table 1 Diagnostic criteria in Study of Early Pre-eclampsia in Spain (STEPS)

| Diagnosis                  | Criteria                                                                                   |
|----------------------------|-------------------------------------------------------------------------------------------|
| Hypertension               | Systolic blood pressure $\geq 140$ mmHg and/or diastolic blood pressure $\geq 90$ mmHg (on two occasions at least 6 h apart) |
| Chronic hypertension       | Hypertension (systolic blood pressure $\geq 140$ mmHg and/or diastolic blood pressure $\geq 90$ mmHg) diagnosed before pregnancy or in first half of pregnancy (≤ 20 weeks) and continued for > 12 weeks after delivery |
| Proteinuria                | For determination of urinary protein using test strips, a value of 1+ was not considered reliable for diagnosis of PE. Data were reconfirmed with protein test on 24-h urine ($\geq 0.3$ g protein/24 h); in an emergency, if it was not possible to determine protein in 24-h urine, protein determination was carried out on isolated urine sample ($\geq 30$ mg protein/dL or protein/creatinine ratio $\geq 30$ mg protein/mmol creatinine). |
| Gestational hypertension   | New-onset hypertension (systolic blood pressure $\geq 140$ mmHg and/or diastolic blood pressure $\geq 90$ mmHg) after 20 weeks of pregnancy, which resolved by 12 weeks postpartum |
| PE                         | New-onset hypertension (systolic blood pressure $\geq 140$ mmHg and/or diastolic blood pressure $\geq 90$ mmHg) and new-onset proteinuria after 20 weeks of pregnancy |
| Severe PE                  | PE plus one or more of the following: systolic blood pressure $\geq 160$ mmHg and/or diastolic blood pressure $\geq 110$ mmHg (on two occasions at least 6 h apart); proteinuria ($\geq 5$ g protein/24 h or test strip ≥ 3+ in two urine samples collected at random at least 4 h apart); impairment of renal function (serum creatinine ≥ 1.2 mg/dL unless known to be elevated previously or oliguria < 500 mL/24 h); pulmonary edema; impairment of hepatic function (elevated liver enzymes, epigastric pain or right upper quadrant pain caused by distension of Glisson’s capsule); neurological symptoms (cerebral or visual disturbances, severe headache); hematological disturbances (thrombocytopenia, hemolysis); IUGR |
| Eclampsia                  | New-onset tonic–clonic convulsions in women with PE, not attributable to any other cause |
| Early- and late-onset PE   | Early onset: PE developing < 34 + 0 weeks; late onset: PE developing ≥ 34 + 0 weeks |
| HELLP syndrome             | Increased ASAT ($> 70$ IU/L); decreased platelet count ($< 100,000$ µL); increased LDH ($> 600$ IU/L) |
| IUGR                       | Estimated fetal weight or abdominal circumference < $10^{th}$ percentile (adjusted for gender/race in accordance with tables normally used by study center); Presence of pathological process that inhibits expression of normal intrinsic growth potential. Pathological process must be demonstrated at least once after 22 weeks, according to either oligohydramnios (amniotic fluid index $< 10^{th}$ percentile) or pathological flow in umbilical artery (pulsatility index $> 95^{th}$ percentile) |
| SGA neonate                | Estimated fetal weight or abdominal circumference < $10^{th}$ percentile (adjusted for gender/race in accordance with tables normally used by study center); no pathological process |
| Preterm birth              | Delivery before end of 37 weeks (e.g. gestational age of 36 + 6 weeks would be recorded as 36 completed weeks of pregnancy and baby would be defined as preterm) |

ASAT, aspartate aminotransferase; HELLP, hemolysis, elevated liver enzymes and low platelet count; IUGR, intrauterine growth restriction; LDH, lactate dehydrogenase; PE, pre-eclampsia; SGA, small-for-gestational-age.

Statistical analysis

To obtain 100 cases of PE, it was calculated that 800 pregnant women would need to be included in the study, based on a presumed prevalence of PE of 12% (including both singleton and multiple pregnancies). The sFlt-1/PIGF ratio was log-transformed to correct for right skewness prior to any calculation. Differences in means between independent groups were assessed using analysis of variance (ANOVA) or Student’s t-test in the case of homogeneity of variances, and using generalized least squares in the case of heteroscedasticity. Appropriateness of the methods was assessed by evaluation of the plots of residuals.

To develop a predictive model of PE, multivariate logistic regression was used considering maternal characteristics, medical history and biomarkers as potential predictors. The variables that were finally included in the early-PE prediction model were selected according to the results of a logistic regression model with L1 penalization (‘lasso’ technique)\(^{34}\). The coefficients derived from the multivariate analysis were used as weights in a nomogram to predict early PE. Performances of the models were evaluated by receiver−operating characteristics (ROC) curves and areas under the curve (AUC) with 95% CIs.

All statistical analyses were performed using R (version 3.1.2) and R-packages rms (version 4.2-1) and ROCR (version 1.0-5).

RESULTS

Study participants

Overall, 729 women were eligible for analysis, including 447 with singleton pregnancy and 282 with multiple pregnancy (twin pregnancy, \(n = 276\); triplet pregnancy, \(n = 6\)). A total of 78 (10.7%) women developed PE (singleton pregnancy, \(n = 42\); multiple pregnancy, \(n = 36\)), of which 24 were early-onset PE (singleton pregnancy, \(n = 14\); multiple pregnancy, \(n = 10\)) and 54 were late-onset PE (singleton pregnancy, \(n = 28\); multiple pregnancy, \(n = 26\)) (Figure 1). The number of participants per study site is reported in Table S2. Women who developed early-onset PE had higher systolic and diastolic blood pressures, mean arterial blood pressure (MAP) and lower gestational age at delivery compared with the control group (women who did not develop PE/hypertension during the entire pregnancy) (Table 2).

sFlt-1, PIGF and sFlt-1/PIGF ratio measurements

In the control group, median sFlt-1/PIGF ratio remained low (< 7) between 20 and 28 weeks’ gestation (Table 3). In women who developed early-onset PE, median sFlt-1/PIGF ratio was already higher (14.5) at 20 weeks’ gestation and increased further to 18.4 at 24 weeks and
Figure 1 Flowchart of participants in Study of Early Pre-eclampsia in Spain (STEPS). *Reasons for exclusion: inclusion criteria not met (n = 4); signed consent given but did not start study (n = 28); miscarriage (n = 7); termination of pregnancy due to fetal malformations (n = 8); lost to follow-up (n = 13); placental abruption at 26 weeks (n = 1); completed follow-up until 28 weeks but data could not be retrieved because of delivery in another setting (n = 29). IUGR, intrauterine growth restriction; M, multiple pregnancy; PE, pre-eclampsia; S, singleton pregnancy.

Table 2 Baseline characteristics of women who developed early- or late-onset pre-eclampsia (PE) and those who did not develop PE (controls)

| Characteristic                         | Controls (n = 651) | Early-onset PE (n = 24) | Late-onset PE (n = 54) |
|---------------------------------------|-------------------|-------------------------|------------------------|
| Age (years)                           | 34.6 ± 5.3        | 35.6 ± 3.9              | 34.7 ± 0.7             |
| Body mass index (kg/m²)               | 26.7 ± 6.0        | 28.5 ± 6.4              | 27.9 ± 7.3             |
| Systolic blood pressure (mmHg)        | 119.1 ± 13.7      | 127.9 ± 13.5*           | 125.0 ± 16.0*          |
| Diastolic blood pressure (mmHg)       | 73.7 ± 11.3       | 77.5 ± 8.5*             | 77.7 ± 12.0*           |
| Mean arterial pressure (mmHg)         | 88.9 ± 11.1       | 94.3 ± 8.1*             | 93.4 ± 12.2*           |
| Multiple pregnancy                    | 246 (37.8)        | 10 (41.7)               | 26 (48.1)              |
| Gestational age at delivery (weeks)   | 37.5 ± 2.7        | 31.8 ± 3.5*             | 36.6 ± 1.4             |
| Birth weight of first infant (g)      | 2911 ± 721 (n = 646) | 1745 ± 830 (n = 22)*   | 2759 ± 584 (n = 54)   |
| Birth weight of second infant (g)     | 2303 ± 552 (n = 243) | 1807 ± 378 (n = 9)†    | 2285 ± 364 (n = 26)   |
| Birth weight of third infant (g)      | 1221 ± 689 (n = 4) | 1445 ± 304 (n = 2)     | — (n = 0)              |
| Nulliparous                           | 272 (41.8)        | 15 (62.5)               | 31 (57.4)              |
| Previous PE                           | 101 (15.5)        | 9 (37.5)*               | 14 (25.9)              |
| Family history of PE                  | 23 (3.5)          | 1 (4.2)                 | 5 (9.3)                |
| Previous IUGR                         | 55 (8.4)          | 3 (12.5)                | 5 (9.3)                |
| Chronic hypertension                  | 81 (12.4)         | 6 (25.0)                | 12 (22.2)              |
| Gestational hypertension              | 4 (0.6)           | 0 (0)                   | 3 (5.6)                |
| Nephropathy                           | 6 (0.9)           | 0 (0)                   | 1 (1.9)                |
| Diabetes mellitus Type 1              | 42 (6.5)          | 1 (4.2)                 | 1 (1.9)                |
| Thrombophilia                         | 50 (7.7)          | 1 (4.2)                 | 3 (5.6)                |
| Conceived by assisted reproduction    | 93 (14.3)         | 5 (20.8)                | 11 (20.4)              |
| Smoker at enrollment                  | 80 (12.3)         | 1 (4.2)                 | 3 (5.6)                |
| Abnormal UtA Doppler                   | 8 (1.2)           | 1 (4.2)                 | 2 (3.7)                |

Data are given as mean ± SD or n (%). PE groups compared with controls using Dunnett’s test: *P < 0.001; †P < 0.05, after adjustment by Bonferroni correction. IUGR, intrauterine growth restriction; UtA, uterine artery.
51.9 at 28 weeks. There was little change in the median sFlt-1/PlGF ratio between 20 and 28 weeks in women who developed late-onset PE, remaining low throughout at < 7.

Mean sFlt-1 levels and PlGF levels were significantly different between singleton and multiple pregnancies at 20, 24 and 28 weeks (P = 0.001). However, the mean sFlt-1/PlGF ratio was only significantly different at 28 weeks’ gestation (P = 0.001) (Table S3) and, at all timepoints, the difference between median values in singleton and multiple pregnancies was small.

**sFlt-1/PIGF ratio: prediction of PE**

Compared with control participants, the sFlt-1/PIGF ratio was consistently significantly higher in women with early-onset PE (P < 0.001 at all timepoints) (Figure 2). Women with early-onset PE also had significantly higher sFlt-1/PIGF ratios at 20, 24 and 28 weeks relative to women with chronic or gestational hypertension and women with late-onset PE (Figure 3). Differences between early-onset PE and control/hypertension/late-onset PE became more pronounced as the pregnancy progressed.

Women with late-onset PE were not easily differentiated from control participants by the sFlt-1/PIGF ratio at 20 and 24 weeks (difference was non-significant at 20 (P = 0.15) and 24 (P = 0.21) weeks, Figure 2). At 28 weeks, there was a statistically significant difference in the sFlt-1/PIGF ratio between women with late-onset PE and control participants (P < 0.001), although the numerical difference in the median ratio was small (2.7) (Table 3).

**Development of a prediction model for early-onset PE**

Prediction models for early-onset PE were developed, which included variations of the following factors: sFlt-1/PIGF ratio, PI GF, UtA-PI, MAP, being parous, previous PE and use of ART. The AUC was optimal for a model including the sFlt-1/PIGF ratio, MAP, being parous and previous PE (hereafter referred to as the ‘early-onset PE prediction model’) (Figure S1) compared with models that used the sFlt-1/PIGF ratio alone or UtA-PI alone (Table 4). The accuracy of the prediction model was not substantially improved by including ART or UtA-PI and ART in the model. At 20 and 24 weeks, including these two parameters in the model increased the AUC from 0.86 (95% CI, 0.77–0.95) to 0.87 (95% CI, 0.79–0.96), and from 0.91 (95% CI, 0.85–0.97) to 0.92 (95% CI, 0.85–0.97), respectively. However, at 28 weeks, including UtA-PI and ART in the model reduced the AUC from 0.93 (95% CI, 0.86–0.99) to 0.91 (95% CI, 0.82–0.99) (Figure 4). A nomogram for prediction risk is presented in Figures S2 and S3. The detection rate for early-onset PE using different prediction models is reported in Table 5.

We also compared the performance of a standard prediction model (maternal history, MAP and UtA-PI)
A comparison of prediction models that included the sFlt-1/PlGF ratio with models that used sFlt-1 or PlGF alone was performed by evaluating their respective AUCs and Akaike information criterion (AIC), which measures goodness of fit. The AUC for prediction models that included the sFlt-1/PlGF ratio (0.86–0.87, 0.91–0.92 and 0.91–0.93 at 20, 24 and 28 weeks' gestation, respectively) was greater than that of models that used single biomarkers (0.81–0.83, 0.88–0.90 and 0.88–0.91 for sFlt-1 and 0.79–0.83, 0.85–0.89 and 0.86–0.89 for PlGF at 20, 24 and 28 weeks' gestation, respectively) (Table S4).

Data consistency

No inconsistencies were found between site and central testing (data not shown).

DISCUSSION

Substantial evidence supports the use of the sFlt-1/PlGF ratio in PE diagnosis and prediction. However, differences between early- and late-onset PE suggest different etiologies; thus, different 'rules' for the sFlt-1/PlGF ratio could be applied. In a study of 275 women with suspected PE, the optimal sFlt-1/PlGF ratio cut-off to diagnose PE < 34 weeks' and ≥ 34 weeks' gestation was 26 (92.0% sensitivity, 81.1% specificity) and 45 (83.7% sensitivity, 72.6% specificity), respectively. In PROGNOSIS, a sFlt-1/PlGF ratio cut-off ≤ 38 ruled out PE within 1 week in women with suspected PE and singleton pregnancy at 24 + 0 to 36 + 6 weeks' gestation. PROGNOSIS had a higher prevalence of PE compared with our study (19% vs 11%, respectively), possibly due to the fact that PROGNOSIS enrolled women with suspicion of PE, while we enrolled women with a moderate or high risk of developing PE. The prevalence of PE in our study falls between the estimated ranges for women with moderate or high risk for PE (5.29-6.19% and 16.09-19.49%, respectively).

In STEPS, the sFlt-1/PlGF ratio was significantly different between women who did not develop PE and those who did. The combination of the sFlt-1/PlGF ratio with other clinical measures produced a predictive model with considerably increased specificity and sensitivity compared with using UtA-PI or sFlt-1/PlGF ratio alone. We also evaluated how the models used to estimate early-onset PE risk would perform when using the single biomarkers, instead of the sFlt-1/PlGF ratio. Based on both AUC and AIC, models with the sFlt-1/PlGF ratio demonstrated consistently the highest predictive performance. Using our early-onset PE prediction model (sFlt-1/PlGF ratio, MAP, being parous, previous PE), early-onset PE could be predicted from 20 weeks onward, with an AUC of 0.86 and 60% sensitivity for a false-positive rate of 10%. A previous model developed without serum biomarkers, which used a history of diabetes, hypertension and MAP, reported an AUC of 0.83 with 55% sensitivity for a false-positive rate of 10% (these were not high-risk women).

sFlt-1 and PlGF as single biomarkers: development of PE

Women with early-onset PE had lower PlGF (P < 0.001 at 20, 24 and 28 weeks) and higher sFlt-1 (P = 0.018, P < 0.001 and P < 0.001 at 20, 24 and 28 weeks, respectively) compared with those who did not develop early-onset PE (women who developed late-onset PE and those who did not develop any PE combined) (Figure S4).
Table 4 Prediction of early-onset pre-eclampsia (PE) at 20, 24 and 28 weeks using different individual parameters and early-onset PE prediction model

| Prediction parameter | AUC (95% CI) |
|----------------------|--------------|
| 20 weeks             |              |
| Early-onset PE prediction model | 0.86 (0.77–0.95) |
| MAP                  | 0.67 (0.55–0.79) |
| UtA-PI               | 0.50 (0.35–0.66) |
| PlGF                 | 0.70 (0.58–0.82) |
| sFlt-1               | 0.61 (0.49–0.74) |
| sFlt-1/PlGF ratio    | 0.77 (0.65–0.89) |
| 24 weeks             |              |
| Early-onset PE prediction model | 0.91 (0.85–0.97) |
| MAP                  | 0.72 (0.62–0.83) |
| UtA-PI               | 0.55 (0.39–0.72) |
| PlGF                 | 0.81 (0.72–0.90) |
| sFlt-1               | 0.71 (0.58–0.84) |
| sFlt-1/PlGF ratio    | 0.86 (0.76–0.96) |
| 28 weeks             |              |
| Early-onset PE prediction model | 0.93 (0.86–0.99) |
| MAP                  | 0.77 (0.66–0.89) |
| UtA-PI               | 0.63 (0.45–0.80) |
| PlGF                 | 0.86 (0.78–0.94) |
| sFlt-1               | 0.81 (0.67–0.95) |
| sFlt-1/PlGF ratio    | 0.89 (0.79–0.98) |

Early-onset PE prediction model includes soluble fms-like tyrosine kinase 1 (sFlt-1)/placental growth factor (PlGF) ratio, mean arterial pressure (MAP), being parous and previous PE. AUC, area under the receiver–operating characteristics curve; UtA-PI, uterine artery pulsatility index.

Other studies have included sFlt-1 and PlGF in their models. An observational study of women at high risk of PE developed a prediction model for early-onset PE using sFlt-1 at 28 + 0 to 31 + 6 weeks’ gestation, which had an AUC of 0.85 (67% sensitivity, 96% specificity)44. A model including gestational age, UtA-PI and sFlt-1/PlGF ratio showed an association with perinatal complications with an AUC of 0.89 (64% sensitivity, 95% specificity)45. Another model combined PlGF with maternal characteristics, obstetric history and UtA-PI to predict early-onset PE in the first trimester with an AUC of 0.9446. Although an abnormal UtA-PI was associated with the development of PE in our study, it was not included in our model since it did not substantially improve PE prediction. From a practical perspective, the UtA can be difficult to locate in the first trimester and the International Society of Ultrasound in Obstetrics and Gynecology does not include UtA Doppler as part of the routine first-trimester fetal ultrasound examination47. Other studies have also not included UtA-PI in their models48,49.

A recent study demonstrated that a prospective screening model at 19–24 weeks’ gestation, involving maternal factors, UtA-PI, MAP and PlGF, was superior to screening by maternal factors alone. The performance of the model was inversely related to the gestational age at which delivery became necessary; detection rates (false-positive rate of 10%) for PE < 32 weeks, between 32 + 0 and 36 + 6 weeks, and ≥ 37 weeks were 99$, 85% and 46%, respectively. However, this study evaluated PlGF and sFlt-1 separately; it did not assess the sFlt-1/PlGF ratio50. Of note, the study defined early-onset PE as requiring delivery before 32 weeks’ gestation, rather than before 34 weeks. A related study showed that a two-stage screening model, in which UtA-PI and PlGF measurements were reserved for at-risk individuals, achieved similar detection rates for preterm PE (< 37 weeks’ gestation), compared with screening the whole population by maternal factors, MAP, UtA-PI and PlGF51.

Various guidelines recommend PE screening based on maternal history52–54. However, the addition of MAP,
Table 5 Prediction rates of early-onset pre-eclampsia (PE) using different models at 20, 24 and 28 weeks

| Prediction model | 20 weeks | 24 weeks | 28 weeks |
|------------------|----------|----------|----------|
|                  | FPR = 5% | FPR = 10% | FPR = 5% | FPR = 10% | FPR = 5% | FPR = 10% |
| Early-onset PE prediction model | 45 | 60 | 60 | 60 | 60 | 60 |
| sFlt-1/PlGF ratio, MAP, being parous, previous PE, UtA-PI and ART | 50 | 60 | 60 | 60 | 60 | 60 |
| sFlt-1/PlGF ratio, MAP, being parous, previous PE and ART | 55 | 60 | 60 | 60 | 60 | 60 |
| MAP, being parous, previous PE, UtA-PI and ART | 35 | 55 | 55 | 55 | 55 | 55 |
| MAP, being parous, previous PE, ART and PlGF | 45 | 45 | 45 | 45 | 45 | 45 |

Early-onset PE prediction model includes soluble fms-like tyrosine kinase 1 (sFlt-1)/placental growth factor (PlGF) ratio, mean arterial pressure (MAP), being parous and previous PE. Areas under receiver operating characteristics curves for each model are provided in Table S4. ART, assisted reproductive technologies; FPR, false-positive rate; UtA-PI, uterine artery pulsatility index.

Table 6 Performance of standard prediction model and same model plus soluble fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PlGF) ratio to estimate risk of early-onset pre-eclampsia at 20, 24 and 28 weeks

| Prediction model | Detection rate (%) | AUC (95% CI) |
|------------------|-------------------|-------------|
|                  | FPR = 5% | FPR = 10% | 20 weeks | 24 weeks | 28 weeks |
| Standard prediction model (maternal history, MAP, UtA-PI) | 0.81 (0.71–0.89) | 0.81 (0.71–0.89) | 0.81 (0.71–0.89) | 0.81 (0.71–0.89) | 0.81 (0.71–0.89) |
| Standard prediction model plus sFlt-1/PlGF ratio | 0.91 (0.85–0.97) | 0.91 (0.85–0.97) | 0.91 (0.85–0.97) | 0.91 (0.85–0.97) | 0.91 (0.85–0.97) |
| Standard prediction model (maternal history, MAP, UtA-PI) | 0.87 (0.79–0.94) | 0.87 (0.79–0.94) | 0.87 (0.79–0.94) | 0.87 (0.79–0.94) | 0.87 (0.79–0.94) |
| Standard prediction model plus sFlt-1/PlGF ratio | 0.95 (0.90–0.99) | 0.95 (0.90–0.99) | 0.95 (0.90–0.99) | 0.95 (0.90–0.99) | 0.95 (0.90–0.99) |
| Standard prediction model (maternal history, MAP, UtA-PI) | 0.89 (0.83–0.95) | 0.89 (0.83–0.95) | 0.89 (0.83–0.95) | 0.89 (0.83–0.95) | 0.89 (0.83–0.95) |
| Standard prediction model plus sFlt-1/PlGF ratio | 0.95 (0.90–1.00) | 0.95 (0.90–1.00) | 0.95 (0.90–1.00) | 0.95 (0.90–1.00) | 0.95 (0.90–1.00) |

AUC, area under receiver operating characteristics curve; FPR, false-positive rate; MAP, mean arterial pressure; UtA-PI, uterine artery pulsatility index.

UtA-PI and angiogenic serum markers to the assessment of maternal history has been shown to increase the PE detection rate between 12 and 36 weeks’ gestation. In STEPS, the addition of the sFlt-1/PlGF ratio increased the detection rate at all gestational ages studied, supporting the inclusion of the sFlt-1/PlGF ratio in the risk estimation of early-onset PE.

The prospective, longitudinal design and large cohort in our study provided a robust dataset and the angiogenic marker results were hidden from the investigators to avoid bias in the diagnosis of outcomes. However, despite the large sample size, there were relatively small numbers of women in the early-onset PE group and the results of this study, which included women at risk of developing PE, cannot be applied to a low-risk population, i.e. in screening for PE. The data were validated using the Elecsys immunoassay sFlt-1/PlGF ratio and the predictive value may differ when other assays are used. The developed prediction model for early-onset PE has to be validated in an independent prospective study cohort in a comparable target population, and interventional studies are required to confirm the clinical utility of the results.

STEPS provides further evidence that the addition of the sFlt-1/PlGF ratio to clinical protocols for women at risk of PE improves prediction of early-onset PE in the second trimester. This complements the findings of PROGNOSIS, which showed that, in women with signs and/or symptoms of PE and a sFlt-1/PlGF ratio above 38, the positive predictive value for PE within the following 4 weeks was 36.7%32. In STEPS, women who developed early-onset PE had a median sFlt-1/PlGF ratio at 28 weeks’ gestation of 51.9, indicating that these women had an increased risk of developing PE in the following 4 weeks before 34 + 0 weeks’ gestation. Better prediction of PE could facilitate targeting of monitoring and therapeutic procedures towards at-risk women and allow better utilization of healthcare resources.
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DISCLOSURES

The STEPS study was sponsored by Roche Diagnostics Spain who were involved in study design, interpretation of the data and writing of the manuscript. ELECSYS and cobas are trademarks of Roche. A.P is a consultant for Roche, GE Healthcare, Ferring, Italfarma, EFFIK, Merck and Gynea. J.L.D is a consultant for Roche and Italfarma. M.H. is employed by Roche Diagnostics and has shares in F. Hoffmann-La Roche.

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SUPPORTING INFORMATION ON THE INTERNET
The following supporting information may be found in the online version of this article:

Table S1 Ethics Committee approval details
Table S2 Participant recruitment by study site
Table S3 Comparison of angiogenic factors at 20, 24 and 28 weeks in women with singleton vs multiple pregnancy
Table S4 Performance of different prediction models using soluble fms-like tyrosine kinase-1 (sFlt-1)/placental growth factor (PIGF) ratio, sFlt-1 or PIGF to estimate risk of early-onset pre-eclampsia (PE) at 20, 24 and 28 weeks

Figure S1 Formulae used in prediction model for early-onset pre-eclampsia (PE). MAP, mean arterial pressure.

Figure S2 Nomogram for estimation of risk model of early-onset pre-eclampsia (PE) at 24 weeks. To calculate probability of early-onset PE for a given patient, the value for each predictor is obtained by drawing a vertical line straight upward from that factor to the ‘points’ axis. The points are then summed and the sum located on the total points nomogram, and the probability of early-onset PE is located by drawing a vertical line downward to the ‘risk of PE’ line. MAP, mean arterial pressure; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

Figure S3 Worked example of the use of the nomogram for estimation of risk of early-onset pre-eclampsia (PE) at 24 weeks. To calculate visually risk of early-onset PE in a parous patient with previous PE at 24 weeks, with mean arterial pressure (MAP) of 100 mmHg and soluble fms-like tyrosine kinases-1 (sFlt-1)/placental growth factor (PIGF) ratio of 50, points are assigned for each item by plotting a line from the item to the points line. Being parous equates to 0 points; 100 mmHg MAP corresponds to 9 points; previous PE equates to 38 points; and sFlt-1/PIGF ratio of 50 (In = 3.91) gives 62 points. Total number of points is 109; drawing a vertical line downward from total points axis to ‘risk of PE’ line, the risk is estimated at approximately 80%.

Figure S4 Placental growth factor (PIGF) (a) and soluble fms-like tyrosine kinase-1 (sFlt-1) (b) at 20, 24 and 28 weeks in women who developed early-onset pre-eclampsia (PE) and in control participants who did not develop PE.