


Soluble fms-like tyrosine kinase-1 to placental growth factor ratio: ruling out pre-eclampsia for up to 4 weeks and value of retesting

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ABSTRACT

Objectives The soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio is generally elevated some time before and at the clinical onset of pre-eclampsia. The PROGNOSIS study validated a sFlt-1/PlGF ratio cut-off of ≤ 38 to rule out the onset of pre-eclampsia within 1 week of testing in women with suspected disease. The aim of this study was to assess the predictive value of the sFlt-1/PlGF ratio to rule out the onset of pre-eclampsia for up to 4 weeks, and to assess the value of repeat measurements.

Methods This was an exploratory post-hoc analysis of data from the PROGNOSIS study performed in pregnant women aged ≥ 18 years with suspected pre-eclampsia, who were at 24+0 to 36+6 weeks' gestation at their first clinic visit. Serum samples were collected at the first visit and weekly thereafter. sFlt-1 and PlGF levels were measured using Elecsys[®] sFlt-1 and PlGF immunoassays. Whether the sFlt-1/PlGF ratio cut-off of ≤ 38 used to rule out the onset of pre-eclampsia within 1 week could predict the absence of pre-eclampsia 2, 3, and 4 weeks post-baseline was assessed. The value of repeat sFlt-1/PlGF testing was assessed by examining the difference in sFlt-1/PlGF ratio 2 and 3 weeks after the first measurement in women with, and those without, pre-eclampsia or adverse fetal outcome.

Results On analysis of 550 women, sFlt-1/PlGF ratio ≤ 38 ruled out the onset of pre-eclampsia 2 and 3 weeks

post-baseline with high negative predictive values (NPV) of 97.9% and 95.7%, respectively. The onset of pre-eclampsia within 4 weeks was ruled out with a high NPV (94.3%) and high sensitivity and specificity (66.2% and 83.1%, respectively). Compared with women who did not develop pre-eclampsia, those who developed pre-eclampsia had significantly larger median increases in sFlt-1/PlGF ratio at 2 weeks (Δ , 31.22 vs 1.45; $P < 0.001$) and at 3 weeks (Δ , 48.97 vs 2.39; $P < 0.001$) after their initial visit. Women who developed pre-eclampsia and/or adverse fetal outcome compared with those who did not had a significantly greater median increase in sFlt-1/PlGF ratio over the same period (Δ , 21.22 vs 1.40; $P < 0.001$ at 2 weeks; Δ , 34.95 vs 2.30; $P < 0.001$ at 3 weeks).

Conclusion The Elecsys[®] immunoassay sFlt-1/PlGF ratio can help to rule out the onset of pre-eclampsia for 4 weeks in women with suspected pre-eclampsia. © 2018 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 affects 2–5% of pregnancies and is a major cause of perinatal and maternal morbidity and mortality^{1,2}, and the disease has an unpredictable

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course, with few specific or sensitive methods for diagnosis and prediction^{1,3,4}.

Pre-eclampsia is associated with a change in circulating maternal levels of pro- and antiangiogenic proteins, which have been investigated as biomarkers across gestational age⁵. For example, the soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio is elevated before and during the clinical onset of pre-eclampsia^{6–8}, so the sFlt-1/PlGF ratio has been established as an aid in the diagnosis and prediction of pre-eclampsia^{7,9}. Pre-eclampsia and pro- and anti-angiogenic factor imbalances are indicative of wider placental dysfunction^{10,11}.

The PROGNOSIS study validated the sFlt-1/PlGF ratio for the short-term prediction of pre-eclampsia in women preterm (before 37 + 0 weeks' gestation)⁹. PROGNOSIS identified a sFlt-1/PlGF ratio cut-off of ≤ 38 to optimally rule out pre-eclampsia within 1 week, with a negative predictive value (NPV) of 99.3% (95% CI, 97.9–99.9%). PROGNOSIS also reported a positive predictive value (PPV) for sFlt-1/PlGF ratio > 38 of 36.7% (95% CI, 28.4–45.7%) to establish the onset of pre-eclampsia within 4 weeks. A sFlt-1/PlGF ratio > 38 also identified women at risk for shorter remaining duration of pregnancy and at higher risk of preterm delivery (< 37 weeks)¹².

The aims of this study were to establish whether the sFlt-1/PlGF ratio can rule out the onset of pre-eclampsia for up to 4 weeks and to determine whether retesting at 2 and 3 weeks post-baseline can further elucidate the risk of developing pre-eclampsia.

METHODS

Study design

PROGNOSIS was a prospective observational study carried out at 30 sites across 14 countries; full methodological details have been published elsewhere^{9,13}. PROGNOSIS was conducted in accordance with the Guidelines for Good Clinical Practice and was funded by Roche Diagnostics. Pregnant women aged ≥ 18 years who were at 24 + 0 to 36 + 6 weeks' gestation at the baseline visit (Visit 1) and suspected to have pre-eclampsia as per protocol-defined criteria (Table S1), were enrolled. Exclusion criteria included manifest pre-eclampsia or eclampsia, confirmed diagnosis of HELLP syndrome or treatment with an investigational medicine within 90 days of enrollment. All participants provided written informed consent.

Study assessments

Women were assessed at Visit 1 (baseline visit; first evaluation of suspected pre-eclampsia and first sample collected), Visit 2 (7–9 days from Visit 1) and Visits 3–5 (7 \pm 2 days after the previous visit). Serum samples (≥ 2 mL) were collected according to a standard operating procedure and were analyzed retrospectively at an independent laboratory (Kreiskliniken Altoetting-Burghausen, Zentrallabor,

Altoetting, Germany). Maternal serum levels of sFlt-1 and PlGF were determined using the fully automated Elecsys[®] sFlt-1 and Elecsys[®] PlGF assays on the cobas e electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany), and were used to calculate the sFlt-1/PlGF ratio. Neither investigator nor participants were informed of the result during the study.

Study objectives/endpoints

This exploratory *post-hoc* analysis examined whether the sFlt-1/PlGF ratio cut-off of ≤ 38 , which has been validated previously for ruling out the onset of pre-eclampsia within 1 week, could predict the absence of pre-eclampsia/eclampsia/HELLP syndrome (referred to as pre-eclampsia hereafter) for 2, 3 and 4 weeks after the baseline visit. A second exploratory objective was to assess the value of repeat sFlt-1/PlGF ratio tests 2 and 3 weeks after Visit 1, by examining correlations between changes in sFlt-1/PlGF ratio and the onset of pre-eclampsia and the onset of pre-eclampsia and/or adverse fetal outcome within 4 weeks. A combined endpoint included pre-eclampsia and adverse fetal outcome within 4 weeks; combined endpoint status was 'yes' if pre-eclampsia was diagnosed within 4 weeks or adverse fetal outcome occurred within 4 weeks, and 'no' if neither pre-eclampsia nor any adverse fetal outcome occurred within 4 weeks. Adverse fetal outcome was defined as perinatal/fetal death, delivery before 34 weeks' gestation, intrauterine growth restriction, placental abruption, respiratory distress syndrome, necrotizing enterocolitis or intraventricular hemorrhage.

Analysis populations

Of the 1273 women enrolled in PROGNOSIS, 500 were included in the development cohort and 550 in the validation cohort (Figure 1). To calculate NPVs for the sFlt-1/PlGF ratio cut-off of ≤ 38 to rule out pre-eclampsia within 2, 3 and 4 weeks after Visit 1, data from women in the PROGNOSIS validation cohort ($n = 550$) were used; sensitivity and specificity were also calculated in this population. To calculate delta sFlt-1/PlGF ratio values between Visit 1 and Visits 3 and 4 (i.e. after 2 and 3 weeks), data were used both from women in the PROGNOSIS development cohort ($n = 500$) and from those in the PROGNOSIS validation cohort ($n = 550$) who had measurements available at these time points. To determine whether the delta sFlt-1/PlGF ratio values predict pre-eclampsia within 4 weeks, only women whose pre-eclampsia status within 4 weeks was known were considered. The only visits considered were within 28 days after Visit 1 and when pre-eclampsia was not diagnosed but still suspected. Visits at which pre-eclampsia was diagnosed or present after 28 days were excluded. Only consecutive visits at which pre-eclampsia was still suspected, as per protocol-defined criteria, were considered.

Statistical analysis

Delta sFlt-1/PIGF ratio values were calculated as the difference in sFlt-1/PIGF ratio between Visit 3 or 4 and Visit 1. In addition to a cut-off of 38, a cut-off of 85 was used, as a sFlt-1/PIGF ratio of ≥ 85 has been associated previously with adverse outcome and imminent delivery (within 2 weeks)¹⁴. To compare delta sFlt-1/PIGF ratio values between groups, an exact Wilcoxon–Mann–Whitney test was used. Box plots presenting delta sFlt-1/PIGF ratios were absolute

log transformed (positive values were transformed as $\log(x + 1)$, negative values as $-\log(-x + 1)$, and values of zero remained zero). To evaluate if the risk of developing pre-eclampsia, according to change in sFlt-1/PIGF ratio, was different if women had a baseline sFlt-1/PIGF ratio of ≤ 38 compared with a baseline value of > 38 to < 85 , these subgroups were analyzed separately. The PROGNOSIS study was powered for the primary analysis^{9,13} rather than for this exploratory *post-hoc* analysis; the results should be interpreted accordingly.

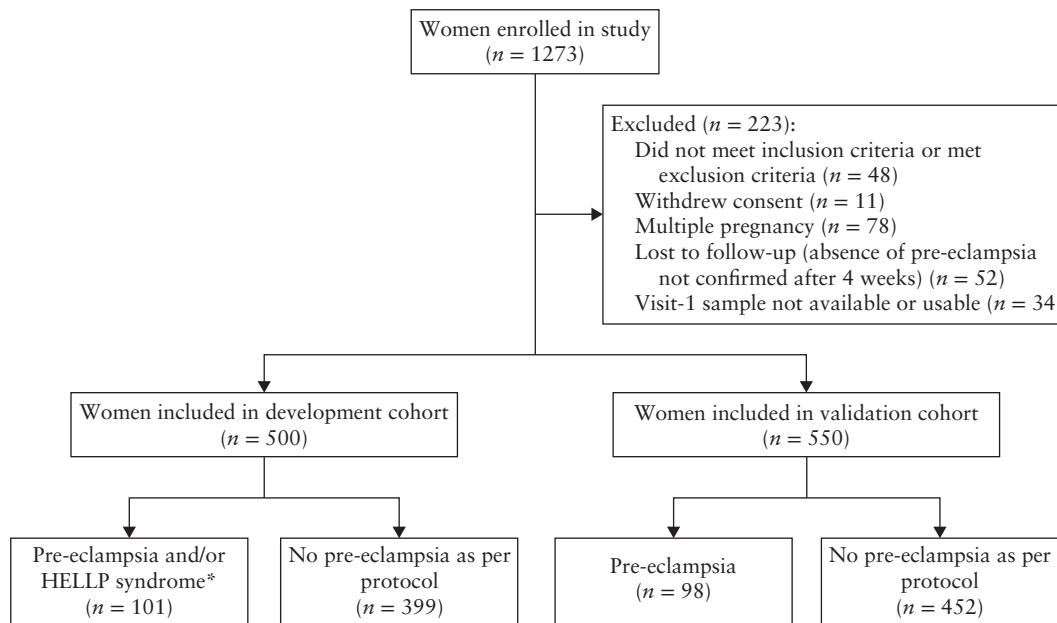


Figure 1 Flowchart showing enrolment and outcome of women with suspected pre-eclampsia in PROGNOSIS development and validation cohorts. *Ninety-nine women had pre-eclampsia only, one had pre-eclampsia and HELLP syndrome, and one had HELLP syndrome only.

Table 1 Baseline characteristics of women with suspected pre-eclampsia in development and validation cohorts of PROGNOSIS study, according to development of pre-eclampsia as per protocol

Characteristic	Development cohort		Validation cohort	
	No pre-eclampsia (n = 399)	Pre-eclampsia (n = 101)	No pre-eclampsia (n = 452)	Pre-eclampsia (n = 98)
Age (years)	32 (27–36)	32 (28–36)	31 (26–36)	32 (25–36)
Gestational age (weeks)	31.6 (27.3–34.7)	32.1 (27.7–34.4)	31.4 (27.6–34.3)	31.6 (28.0–34.6)
Prepregnancy BMI (kg/m ²)	27.0 (22.3–32.0)	24.9 (21.5–31.2)	26.1 (22.5–30.6)	26.4 (22.8–29.4)
Systolic BP (mmHg)	128 (115–140)*	137 (130–149)	125 (110–137)*	137 (126–146)
Diastolic BP (mmHg)	80 (70–90)*	85 (80–94)	78 (70–86)*	90 (80–95)
Smoker				
Past	71 (17.8)	22 (21.8)	105 (23.2)	18 (18.4)
Current	60 (15.0)	13 (12.9)	70 (15.5)	9 (9.2)
Ethnicity				
Asian	14 (3.5)	7 (6.9)	24 (5.3)	9 (9.2)
Black	26 (6.5)	7 (6.9)	20 (4.4)	8 (8.2)
Caucasian	355 (89.0)	87 (86.1)	345 (76.3)	73 (74.5)
Other	4 (1.0)	0 (0.0)	63 (13.9)	8 (8.2)

Data are given as median (interquartile range) or n (%). *Significantly different compared with those in cohort who developed pre-eclampsia ($P < 0.01$) (calculated using Mann–Whitney *U*-test for continuous variables and Fisher's exact test for categorical variables). BMI, body mass index; BP, blood pressure.

RESULTS

Baseline characteristics

Age, gestational age, prepregnancy body mass index and smoking status were not significantly different between participants who developed clinically confirmed pre-eclampsia and those who did not (Table 1). For Visits 3 (2 weeks after Visit 1) and 4 (3 weeks after Visit 1), data were available for 652 and 528 participants, respectively. The median interval between Visits 1 and 3 was 14 ± 1.45 days and between Visits 1 and 4 it was 21 ± 1.63 days.

Ruling out pre-eclampsia using baseline sFlt-1/PIGF ratio

Of the 550 women in the validation cohort, 98 (17.8%) developed pre-eclampsia, which was seen in 15 (2.7%) within 1 week, 41 (7.5%) within 2 weeks, 60 (10.9%)

within 3 weeks and 71 (12.9%) within 4 weeks; Table S2. A low sFlt-1/PIGF ratio of ≤ 38 ruled out the development of pre-eclampsia within 2 and 3 weeks with a high NPV (97.9% and 95.7%, respectively) in women suspected to be developing pre-eclampsia before 37+0 weeks. Development of pre-eclampsia within 4 weeks was also ruled out using a sFlt-1/PIGF ratio of ≤ 38 , with a NPV of 94.3%, and sensitivity and specificity of 66.2% (95% CI, 54.0–77.0%) and 83.1% (95% CI, 79.4–86.3%), respectively (Table 2).

Ruling out pre-eclampsia using change in sFlt-1/PIGF ratio after 2 and 3 weeks

On analysis conducted in women with available data from both the validation and development cohorts, those who did not develop pre-eclampsia had a lower median increase in sFlt-1/PIGF ratio after 2 weeks (Δ , 1.45 (interquartile range (IQR), –0.12

Table 2 Performance of soluble fms-like tyrosine kinase-1 to placental growth factor ratio cut-off of ≤ 38 to rule out onset of pre-eclampsia/HELLP syndrome within 1 to 4 weeks after baseline, in 550 women with suspected pre-eclampsia in PROGNOSIS validation cohort

Parameter	Rule out onset of pre-eclampsia within:			
	1 week	2 weeks	3 weeks	4 weeks
NPV (%)	99.3 (97.9–99.9)	97.9 (96.0–99.0)	95.7 (93.3–97.5)	94.3 (91.7–96.3)
PPV (%)	9.4 (4.9–15.8)	25.0 (17.8–33.4)	32.8 (24.8–41.7)	36.7 (28.4–45.7)
Sensitivity (%)	80.0 (51.9–95.7)	78.0 (62.4–89.4)	70.0 (56.8–81.2)	66.2 (54.0–77.0)
Specificity (%)	78.3 (74.6–81.7)	81.1 (77.5–84.4)	82.4 (78.8–85.7)	83.1 (79.4–86.3)
Positive likelihood ratio	3.69 (2.73–4.98)	4.14 (3.25–5.27)	3.99 (3.1–5.14)	3.91 (3.02–5.07)
Negative likelihood ratio	0.26 (0.09–0.7)	0.27 (0.15–0.48)	0.36 (0.25–0.54)	0.41 (0.29–0.56)

Data in parentheses are 95% CI. NPV, negative predictive value; PPV, positive predictive value.

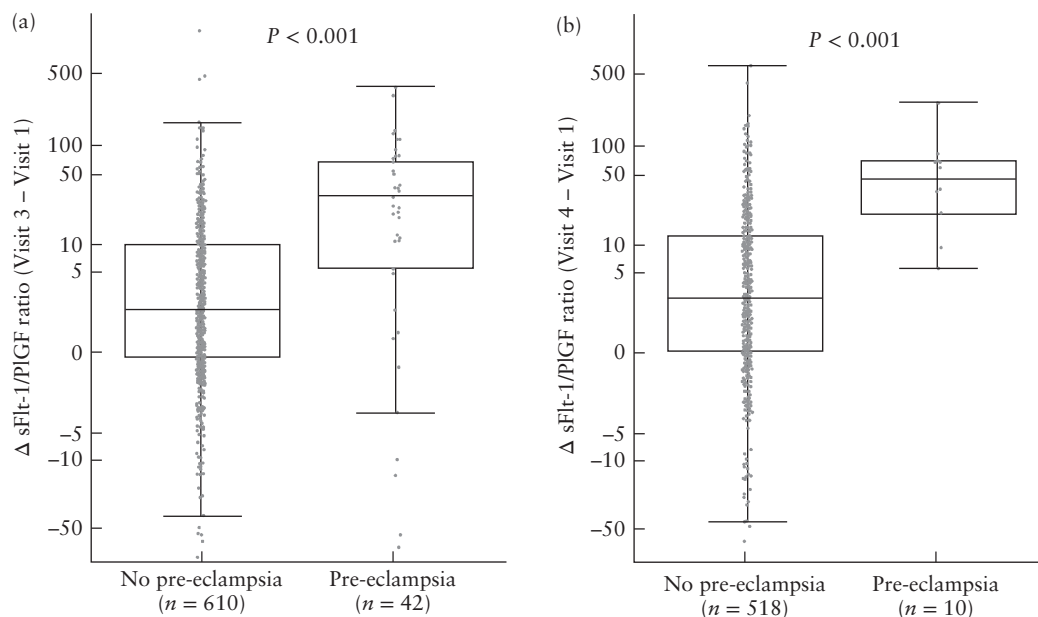


Figure 2 Change in soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PIGF) ratio 2 (a) and 3 (b) weeks after baseline measurement in women with suspected pre-eclampsia in PROGNOSIS development and validation cohorts, according to development of pre-eclampsia. Boxes represent median and interquartile range and whiskers represent range excluding outliers more than $1.5 \times$ interquartile range from upper or lower quartile. y-axis is in log scale.

to 9.41) ($n = 610$; Figure 2a)) and after 3 weeks (Δ , 2.39 (IQR, 0.04–12.49) ($n = 518$; Figure 2b)). In contrast, in women who developed pre-eclampsia, the median difference in sFlt-1/PlGF ratio was significantly higher after 2 weeks (Δ , 31.22 (IQR, 6.48–62.36)

($n = 42$) ($P < 0.001$) (Figure 2a)) and after 3 weeks (Δ , 48.97 (IQR, 25.01–69.67) ($n = 10$) ($P < 0.001$) (Figure 2b)).

For women who had a sFlt-1/PlGF ratio of ≤ 38 at Visit 1 and who did not develop pre-eclampsia, the median

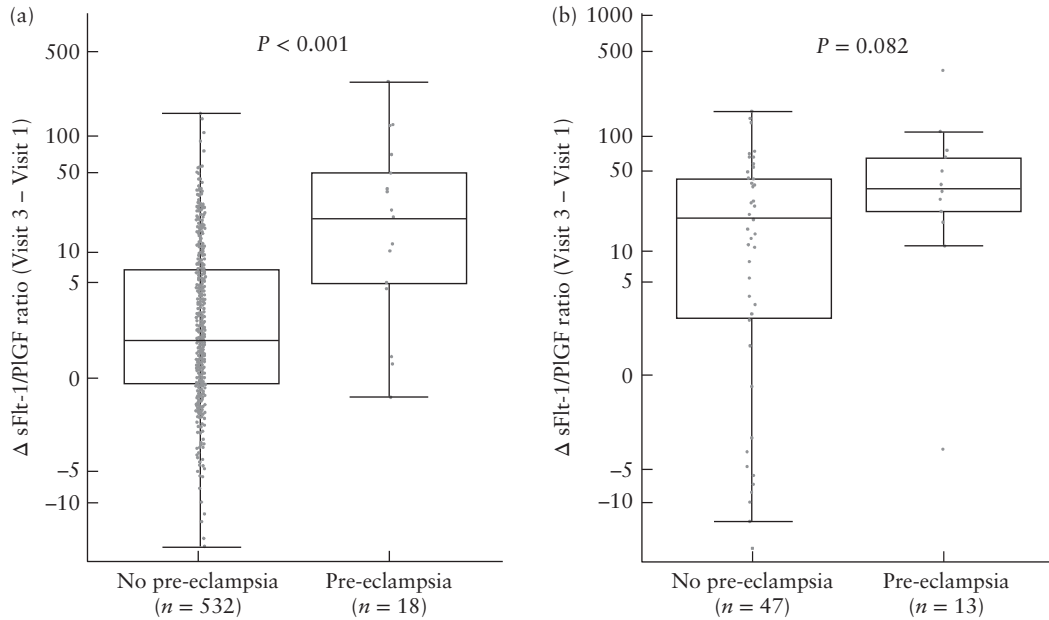


Figure 3 Change in soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio 2 weeks after baseline measurement in women with suspected pre-eclampsia in PROGNOSIS development and validation cohorts, in those with baseline sFlt-1/PlGF ratio ≤ 38 (a) and in those with baseline sFlt-1/PlGF ratio > 38 to < 85 (b), according to development of pre-eclampsia. Boxes represent median and interquartile range and whiskers represent range excluding outliers more than $1.5 \times$ interquartile range from upper or lower quartile. y-axis is in log scale.

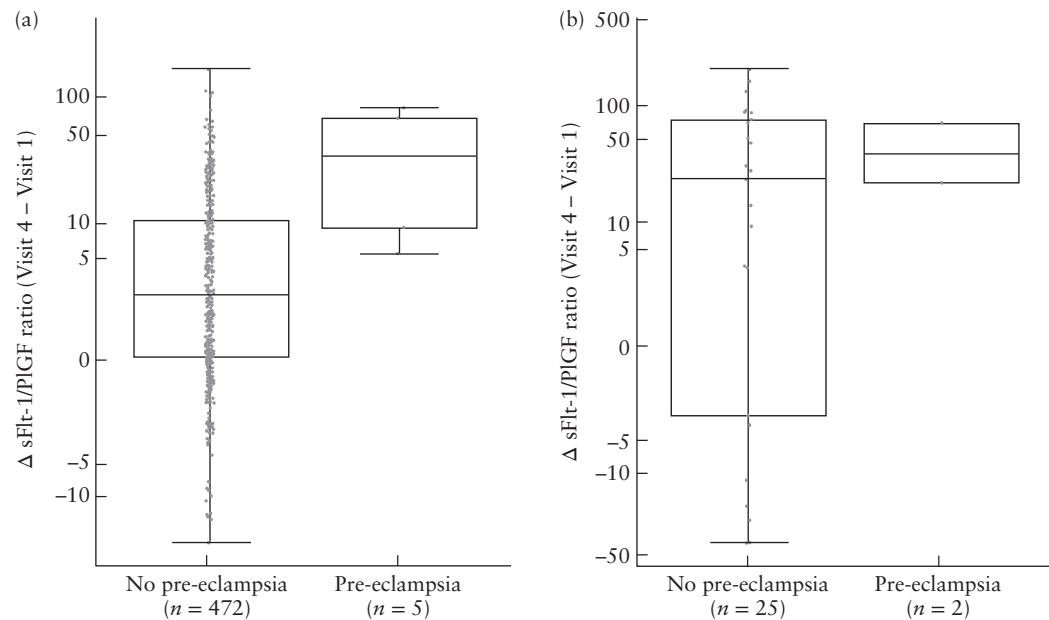


Figure 4 Change in soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio 3 weeks after baseline measurement in women with suspected pre-eclampsia in PROGNOSIS development and validation cohorts, in those with baseline sFlt-1/PlGF ratio ≤ 38 (a) and in those with baseline sFlt-1/PlGF ratio > 38 to < 85 (b), according to development of pre-eclampsia. Boxes represent median and interquartile range and whiskers represent range excluding outliers more than $1.5 \times$ interquartile range from upper or lower quartile. y-axis is in log scale.

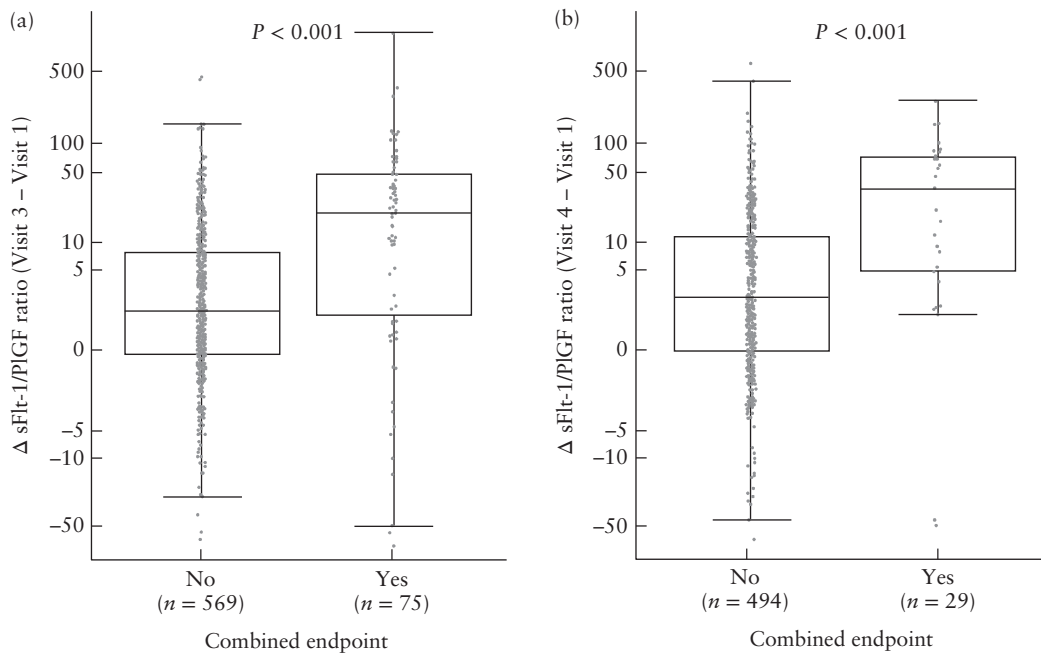


Figure 5 Change in soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PIGF) ratio 2 (a) and 3 (b) weeks after baseline measurement in women with suspected pre-eclampsia in PROGNOSIS development and validation cohorts, according to development of pre-eclampsia and/or adverse fetal outcome (combined endpoint). Boxes represent median and interquartile range and whiskers represent range excluding outliers more than $1.5 \times$ interquartile range from upper or lower quartile. y-axis is in log scale.

difference in sFlt-1/PIGF ratio was Δ , 1.12 (IQR, -0.11 to 6.94) after 2 weeks ($n = 532$) (Figure 3a) and Δ , 2.10 (IQR, 0.05 – 10.48) after 3 weeks ($n = 472$) (Figure 4a). In comparison, women who developed pre-eclampsia had a higher median increase in sFlt-1/PIGF ratio after 2 weeks (Δ , 20.30 (IQR, 6.48 – 46.21) ($n = 18$) ($P < 0.001$) (Figure 3a)) and after 3 weeks (Δ , 34.95 (IQR, 9.26 – 68.93) ($n = 5$) (sample size too low for statistical analysis) (Figure 4a)).

Women who had an initial sFlt-1/PIGF ratio of > 38 to < 85 and who did not develop pre-eclampsia had a median increase in sFlt-1/PIGF ratio of Δ , 19.77 (IQR, 2.16 – 43.84) ($n = 47$) after 2 weeks (Figure 3b) and Δ , 23.84 (IQR, -2.77 to 74.56) ($n = 25$) after 3 weeks (Figure 4b). In contrast, those who developed pre-eclampsia had a higher median increase in sFlt-1/PIGF ratio after 2 weeks (Δ , 35.80 (IQR, 22.87 – 65.54) ($n = 13$) ($P = 0.082$) (Figure 3b)) and after 3 weeks (Δ , 45.81 (IQR, 33.75 – 57.86) ($n = 2$) (sample size too low for statistical analysis) (Figure 4b)). The trend towards a greater difference in sFlt-1/PIGF ratio between visits in women who developed pre-eclampsia *vs* those who did not, was less pronounced in this subgroup (baseline sFlt-1/PIGF ratio of > 38 to < 85) than in women who initially had a sFlt-1/PIGF ratio of ≤ 38 .

Of the 64 women who had a sFlt-1/PIGF ratio of ≤ 38 at Visit 1 (baseline) and a sFlt-1/PIGF ratio > 38 at Visit 3 (after 2 weeks), nine developed pre-eclampsia within 4 weeks and had a median delta sFlt-1/PIGF ratio of 49.5 (Table S3). Of the 69 women with a sFlt-1/PIGF ratio of ≤ 38 at Visit 1 and a sFlt-1/PIGF ratio > 38 at Visit 4 (after 3 weeks), three developed pre-eclampsia within

4 weeks, with a median delta sFlt-1/PIGF ratio of 68.9 (Table S3).

Maternal and adverse fetal outcomes

The median difference in sFlt-1/PIGF ratio after 2 weeks in women who had not developed pre-eclampsia or adverse fetal outcome was Δ , 1.40 (IQR, -0.11 to 8.04) ($n = 569$). In comparison, in women who developed pre-eclampsia or adverse fetal outcome, the median increase in sFlt-1/PIGF ratio after 2 weeks was greater, at Δ , 21.22 (IQR, 1.20 – 49.81) ($n = 75$) ($P < 0.001$) (Figure 5a). The median increase in sFlt-1/PIGF ratio in women with suspected pre-eclampsia after 3 weeks who had not developed pre-eclampsia or adverse fetal outcome was Δ , 2.30 (IQR, 0.01 – 11.79) ($n = 494$). In women who developed pre-eclampsia or adverse fetal outcome, the median increase in sFlt-1/PIGF ratio after 3 weeks was greater, at Δ , 34.95 (IQR, 4.94 – 74.56) ($n = 29$) ($P < 0.001$) (Figure 5b). After both 2 and 3 weeks, greater increases in median sFlt-1/PIGF ratio were observed in women who developed pre-eclampsia or adverse fetal outcome and had a sFlt-1/PIGF ratio of ≤ 38 at Visit 1 compared with those who did not develop pre-eclampsia or adverse fetal outcome (Figures S1 and S2).

PIGF and sFlt-1 biomarker levels were analyzed individually (Table S4). Changes in the sFlt-1 level were significantly different between women who developed pre-eclampsia and those who did not. In contrast, changes in PIGF level did not significantly differ between the groups.

DISCUSSION

The sFlt-1/PIGF ratio cut-off of ≤ 38 was validated previously to rule out the onset of pre-eclampsia within 1 week in women suspected to have the syndrome, when tested before 37 + 0 weeks' gestation⁹. The present *post-hoc* analysis shows that a sFlt-1/PIGF ratio of ≤ 38 has a high NPV to rule out pre-eclampsia for up to 4 weeks after the initial test, with high sensitivity and specificity. The ability to rule out development of pre-eclampsia/eclampsia/HELLP syndrome within the following 4 weeks of pregnancy could help clinicians make informed decisions regarding patient monitoring.

sFlt-1/PIGF levels are dynamic^{6,9,11}; thus, we hypothesized that, in women with suspected pre-eclampsia who initially had a sFlt-1/PIGF ratio of > 38 to < 85 , an increase in sFlt-1/PIGF ratio over the following 2 or 3 weeks might indicate impending pre-eclampsia or a higher risk of developing it. There was a significantly greater increase in the sFlt-1/PIGF ratio between Visit 1 and a visit 2 or 3 weeks later in women who developed pre-eclampsia and/or adverse fetal outcome within 4 weeks, compared with those who did not develop pre-eclampsia and/or adverse fetal outcome. This was most apparent in women with a sFlt-1/PIGF ratio of ≤ 38 at Visit 1, compared with those with a ratio of > 38 to < 85 at Visit 1, possibly owing to a higher starting level meaning there was less scope for increase. Among women with a sFlt-1/PIGF ratio of ≤ 38 at Visit 1, nine had developed pre-eclampsia at Visit 3 and three had developed pre-eclampsia at Visit 4; these women were ruled in with a sFlt-1/PIGF ratio > 38 following retesting after 2–3 weeks, demonstrating the value of retesting.

The prevalence of pre-eclampsia in the PROGNOSIS study was relatively low (2.7% within 1 week; 12.9% within 4 weeks; 17.8% overall during pregnancy in the validation cohort) compared with in previous studies; Sabrià *et al.*¹⁵ observed that 33.3% of pregnant women developed pre-eclampsia or HELLP syndrome. The predictive performance of the sFlt-1/PIGF ratio may vary between target populations with differing prevalences of the disease. With a higher prevalence of pre-eclampsia, NPV will be slightly lower and PPV slightly higher.

Assessment of the change in sFlt-1/PIGF ratio between visits can provide further information to clinicians, allowing additional confidence in decision-making. These findings are in agreement with those reported in a general population of pregnant women, in which women with an increase in sFlt-1/PIGF ratio between the first and second trimesters had a higher risk of developing preterm pre-eclampsia⁵.

The finding that the sFlt-1 level increases significantly in women developing pre-eclampsia in the short term, compared with those who do not develop pre-eclampsia, suggests that the increase in sFlt-1 level reflects the development of imminent disease, in contrast to PIGF. These results are consistent with

previous findings that sFlt-1 increases significantly in early-onset pre-eclampsia, compared with PIGF, which does not decrease significantly as early-onset disease progresses¹⁶.

Previous studies have observed a strong link between sequential changes in sFlt-1/PIGF ratio and the development of pre-eclampsia^{5,14–18}. These studies, alongside our data, suggest that there is value in remeasuring the sFlt-1/PIGF ratio in women with suspected pre-eclampsia, especially in women with a sFlt-1/PIGF ratio between 38 and 85 or 110.

Current UK National Institute for Health and Care Excellence guidelines recommend that the Elecsys[®] immunoassay sFlt-1/PIGF ratio, alongside standard clinical assessment and follow-up, can be used to help rule out pre-eclampsia in preterm women presenting with symptoms of the condition¹⁹. Our study suggests that the initially recommended 1-week rule-out period could be confidently extended to at least 2 and up to 4 weeks, albeit with slightly lower confidence depending on the clinical presentation of the patient. For women with suspected pre-eclampsia and a sFlt-1/PIGF ratio of > 38 to < 85 , Stepan *et al.*²⁰ recommend considering a follow-up test after 1 or 2 weeks, as these women do not have a definite diagnosis of pre-eclampsia but are highly likely to develop it. Our study suggests that identifying changes in the sFlt-1/PIGF ratio by retesting can stratify a patient's risk and help guide clinicians in the management of at-risk groups. Currently, the rate of retesting may be low; in a study of real-world decision-making based on the sFlt-1/PIGF ratio test²¹, the actual frequency of retesting was only 6.5%²². Increasing the retesting rate may help identify additional at-risk women.

The clinical presentation of pre-eclampsia is diverse and the diagnosis of atypical cases of pre-eclampsia is a daily challenge for obstetricians. The data presented here highlight the value of the sFlt-1/PIGF ratio in ruling out pre-eclampsia for up to 4 weeks in women with suspected pre-eclampsia. Improved triage of patients with suspected pre-eclampsia will ensure appropriate treatment and reduce unnecessary hospitalization.

The strengths of this study include the large sample size, well-characterized participants and the use of prospective longitudinal sequential sampling at defined time intervals. The limitations include potential variation in assay cut-off value from that used in the current study, sample size being based on power calculations for the primary PROGNOSIS analysis rather than for this exploratory *post-hoc* analysis, small sample sizes used for some retesting analyses, lack of data on the need for retesting if the initial test score is ≥ 85 and, finally, the inclusion of patients who may not be representative of the general population. This study does not validate the optimal time interval for retesting, nor does it assess the predictive performance of the sFlt-1/PIGF ratio across target populations with differing prevalences of pre-eclampsia; these should be the objectives of future studies.

In conclusion, this *post-hoc* analysis demonstrates the additional value of the Elecsys® sFlt-1/PlGF ratio for the prediction of pre-eclampsia in women clinically suspected to have the syndrome. In women with a sFlt-1/PlGF ratio of ≤ 38 , pre-eclampsia can be ruled out for up to 4 weeks with a NPV of $\geq 94\%$. Retesting 2 or 3 weeks after the initial test improves risk stratification of pre-eclampsia in women with suspected pre-eclampsia. Repeat testing of the sFlt-1/PlGF ratio in women with suspected pre-eclampsia should increase confidence in decision-making for clinicians.

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Disclosures

H.Z. reports receiving lecture fees from Ferring and Roche Diagnostics and travel support from Ferring; E.L. reports receiving fees from Roche Diagnostics for lectures and serving on advisory boards; F.J.C. reports receiving fees from Roche Diagnostics for serving on advisory boards; M.V., M.Se. and S.P.B. report receiving lecture fees from Roche Diagnostics; H.S. reports receiving consulting and lecture fees from Roche Diagnostics; D.A., M.Sc., S.G. and M.H. report being employees of Roche Diagnostics; D.A., M.Sc. and M.H. report holding stock in Roche; M.Sc. and M.H. report holding a pending patent related to the sFlt-1:PlGF or endoglin:PlGF ratio to rule out onset of pre-eclampsia in pregnant women within a certain time period (PCT/EP2013/063115); M.H. reports holding pending patents related to the dynamics of sFlt-1 or endoglin:PlGF ratio as an indicator for imminent pre-eclampsia or the HELLP syndrome or both (PCT/EP2012/072157) and the prediction of postpartum HELLP syndrome, postpartum eclampsia, or postpartum pre-eclampsia (PCT/EP2015/051457); and S.V. reports receiving consulting fees from Roche Diagnostics, Thermo Fisher, Ferring, and Novartis, and lecture fees from Roche Diagnostics and Thermo Fisher. No other potential conflict of interest relevant to this article was reported.

REFERENCES

- Ananth CV, Keyes KM, Wapner RJ. Pre-eclampsia rates in the United States, 1980–2010: age-period-cohort analysis. *BMJ* 2013; **347**: f6564.
- World Health Organization (WHO). The World Health Report 2005 – Make every mother and child count. 2005; <http://www.who.int/whr/2005/en/>. [Accessed October 2016.]
- Hernández-Díaz S, Toh S, Cnattingius S. Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. *BMJ* 2009; **338**: b2255.
- Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol* 2009; **33**: 130–137.
- Vatten LJ, Eskild A, Nilsen TIL, Jeansson S, Jenum PA, Staff AC. Changes in circulating level of angiogenic factors from first to second trimester as predictors of preeclampsia. *Am J Obstet Gynecol* 2007; **196**: 239–241.
- Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA; CPEP Study Group. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006; **355**: 992–1005.
- Verlohren S, Herraiz I, Lapaire O, Schlembach D, Zeisler H, Calda P, Sabria J, Markfeld-Erol F, Galindo A, Schoofs K, Denk B, Stepan H. New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. *Hypertension* 2014; **63**: 346–352.
- Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, Pape J, Dudenhausen JW, Denk B, Stepan H. An automated method for the determination of the sFlt-1/PlGF ratio in the assessment of preeclampsia. *Am J Obstet Gynecol* 2010; **202**: 161.e1–11.
- Zeisler H, Llorca E, Chantraine F, Vatis M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M, Verlohren S. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. *N Engl J Med* 2016; **374**: 13–22.
- Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. *Am J Obstet Gynecol* 2015; **213**: S9.e1, S9–11.
- Staff AC, Benton SJ, von Dadelsen P, Roberts JM, Taylor RN, Powers RW, Charnock-Jones DS, Redman CW. Redefining preeclampsia using placenta-derived biomarkers. *Hypertension* 2013; **61**: 932–942.
- Zeisler H, Llorca E, Chantraine F, Vatis M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dinkel C, Schoedl M, Dilba P, Hund M, Verlohren S. Soluble fms-like tyrosine kinase-1-to-placental growth factor ratio and time to delivery in women with suspected preeclampsia. *Obstet Gynecol* 2016; **128**: 261–269.
- Hund M, Allegranza D, Schoedl M, Dilba P, Verhagen-Kamerbeek W, Stepan H. Multicenter prospective clinical study to evaluate the prediction of short-term outcome in pregnant women with suspected preeclampsia (PROGNOSIS): study protocol. *BMC Pregnancy Childbirth* 2014; **14**: 324.
- Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, Lim KH, Wenger JB, Thadhani R, Karumanchi SA. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation* 2012; **125**: 911–919.
- Sabriá E, Lequerica-Fernández P, Ganuza PL, Ángeles EE, Escudero AI, Martínez-Morillo E, Álvarez FV. Use of the sFlt-1/PlGF ratio to rule out preeclampsia requiring delivery in women with suspected disease. Is the evidence reproducible? *Clin Chem Lab Med* 2018; **56**: 303–311.
- Schaarschmidt W, Rana S, Stepan H. The course of angiogenic factors in early- vs. late-onset preeclampsia and HELLP syndrome. *J Perinat Med* 2013; **41**: 511–516.
- Baltajian K, Bajracharya S, Salahuddin S, Berg AH, Geahchan C, Wenger JB, Thadhani R, Karumanchi SA, Rana S. Sequential plasma angiogenic factors levels in women with suspected preeclampsia. *Am J Obstet Gynecol* 2016; **215**: 89.e1–10.
- Holmes VA, Young IS, Patterson CC, Maresh MJ, Pearson DW, Walker JD, McCance DR; Diabetes and Preeclampsia Intervention Trial (DAPIT) Study Group. The role of angiogenic and antiangiogenic factors in the second trimester in the prediction of preeclampsia in pregnant women with type 1 diabetes. *Diabetes Care* 2013; **36**: 3671–3677.
- National Institute of Health and Care Excellence (NICE). NICE Guidelines. PlGF-based testing to help diagnose suspected pre-eclampsia (Triage PlGF test, Elecsys immunoassay sFlt-1/PlGF ratio, DELFIA Xpress PlGF 1-2-3 test, and BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio). <https://www.nice.org.uk/guidance/dg23> [May 2016].
- Stepan H, Herraiz I, Schlembach D, Verlohren S, Brennecke S, Chantraine F, Klein E, Lapaire O, Llorca E, Ramoni A, Vatis M, Wertaschnigg D, Galindo A. Implementation of the sFlt-1/PlGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: implications for clinical practice. *Ultrasound Obstet Gynecol* 2015; **45**: 241–246.
- Hund M, Verhagen-Kamerbeek W, Reim M, Messinger D, van der Does R, Stepan H. Influence of the sFlt-1/PlGF ratio on clinical decision-making in women with suspected preeclampsia – the PreOS study protocol. *Hypertens Pregnancy* 2015; **34**: 102–115.
- Klein E, Schlembach D, Ramoni A, Langer E, Bahlmann F, Grill S, Schaffnerath H, van der Does R, Messinger D, Verhagen-Kamerbeek WD, Reim M, Hund M, Stepan H. Influence of the sFlt-1/PlGF ratio on clinical decision-making in women with suspected preeclampsia. *PLoS One* 2016; **11**: e0156013.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:



Figure S1 Change in sFlt-1/PIGF ratio 2 weeks after baseline measurement in women with suspected pre-eclampsia in PROGNOSIS development and validation cohorts with sFlt-1/PIGF ratio ≤ 38 at baseline (a) and those with sFlt-1/PIGF ratio > 38 to < 85 at baseline (b), according to development of pre-eclampsia and/or adverse fetal outcome.

Figure S2 Change in sFlt-1/PIGF ratio 3 weeks after baseline measurement in women with suspected pre-eclampsia in PROGNOSIS development and validation cohorts with sFlt-1/PIGF ratio ≤ 38 at baseline (a) and those with sFlt-1/PIGF ratio > 38 to < 85 at baseline (b), according to development of pre-eclampsia and/or adverse fetal outcome.

Table S1 Criteria for suspected pre-eclampsia. Adapted from Hund *et al.*, 2014¹³

Table S2 Contingency table for performance of sFlt-1/PIGF ratio for ruling out onset of pre-eclampsia within 1 to 4 weeks from baseline visit in women with suspected pre-eclampsia

Table S3 Categorization of women with suspected pre-eclampsia, based on sFlt-1/PIGF ratio cut-off value at baseline *vs* after 2 and 3 weeks

Table S4 Analysis of change in sFlt-1 and PIGF as single biomarkers between visits and relation to occurrence of pre-eclampsia and/or adverse fetal outcome within 4 weeks from baseline visit in women with suspected pre-eclampsia