

# Preeclampsia

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## Prediction of Preeclampsia Using the Soluble fms-Like Tyrosine Kinase 1 to Placental Growth Factor Ratio A Prospective Cohort Study of Unselected Nulliparous Women

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See Editorial Commentary, pp 578–579

**Abstract**—We sought to assess the ratio of sFlt-1 (soluble fms-like tyrosine kinase 1) to PIGF (placental growth factor) in maternal serum as a screening test for preeclampsia in unselected nulliparous women with a singleton pregnancy. We studied 4099 women recruited to the POP study (Pregnancy Outcome Prediction) (Cambridge, United Kingdom). The sFlt-1:PIGF ratio was measured using the Roche Cobas e411 platform at  $\approx 20$ ,  $\approx 28$ , and  $\approx 36$  weeks of gestational age (wkGA). Screen positive was defined as an sFlt-1:PIGF ratio  $>38$ , but higher thresholds were also studied. At 28 wkGA, an sFlt-1:PIGF ratio  $>38$  had a positive predictive value (PPV) of 32% for preeclampsia and preterm birth, and the PPV was similar comparing women with low and high prior risk of disease. At 36 wkGA, an sFlt-1:PIGF ratio  $>38$  had a PPV for severe preeclampsia of 20% in high-risk women and 6.4% in low-risk women. At 36 wkGA, an sFlt-1:PIGF ratio  $>110$  had a PPV of 30% for severe preeclampsia, and the PPV was similar comparing low- and high-risk women. Overall, at 36 wkGA, 195 (5.2%) women either had an sFlt-1:PIGF ratio of  $>110$  or an sFlt-1:PIGF ratio  $>38$  plus maternal risk factors: 43% of these women developed preeclampsia, about half with severe features. Among low-risk women at 36 wkGA, an sFlt-1:PIGF ratio  $\leq 38$  had a negative predictive value for severe preeclampsia of 99.2%. The sFlt-1:PIGF ratio provided clinically useful prediction of the risk of the most important manifestations of preeclampsia in a cohort of unselected nulliparous women. (*Hypertension*. 2017;69:731-738. DOI: 10.1161/HYPERTENSIONAHA.116.08620.) • [Online Data Supplement](#)

**Key Words:** clinical markers ■ cohort studies ■ immunoassay ■ pregnancy ■ risk factors

Preeclampsia is one of the most common adverse outcomes of pregnancy.<sup>1</sup> The condition consists of new onset hypertension and proteinuria in the second half of pregnancy but can also be superimposed on preexisting hypertension or renal disease. It is associated with increased risks of maternal and perinatal morbidity and mortality. A substantial proportion of severe adverse perinatal outcomes occur as a consequence of preterm birth because of preeclampsia, and a substantial proportion of adverse maternal outcomes occurs in severe preeclampsia.<sup>1</sup>

Preeclampsia is associated with an altered maternal pattern of circulating placentally derived proteins regulating angiogenesis,<sup>2,3</sup> such as sFlt-1 (soluble fms-like tyrosine

kinase 1) and PIGF (placental growth factor). One of the simplest methods to quantify the pattern is to calculate the ratio between these 2 angiogenic factors in maternal serum. A recent study of women with clinically suspected disease demonstrated that an sFlt-1:PIGF ratio cutoff of 38 provided clinically useful prediction of the risk of preeclampsia.<sup>4</sup> Higher sFlt-1:PIGF ratios, namely  $>85$  at 28 weeks of gestational age (wkGA) and  $>110$  at 36 wkGA, have been shown to be more strongly associated with the risk of preeclampsia.<sup>5</sup> However, evidence for the diagnostic effectiveness of the ratio in screening women without clinical suspicion of the disease is poor. A meta-analysis published in 2015 concluded that further studies were required.<sup>6</sup>

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The aim of the present analysis was to evaluate the effectiveness of the sFlt-1:PIGF ratio as a screening test for preeclampsia in unselected nulliparous women recruited to the POP study (Pregnancy Outcome Prediction).<sup>7,8</sup> Most of the participants were healthy because the cohort selection was solely based on nulliparity, singleton pregnancy, and the study catchment area. We analyzed the sFlt-1:PIGF ratio measured repeatedly at 20, 28, and 36 wkGA using the Roche Cobas e411 Elecsys immunoassay system, which has been certified by the Conformité Européenne mark for use as an in vitro medical device. Screen positive was defined on the basis of the previously described and validated cutoff of  $>38$ .<sup>4</sup> We studied the most clinically important manifestations of preeclampsia, namely any severity of the disease leading to preterm birth or preeclampsia with severe features.

## Methods

### Study Design

The POP study was conducted at the Rosie Hospital, Cambridge, United Kingdom, as previously described.<sup>7,8</sup> In brief, it was a prospective cohort study of nulliparous women attending the hospital for their dating ultrasound scan between January 14, 2008 and July 31, 2012 with a viable singleton pregnancy. The only clinical exclusion criterion was multiple pregnancy. The population was drawn from Cambridge and surrounding areas, with low rates of severe socioeconomic deprivation. Therefore, the cohort can be considered a population with low prior risk of disease and homogeneous from a socioeconomic perspective. Blood was obtained at the time of recruitment (not analyzed in this study). Study participants attended the NIHR (National Institute for Health Research) Cambridge Clinical Research Facility at  $\approx 20$ ,  $\approx 28$ , and  $\approx 36$  wkGA for blood sampling and ultrasound scans. Ethical approval was given by the Cambridgeshire 2 Research Ethics Committee (reference number 07/H0308/163) and all participants provided written informed Consent.

### Outcome Data

Outcome data were ascertained by review of each woman's paper case record by research midwives and by record linkage to clinical electronic databases of ultrasonography (Astraia, Munchen, Germany), delivery (Protos; iSoft, Banbury, United Kingdom), biochemical tests (Meditech, Westwood, MA), and neonatal intensive care (Badgernet; Clevermed Ltd, Edinburgh, United Kingdom). Where preeclampsia was suspected on the basis of these data, there was a second review of the clinical case record to confirm the diagnosis and classification (ie, with or without severe features) on the basis of the objective criteria of the 2013 ACOG Guideline (The American Congress of Obstetricians and Gynecologists; [online-only Data Supplement](#)).<sup>9</sup> Superimposed preeclampsia was defined as preeclampsia in women with preexisting renal disease or hypertension. Socioeconomic status was quantified using the Index of Multiple Deprivation,<sup>10</sup> and birth weight percentiles were calculated using a population-based United Kingdom reference.<sup>11</sup>

### Analysis Plan and Reporting

The definition of exposures, primary outcomes, secondary outcomes, and sensitivity analyses were agreed in an analysis plan ([online-only Data Supplement](#)) before performing any analysis of the sFlt-1 and PIGF data. Analyses which were not predefined are identified as such. The primary outcome for the 20 wkGA measurement was a composite of (1) preeclampsia with delivery before 28 wkGA or (2) preeclampsia with delivery before 37 wkGA where the onset of hypertension was before 28 wkGA. The primary outcome for the 28 wkGA measurement was preeclampsia with delivery before 37 wkGA. The primary outcome for the 36 wkGA measurement was preeclampsia with severe features

(ie, preeclampsia with either severe hypertension or evidence of hepatic, renal, hematologic, cerebral, or pulmonary complications; [online-only Data Supplement](#)). Secondary outcomes are defined in the [online-only Data Supplement](#). High risk of preeclampsia was defined as either (1) maternal characteristics, using the UK National Institute for Health and Care Excellence Guideline ([online-only Data Supplement](#))<sup>12</sup> or (2) elevated 20 wkGA uterine artery Doppler, defined as a mean pulsatility index in the highest decile, as previously described.<sup>8</sup> Family history of preeclampsia was not included in the definition of risk status because this information was not available. The reporting of this study conforms to the STROBE (The Strengthening the Reporting of Observational Studies in Epidemiology) statement.

### Samples and Immunoassays

Serum samples were collected as previously reported<sup>6</sup> and stored at  $-80^{\circ}\text{C}$ . All samples used in the current analysis had not previously been thawed before the day of analysis. Researchers performing the assays were blinded to the patients' clinical information and pregnancy outcome. Maternal serum levels of sFlt-1 and PIGF were measured using Roche Elecsys assays on the electrochemiluminescence immunoassay platform, Cobas e411 (Roche Diagnostics). Using this system, the intra-assay coefficient of variation for human serum samples is  $<2\%$  for sFlt-1 and PIGF, and the interassay coefficients of variation are 2.3% to 4.3% for the sFlt-1 assay and 2.7% to 4.1% for the PIGF assay. Screen positive was defined as sFlt-1:PIGF ratio of  $>38$ .<sup>4</sup> We also studied more severe elevation of the ratio, namely,  $>85$  at 28 wkGA and  $>110$  at 36 wkGA.<sup>5</sup>

### Statistical Analysis

Full details of the statistical analysis are described in the Analysis plan ([online-only Data Supplement](#)). In brief, standard screening summary statistics were calculated from  $2 \times 2$  tables. In addition, sFlt-1:PIGF ratio was analyzed as a continuous variable using the area under the receiver operating characteristic curve (AUROC). Time-to-event analysis was performed where delivery with the given outcome was the event and delivery where the outcome was treated as a competing risk, and this method was used to generate plots of the cumulative incidence of the outcome from the time of measurement. All analyses were performed using Stata 14.1.

### Exclusions and Missing Data

Of the 4512 women recruited, 67 (1.5%) women withdrew and 233 (5.2%) delivered elsewhere, leaving 4212 eligible women.<sup>8</sup> Of these, 5 (0.1%) did not have preeclampsia status available, and 108 (2.6%) did not have any sFlt-1:PIGF measurements available from the 28 or 36 wkGA visits.

## Results

### Description of the Study Cohort

The study group consisted of 4099 women, of whom 3953 had sFlt-1:PIGF measurement available from the 20 wkGA visit, 3989 from the 28 wkGA visit, and 3776 from the 36 wkGA visit. The overall incidence of preeclampsia was 6.5% (265/4099). The incidence of preterm preeclampsia with onset before 28 wkGA after the 20 wkGA measurement was 0.10% (4/3953). The incidence of preeclampsia leading to preterm delivery following the 28 wkGA measurement was 0.65% (26/3989). The incidence of delivery with severe preeclampsia after the 36 wkGA measurement was 2.8% (106/3776). The characteristics of the cohort are tabulated according to their experience of hypertensive complications of pregnancy (Table 1). In normotensive women, the median sFlt-1:PIGF ratios were 6.5, 2.7, and 11.2 at 20, 28, and 36 wkGA, respectively. The median ratio at 28 wkGA

**Table 1. Characteristics of the Study Cohort (n=4099)**

Characteristic	No Hypertensive Disorder	Preterm Preeclampsia	Preeclampsia With Severe Features*	Preeclampsia Without Severe Features*	Gestational Hypertension
n (%)	3751 (91.5)	26 (0.6)	111 (2.7)	128 (3.1)	83 (2.0)
<b>Maternal characteristics</b>					
Age, y	30 (27 to 33)	29 (25 to 34)	30 (26 to 34)	29 (26 to 33)	29 (25 to 33)
Age stopped FTE, y	21 (18 to 23)	18 (16 to 21)	19 (17 to 22)	21 (18 to 23)	21 (17 to 22)
Missing	105 (2.8)	1 (3.9)	4 (3.6)	7 (5.5)	5 (6.0)
Height, cm	165 (161 to 170)	163 (158 to 167)	165 (160 to 168)	165 (161 to 168)	165 (160 to 168)
<b>Deprivation quartile</b>					
1 (lowest)	917 (24)	7 (27)	26 (23)	27 (21)	20 (24)
2	899 (24)	2 (7.7)	28 (25)	33 (26)	14 (17)
3	897 (24)	12 (46)	26 (23)	28 (22)	21 (25)
4 (highest)	889 (24)	5 (19)	28 (25)	34 (27)	18 (22)
Missing	149 (3.7)	0 (0.0)	3 (2.7)	6 (4.7)	10 (12)
<b>Ethnicity</b>					
Non-white	211 (5.6)	2 (7.7)	5 (4.5)	4 (3.1)	5 (6.0)
White	3478 (93)	23 (88)	105 (95)	124 (97)	73 (88)
Missing	62 (1.7)	1 (3.9)	1 (0.9)	0 (0.0)	5 (6.0)
Married	2558 (68)	21 (81)	74 (67)	80 (63)	56 (67)
Smoker	182 (4.9)	1 (3.9)	3 (2.7)	7 (5.5)	8 (9.6)
Any alcohol consumption	172 (4.6)	0 (0.0)	4 (3.6)	4 (3.1)	6 (7.2)
Missing	1 (<0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Body mass index, kg/m <sup>2</sup>	24 (22 to 27)	28 (26 to 30)	25 (23 to 32)	28 (23 to 32)	27 (24 to 31)
Missing	1 (<0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Type 1 or type 2 DM	7 (0.2)	3 (12)	0 (0.0)	2 (1.6)	2 (2.4)
Chronic hypertension	95 (2.5)	9 (35)	34 (31)	74 (58)	0 (0.0)
Renal disease	28 (0.8)	1 (3.9)	3 (2.7)	8 (6.3)	0 (0.0)
UtA mean PI highest decile	335 (8.9)	11 (42)	23 (21)	21 (16)	9 (11)
Missing	91 (2.4)	2 (7.7)	1 (0.9)	7 (5.5)	3 (3.6)
<b>Birth outcomes</b>					
Birth weight, g	3425 (3110 to 3735)	2210 (1650 to 2660)	3425 (3050 to 3750)	3383 (3100 to 3785)	3501 (3190 to 3830)
Birth weight, z score	-0.15 (-0.71 to 0.41)	-0.61 (-1.15 to 0.06)	-0.14 (-0.73 to 0.63)	-0.03 (-0.69 to 0.40)	-0.15 (-0.59 to 0.71)
Birth weight, centile	44 (24 to 66)	28 (13 to 52)	45 (23 to 73)	49 (25 to 66)	44 (28 to 76)
Missing	1 (<0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gestational age, wk	40 (39 to 41)	35 (34 to 36)	40 (39 to 41)	40 (39 to 41)	40 (39 to 41)
Induction of labor	1115 (30)	6 (23)	70 (63)	82 (64)	37 (45)
<b>Mode of delivery</b>					
Spontaneous vaginal	1896 (51)	6 (23)	25 (23)	42 (33)	31 (37)
Assisted vaginal	873 (23)	0 (0.0)	29 (26)	41 (32)	23 (28)
Intrapartum cesarean	616 (16)	3 (12)	43 (39)	31 (24)	21 (25)
Prelabor caesarean	356 (9.5)	17 (65)	13 (12)	14 (11)	8 (10)
Missing	10 (0.3)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)

(Continued)

Table 1. Continued

Characteristic	No Hypertensive Disorder	Preterm Preeclampsia	Preeclampsia With Severe Features*	Preeclampsia Without Severe Features*	Gestational Hypertension
sFlt-1:PIGF ratio					
At 20 wkGA	6.5 (4.4 to 9.4)	9.1 (5.1 to 15.9)	6.9 (5.0 to 9.1)	6.9 (4.5 to 10.6)	6.3 (4.2 to 8.4)
Missing	125 (3.3)	4 (15)	6 (5.4)	7 (5.5)	4 (4.8)
At 28 wkGA	2.7 (1.7 to 4.2)	6.5 (3.4 to 22.5)	3.9 (2.4 to 7.8)	3.9 (2.3 to 6.9)	2.6 (1.8 to 4.3)
Missing	101 (2.7)	0 (0.0)	4 (3.6)	3 (2.3)	2 (2.4)
At 36 wkGA	11.2 (5.1 to 22.6)	126.2 (64.5 to 187.7)	42.3 (22.6 to 88.2)	35.3 (18.4 to 65.3)	20.2 (8.2 to 38.3)
Missing	274 (7.3)	20 (77)	9 (8.1)	11 (8.6)	9 (11)

Data are expressed as median (IQR) or n (column %) as appropriate. For fields where there is no category labeled missing, data were 100% complete. Maternal age was defined as age at recruitment. All other maternal characteristics were defined by self-report at the 20 wkGA interview, from examination of the clinical case record, or linkage to the hospital's electronic databases. Deprivation was quantified using the Index of Multiple Deprivation 2007,<sup>10</sup> which is based on census data from the area of the mother's postcode. Birth weight percentiles and z scores were calculated using a population-based UK reference.<sup>11</sup> Gestational hypertension was defined as development of hypertension in the second half of pregnancy in a previously normotensive woman who did not fulfill the diagnostic criteria for preeclampsia (online-only Data Supplement). DM indicates diabetes mellitus; FTE, full-time education; IQR, interquartile range; PI, pulsatility index; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; UTA, uterine artery; and wkGA, weeks of gestational age.

\*Confined to women delivering  $\geq 37$  wkGA.

was 6.5 (interquartile range, 3.4–22.5) in women who developed preterm preeclampsia, and the median ratio at 36 wkGA was 42.3 (interquartile range, 22.6–88.2) in women who had severe preeclampsia.

### Screening Performance at 20 wkGA

None of the 4 women who experienced the primary outcome after the 20 wkGA measurement had an sFlt-1:PIGF ratio  $>38$ . The AUROCC for the sFlt-1:PIGF ratio was 0.70 (95% confidence interval, 0.43–0.97; Figure 1A). Ten women had a ratio  $>38$ , and 1 woman had a ratio  $>85$  at 20 wkGA. Further analysis was not performed because of the small number of events.

### Screening Performance at 28 wkGA

The AUROCC for the sFlt-1:PIGF ratio was 0.80 (95% confidence interval, 0.70–0.89; Figure 1B). Women with an sFlt-1:PIGF ratio  $>38$  (n=19) had an incidence of preeclampsia

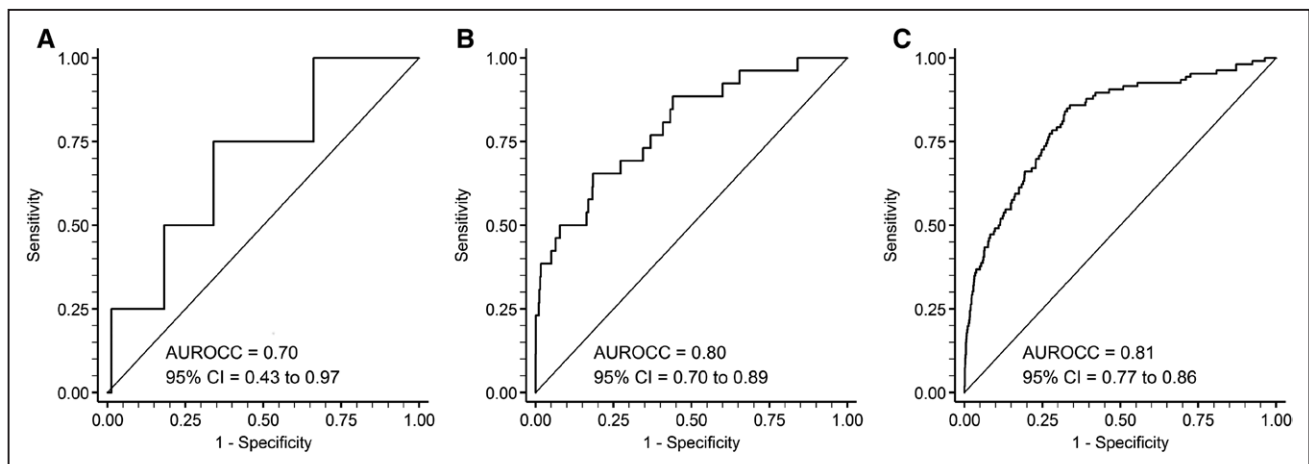
leading to preterm delivery of 32% (Table 2). The positive predictive value (PPV) was similar in low- and high-risk women (33% versus 31%, respectively;  $P=0.91$ ).

### Screening Performance at 36 wkGA

The AUROCC for the sFlt-1:PIGF ratio was 0.81 (95% confidence interval, 0.77–0.86; Figure 1C). Women with an sFlt-1:PIGF ratio  $>38$  (n=566) had an incidence of severe preeclampsia of 10% (Table 2). The PPV was 20% in high-risk women and 6.4% in low-risk women. Among women with no prior risk factors, an sFlt-1:PIGF ratio  $\leq 38$  had a high negative predictive value for subsequent development of severe preeclampsia ( $>99\%$ ).

### Analysis of Severe Elevation of the sFlt-1:PIGF Ratio

We studied more severe elevation of the ratio, using pre-defined thresholds, namely, 85 at 28 wkGA and 110 at 36



**Figure 1.** Receiver operating characteristic curve analysis of the relationship between (A) sFlt-1 (soluble fms-like tyrosine kinase 1) to PIGF (placental growth factor) ratio at 20 weeks of gestational age (wkGA) and (1) preeclampsia with delivery before 28 wkGA or (2) preeclampsia with delivery before 37 weeks where the onset of hypertension was before 28 wkGA (n=4), (B) sFlt-1:PIGF at 28 wkGA and preeclampsia leading to preterm birth (n=26), and (C) sFlt-1:PIGF at 36 wkGA and severe preeclampsia (n=106). The continuous sFlt-1:PIGF ratio is used, and the area under the receiver operating characteristic curve (AUROCC) with 95% confidence interval (CI) is given for each analysis.

**Table 2. Screening Statistics for the Primary Outcomes by Maternal Risk Status Using the Threshold of sFlt-1:PIGF Ratio of >38 at 28 and 36 wkGA**

Screening Statistic (95% CI)	28 wkGA			36 wkGA		
	Preeclampsia With Preterm Delivery			Preeclampsia With Severe Features		
	All	High Risk	Low Risk	All	High Risk	Low Risk
Sensitivity (%)	23.1 (6.9–39.3)	22.2 (3.0–41.4)	25.0 (0.0–55.0)	54.7 (45.2–64.2)	53.3 (40.7–66.0)	56.5 (42.2–70.8)
Specificity (%)	99.7 (99.5–99.8)	98.8 (98.0–99.6)	99.9 (99.8–100.0)	86.2 (85.0–87.3)	80.6 (77.5–83.6)	87.4 (86.2–88.5)
Positive predictive value (%)	31.6 (10.7–52.5)	30.8 (5.7–55.9)	33.3 (0.0–71.1)	10.2 (7.7–12.7)	20.3 (14.0–26.5)	6.4 (4.0–8.7)
Negative predictive value (%)	99.5 (99.3–99.7)	98.2 (97.2–99.1)	99.8 (99.7–100.0)	98.5 (98.1–98.9)	94.9 (93.1–96.7)	99.2 (98.9–99.6)
Positive likelihood ratio*	70.3 (29.0–170.8)	18.6 (6.3–54.9)	200.6 (42.6–944.1)	4.0 (3.3–4.8)	2.7 (2.1–3.6)	4.5 (3.4–5.9)
Negative likelihood ratio†	0.77 (0.63–0.95)	0.79 (0.61–1.01)	0.75 (0.50–1.12)	0.53 (0.43–0.65)	0.58 (0.44–0.76)	0.50 (0.36–0.69)

CI indicates confidence interval; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; and wkGA, weeks of gestational age (of measurement of the sFlt-1:PIGF ratio).

\*Positive likelihood ratio was defined as the ratio between the proportions of screen positives among cases and screen positives among noncases ( $(S+ID+)/[(S+ID-)]$ ).

†Negative likelihood ratio was defined as the ratio between the proportions of screen negatives among cases and screen negatives among noncases ( $[(S-ID+)/(S-ID-)]$ ). Raw data from 2×2 tables are shown in Tables S1 and S2 in the [online-only Data Supplement](#).

wkGA (Table 3). Only 7 women had an sFlt-1:PIGF ratio >85 at 28 wkGA. However, 4 out of 7 delivered preterm with a diagnosis of preeclampsia (PPV=57%). At 36 wkGA, 70 women had an sFlt-1:PIGF ratio >110, and 21 developed severe preeclampsia (PPV=30%). The PPV was similar comparing women with and without prior risk factors (36% and 24%, respectively).

### Screening Performance at 36 wkGA Using the sFlt-1:PIGF Ratio Combined With Maternal Risk Factors

We then analyzed a composite definition of screen positive at 36 wkGA, namely, an sFlt-1:PIGF ratio of >110 irrespective of maternal risk factors or an sFlt-1:PIGF ratio >38 combined with maternal risk factors. A total of 195 (5.2%) women screened positive by this definition and 43% of them subsequently delivered with a diagnosis of preeclampsia: 41 women (PPV=21%) developed preeclampsia with severe features, and 43 (PPV=22%) developed preeclampsia without severe features. The characteristics and outcomes for the 195 women are summarized ([online-only Data Supplement](#)).

### Time-to-Event Analysis

We plotted the cumulative incidence of the primary outcomes after the 28 and 36 wkGA measurements of the sFlt-1:PIGF ratio using a competing risks model (Figure 2A and B). The 36 wkGA measurements were stratified by maternal risk status (Figure 2B). In both cases, the curves started to deviate at least 1 week after the time of measurement, and the proportional increase in risk was maintained over the 7 to 8 weeks after the test. We also plotted the cumulative incidence of preeclampsia for women at 36 wkGA with the composite definition of screen positive (Figure 2C), that is, a ratio >38 plus risk factors or a ratio of >110 irrespective of risk factors. Delivery without the primary outcome was treated as a competing risk in all 3 analyses. In all 3 plots, it is evident that >90% of the deliveries in the highest risk group occurred >1 week from the time of measurement of the ratio.

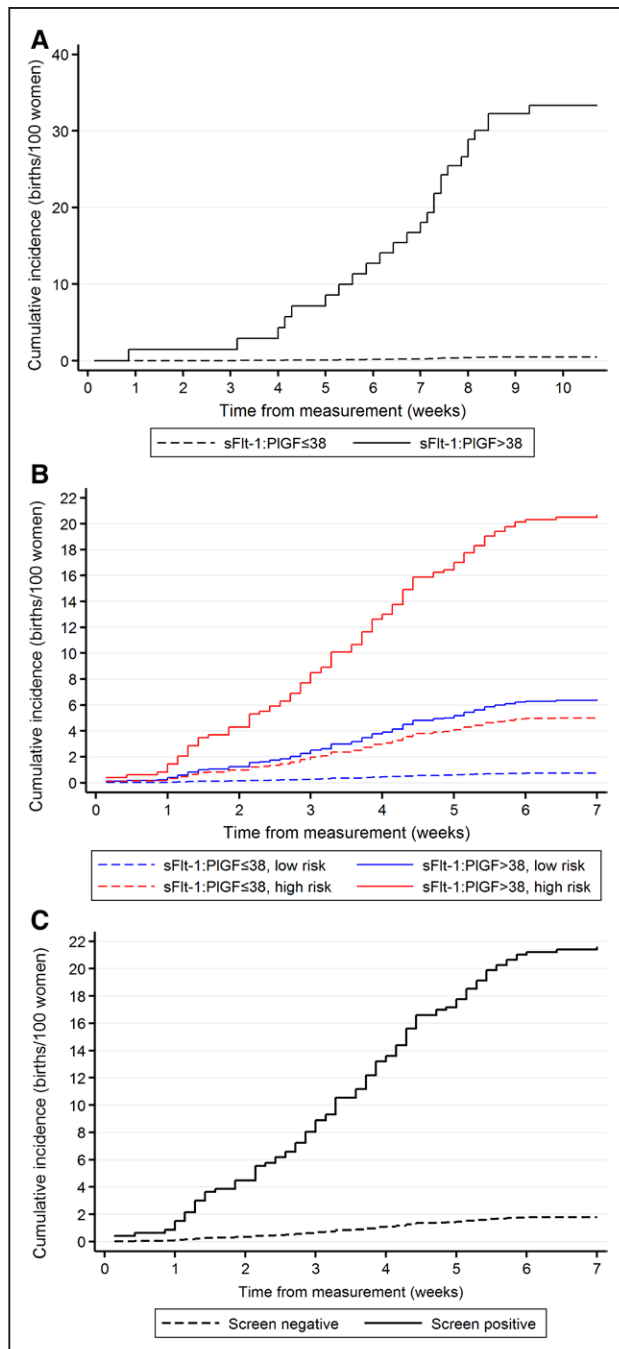
### Discussion

The main finding of this study is that, in a cohort of unselected, first singleton pregnancies, measurement of the sFlt-1:PIGF ratio identified women with a high absolute risk

**Table 3. Screening Statistics for the Primary Outcomes by Maternal Risk Status Using the Threshold of sFlt-1:PIGF Ratio of >85 at 28 wkGA and >110 at 36 wkGA**

Screening Statistic (95% CI)	28 wkGA		36 wkGA		
	Preeclampsia With Preterm Delivery		Preeclampsia With Severe Features		
	All	High Risk	All	High Risk	Low Risk
Sensitivity (%)	15.4 (1.5–29.3)	19.8 (12.2–27.4)	20.0 (9.9–30.1)	19.6 (8.1–31.0)	
Specificity (%)	99.9 (99.8–100.0)	98.7 (98.3–99.0)	96.8 (95.4–98.1)	99.1 (98.7–99.4)	
Positive predictive value (%)	57.1 (20.5–93.8)	30.0 (19.3–40.7)	36.4 (20.0–52.8)	24.3 (10.5–38.1)	
Negative predictive value (%)	99.4 (99.2–99.7)	97.7 (97.2–98.2)	92.9 (90.9–94.8)	98.8 (98.4–99.2)	
Positive likelihood ratio	203.2 (47.8–863.3)	14.8 (9.2–23.8)	6.2 (3.2–11.9)	21.1 (10.6–42.2)	
Negative likelihood ratio	0.85 (0.72–1.00)	0.81 (0.74–0.89)	0.83 (0.73–0.94)	0.81 (0.70–0.94)	

CI indicates confidence interval; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; and wkGA denotes weeks of gestational age (of measurement of the sFlt-1:PIGF ratio). See Methods for definition of risk status and Tables S1 and S2 for raw data from 2×2 tables.



**Figure 2.** Cumulative incidence of the primary outcomes (Methods) by sFlt-1 (soluble fms-like tyrosine kinase 1) to PlGF (placental growth factor) ratio: (A) sFlt-1:PlGF at 28 wkGA and preeclampsia leading to preterm birth, (B) sFlt-1:PlGF at 36 wkGA and severe preeclampsia, stratified by maternal risk. High risk was defined on the basis of maternal risk factors or 20 wkGA uterine artery Doppler (see Methods for details), and (C) composite risk status at 36 wkGA. Screen positive was defined as (1) sFlt-1:PlGF ratio of >38 and maternal risk factors or (2) sFlt-1:PlGF ratio >110 irrespective of maternal risk factors. Screen negative was defined as all other women. Delivery without the given primary outcome was treated as a competing risk in all 3 analyses. Hence, the maximum value of the cumulative incidence is the same as the positive predictive value, and the curve illustrates the distribution of the timing of the deliveries with the outcome in question.

of experiencing the clinically most important manifestations of preeclampsia. At 28 wkGA, an sFlt-1:PlGF ratio >38 identified women with a high risk (>30%) of subsequently delivering preterm with preeclampsia. Women who had a more severe elevation of the ratio (>85) had nearly 60% risk of delivering preterm with preeclampsia, whereas >99% of women who had a ratio of <38 did not develop the outcome. At 36 wkGA, ≈5% of women were identified as high risk on the basis of either an sFlt-1:PlGF ratio of >110 or an sFlt-1:PlGF ratio >38 plus maternal risk factors. Of this group, 43% were subsequently delivered with a diagnosis of preeclampsia, and about half of these cases were severe. Approximately 70% of women were identified as low risk at 36 wkGA, that is, they had no maternal risk factors and an sFlt-1:PlGF ratio ≤38; their risk of developing severe preeclampsia was <1%.

Screening is generally only conducted when there are evidence-based interventions which mitigate the risk. A key element in the study design was to make a measurement close to term (36 wkGA), the rationale being that delivery is the main intervention to prevent preeclampsia. This can more easily and more safely be performed at term.<sup>13</sup> Moreover, a randomized controlled trial has shown improved outcome after immediate induction of labor compared with expectant management in women with gestational hypertension or mild preeclampsia near term.<sup>14</sup> In this study, we evaluated the diagnostic effectiveness of the sFlt-1:PlGF ratio in identifying women at risk of developing preeclampsia. In light of our results, we hypothesize that one approach to reducing the burden of morbidity associated with preeclampsia could be to screen nulliparous women at 36 wkGA using maternal risk factors and sFlt-1:PlGF ratio, monitor screen positive women closely, and perform induction of labor before the development of severe disease. This hypothesis could be readily tested in a randomized controlled trial evaluating whether the introduction of the screening test improves pregnancy outcome (clinical effectiveness). Such an intervention is unlikely to cause harm, and we have recently demonstrated that routine induction of labor at 39 wkGA does not increase the risk of cesarean delivery or perinatal morbidity in another high-risk population of nulliparous women, namely, those aged ≥35.<sup>15</sup>

The sFlt-1:PlGF ratio was also informative for the risk of preterm preeclampsia. Women with a ratio >38 at 28 wkGA had a 32% risk of preterm delivery with preeclampsia, and the ratio >38 had a high positive likelihood ratio (≈70). This may reflect the fact that this threshold represents much more significant elevation at 28 wkGA (99.5th percentile) than 36 wkGA (85th percentile). However, the sensitivity of the sFlt-1:PlGF ratio >38 at 28 wkGA was only 23%. Although the POP study included mostly healthy women, this is consistent with the findings in women clinically suspected to have preeclampsia, where an elevated sFlt-1:PlGF ratio between 24 and 37 wkGA was also associated with an increased risk of developing the disorder within 4 weeks after the measurement.<sup>16</sup> Hence, although the test provides clinically useful prediction of risk for a small proportion of women, the majority of women experiencing the disease would not be identified using the test at 28 wkGA. However, the AUROC for the sFlt-1:PlGF ratio at 28 wkGA for preterm preeclampsia was 0.80. It is possible that combining the ratio with other measurements (clinical,

biomarker, and ultrasonic) in a multivariable model might provide better risk prediction. This is an area of further investigation, which will be hopefully paralleled by studies aiming at the implementation of better treatment options. Currently, the main limitation of the clinical usefulness of the 28 wkGA measurement is the lack of a clearly effective intervention mitigating the risks for those who screen positive, other than close monitoring of the patient. Another important area for further study is to refine the estimation of risk in women whose 36 wkGA assessment identified them as being at intermediate risk (5%–6%) of severe preeclampsia, namely, women with an sFlt-1:PlGF ratio >38 and no risk factors or risk factors but a ratio of ≤38. Possible approaches include identifying other informative biomarkers or repeating measurement of the sFlt-1:PlGF ratio after 36 wkGA.

This study had many methodological strengths over previous studies. First, the size was sufficiently large that we were able to study the variants of preeclampsia associated with the most severe complications. Second, we used a clinically validated assay where the definition of screen positive was based on prior studies which had identified and validated the chosen threshold. Moreover, the sFlt-1:PlGF ratio can be calculated without modeling in relation to gestational age or maternal characteristics, and in this study, clinical care was provided without knowledge of the test result. Finally, the analyses in this study were planned and specified in advance.

A large-scale study on screening women using the sFlt-1:PlGF ratio has recently been reported.<sup>17</sup> However, their findings are difficult to compare to this study because they pooled the results from a wide range of gestational ages (30–37 wkGA). Moreover, almost half of their population consisted of multiparous women who had not previously experienced preeclampsia, and this is a group with a very low prior risk of disease. The PPV of a test depends both on the a priori risk and the positive likelihood ratio. It is difficult to interpret a summary estimate of PPV when almost half the population has a very low prior risk of the outcome. Another large study based on a multiethnic cohort of nulliparous women and high-risk parous women concluded that angiogenic biomarkers measured in the first half of pregnancy performed poorly for predicting later development of preeclampsia.<sup>18</sup> They also observed, however, that the measurements became more strongly predictive when made closer to disease onset, but the analyses of late pregnancy data were limited by high rates of missing biomarker data (20%–30%). The focus of this study on nulliparous women was purposeful. One of the strongest clinical predictors of the risk of preeclampsia is whether a woman has a previous history of pregnancy affected by the condition. This information dominates risk prediction in multiparous women but is necessarily absent among nulliparous women. Another purposeful feature of the design of this study was measurement of biomarkers throughout gestation. Results reported in the current and previous studies<sup>13</sup> suggest that screening tests for pregnancy complications have a better predictive value when performed close to disease onset.

Many studies have evaluated trying to predict preeclampsia solely using measurements made in the first trimester. Statistical models are used to determine prior risk. Further modeling is used to process values of first trimester uterine artery Doppler flow velocimetry and to convert first trimester protein concentrations

into gestational age-corrected multiples of the median. Two models were externally evaluated in a prospective cohort study in Norway, where measurements were performed between 11 and 14 wkGA.<sup>19</sup> A study-derived threshold (10% false-positive rate) yielded PPVs of 5% to 12%, a sensitivity of 40% and positive likelihood ratios of 1.5 to 3.6 for all preeclampsia cases. Although the nature of that study does not allow direct comparison with this analysis, the current approach may be more likely to be clinically applicable, given that (1) the definition of screen positive was externally defined, (2) the PPVs were higher, (3) the outcome was confined to the clinically most significant cases, and (4) the handling of clinical and biochemical predictors is simpler.

## Perspectives

We conclude that measurement of the sFlt-1:PlGF ratio at 36 wkGA, combined with maternal risk factors, provides clinically useful prediction of the risk of preeclampsia at term for ≈3 quarters of unselected nulliparous women, identifying 5% of them as high risk and 70% as low risk. Screening the pregnant nulliparous population in late pregnancy using this measurement could plausibly improve maternal and perinatal outcome when coupled with close monitoring and induction of labor, and this would be an appropriate focus for future randomized controlled trials. Women with extreme elevation of the ratio at 28 wkGA have high absolute risks of preterm disease, and the test may be useful to identify women for the evaluation of candidate disease-modifying therapies as they become available.

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## Disclosures

M. Hund reports being an employee of Roche Diagnostics, holding stock in Roche, having a pending patent related to the sFlt-1 (soluble fms-like tyrosine kinase 1) to PlGF (placental growth factor) or endoglin:PlGF ratio to rule out onset of preeclampsia in pregnant women within a certain time period (PCT/EP2013/063115), holding pending patents related to the dynamic of sFlt-1 or endoglin:PlGF ratio as an indicator for imminent preeclampsia or the HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome or both (PCT/EP2012/072157) and the prediction of postpartum HELLP syndrome, postpartum eclampsia, or postpartum preeclampsia (PCT/EP2015/051457). G.C.S. Smith reports equipment loans and consumable support from Roche Diagnostics. The other authors report no conflicts.

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## Novelty and Significance

## What Is New?

Among a population of nulliparous women at mixed risk of disease:

- An sFlt-1 (soluble fms-like tyrosine kinase 1) to PIGF (placental growth factor) ratio  $>38$  at 28 weeks of gestational age identified women with a high risk ( $>30\%$ ) of subsequently delivering preterm with preeclampsia.
- An sFlt-1:PIGF ratio  $>110$  at 36 weeks of gestational age identified women with a high risk ( $>30\%$ ) of subsequently experiencing severe preeclampsia.
- An sFlt-1:PIGF ratio between  $>38$  and  $<110$  at 36 weeks of gestational age was only associated with a high absolute risk ( $>20\%$ ) of subsequently experiencing severe preeclampsia if the mother had additional risk factors.

## What Is Relevant?

- Measurement of the sFlt-1:PIGF ratio provides clinically useful information on risk of the most clinically important manifestations of preeclampsia among women having first pregnancies.
- This identifies potential new approaches to trials of screening and intervention.

## Summary

Very high elevation of the sFlt-1:PIGF ratio identifies women with high absolute risks of preterm or severe preeclampsia, and moderate elevation is informative of risk when combined with maternal risk factors.



**Prediction of Preeclampsia Using the Soluble fms-Like Tyrosine Kinase 1 to Placental Growth Factor Ratio: A Prospective Cohort Study of Unselected Nulliparous Women**  
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**ONLINE SUPPLEMENTAL MATERIAL**

**PREDICTION OF PREECLAMPSIA USING THE sFlt-1:PIGF RATIO: A PROSPECTIVE COHORT STUDY OF  
UNSELECTED NULLIPAROUS WOMEN**

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## Supplemental Methods

*Diagnosis of preeclampsia (based on the 2013 ACOG Guideline (PMID 24150027))*

Hypertension, for the purposes of diagnosis of preeclampsia, was defined as one or more of the following:

- 2x diastolic blood pressure (DBP) >89 mmHg at least 4 hours apart
- 1x DBP >89 mmHg + (new antihypertensive treatment AND/OR magnesium sulphate)
- 1x DBP >109 mmHg
- 2x systolic blood pressure (SBP) >139 mmHg at least 4 hours apart
- 1x SBP >139 mmHg + (new antihypertensive treatment AND/OR magnesium sulphate)
- 1x SBP >159 mmHg

Severe hypertension, for the purposes of diagnosis of preeclampsia with severe features, was defined as any one of the following:

- 2x DBP >109 mmHg at least 4 hours apart
- 2x SBP >159 mmHg at least 4 hours apart
- 1x DBP >109 mmHg + (new antihypertensive Rx AND/OR magnesium sulphate)
- 1x SBP >159 mmHg + (new antihypertensive Rx AND/OR magnesium sulphate)

Proteinuria was defined as one or more of the following:

- Greater than or equal to 300 mg per L in a 24 hour urine collection
- 2x dipstick reading of 1+ or greater 4 hours apart at >20 weeks gestational age

Low platelets was defined as:

- Platelets <100 at ≥ 24 weeks gestational age up to 48h after delivery in a woman with normal levels (>150) <20 weeks

Elevated creatinine was defined as:

- Creatinine >99mmol/L at ≥ 24 weeks gestational age up to 48h after delivery in women with normal levels or unrecorded <20 weeks. If the first onset of the hypertension was after delivery measurements up to 7d after birth were included in the definition.

Elevated alanine transaminase (ALT) was defined as:

- ALT >49 at ≥ 24 weeks gestational age up to 48h after delivery in a woman with normal levels or unrecorded <20 weeks

Pulmonary oedema was defined on the basis of whether it was documented as being present when reviewing the paper case record

Severe cerebral or visual disturbance was defined as:

- Severe cerebral or visual disturbance documented in the paper case record, plus evidence of significant clinical concern, including 2 or more measures of uric acid

Preeclampsia was defined as:

- Hypertension (defined above) AND (Proteinuria AND/OR low platelets AND/OR elevated ALT AND/OR elevated creatinine AND/OR pulmonary oedema AND/OR severe cerebral/visual symptoms)

Preeclampsia with severe features was defined as:

- Preeclampsia AND (severe hypertension AND/OR low platelets AND/OR elevated ALT AND/OR elevated creatinine AND/OR pulmonary oedema AND/OR severe cerebral/visual symptoms)

Gestational hypertension (GH) was defined as hypertension (as above) but not preeclampsia in a woman who was not hypertensive prior to 20 weeks and had no history of renal disease or essential hypertension.

Severe gestational hypertension was defined as GH AND severe hypertension

Pre-existing hypertension was defined as:

- Record of any documented history of essential hypertension or documented past or present use of anti-hypertensives when booking for antenatal care AND/OR any DBP>89 prior to 20 weeks gestational age AND/OR any SBP>139 prior to 20 weeks gestational age

Pre-existing renal disease was defined as:

- Record of any documented history of renal disease AND/OR heavy proteinuria prior to 20 weeks

Super-imposed preeclampsia

- If there is pre-existing hypertension or renal disease, then preeclampsia is defined as super-imposed. Diagnosis of superimposed preeclampsia required either new proteinuria or evidence of systemic involvement.

### *Analysis plan*

#### **Analysis plan for assessing the diagnostic effectiveness of the sFlt-1/PIGF ratio as a screening test for pre-eclampsia in the Pregnancy Outcome Prediction study (PMID 19019223 & 26360240).**

##### 1.1 Defining exposure

An existing study, PROGNOSIS (Hund et al, PMID 25230734), has evaluated the predictive value of knowing the Elecsys sFlt-1/PIGF immunoassay ratio in women with suspected pre-eclampsia (Zeisler et al, PMID 26735990). In that study, the ratio was treated as elevated when the value was >38 (ruling in preeclampsia within 4 weeks). We will examine the relationship between measurements above and below this threshold and the risk of pre-eclampsia using blood samples collected from a prospective cohort of nulliparous women, conducted in Cambridge, UK, namely, the Pregnancy Outcome Prediction study (PMID 19019223 & 26360240). The analysis will focus on three time points of measurement of the sFlt-1/PIGF ratio: (i) at ~20 weeks, (ii) at ~28 weeks, and (iii) at ~36 weeks. We will also evaluate the screening properties of greater elevation of the ratio (>85 at 20 & 28 weeks and >110 at 36 weeks) which have been previously described as diagnostic for pre-eclampsia (Stepan et al, PMID 25736847).

##### 1.2 Defining outcome

All definitions of outcome will be as per the 2013 ACOG classification (PMID 24150027). Super-imposed pre-eclampsia is defined as pre-eclampsia in women with previous renal disease or hypertension. "Severe" is defined in the ACOG Guideline.

##### 1.3 Primary and secondary outcomes

### *1.3.1 The sFlt-1/PIGF ratio at ~20 weeks*

The primary outcome for the 20 week measurement is pre-eclampsia with delivery prior to 28 weeks or pre-eclampsia with delivery prior to 37 weeks and where the onset of hypertension was prior to 28 weeks.

### *1.3.2 The sFlt-1/PIGF ratio at ~28 weeks*

The primary outcome for the 28 week measurement is pre-eclampsia with delivery prior to 37 weeks. Secondary outcomes for the 28 week measurement are (a) pre-eclampsia, with onset of hypertension prior to 37 weeks, (b) gestational hypertension, with delivery prior to 37 weeks, (c) gestational hypertension, with onset of hypertension prior to 37 weeks, (d) either pre-eclampsia or gestational hypertension with onset of hypertension prior to 37 weeks.

### *1.3.3 The sFlt-1/PIGF ratio at ~36 weeks*

The primary outcome for the 36 week measurement is pre-eclampsia with severe features. Secondary outcomes for the 36 week measurement are (a) pre-eclampsia without severe features, (b) severe gestational hypertension, (c) gestational hypertension, (d) either pre-eclampsia or gestational hypertension.

### 1.3.4 Sensitivity analyses

1.3.4.1 We will compare associations between super-imposed pre-eclampsia and pre-eclampsia in women without prior hypertension or renal disease.

1.3.4.2 We will also compare the predictive ability of the test, stratifying the cohort on the basis of being at high risk of pre-eclampsia. This will be defined on the basis of maternal characteristics (using the NICE Guideline recommendation of 2 moderate or 1 high risk factor) or the 20 week uterine artery Doppler (defined as a mean pulsatility index >90<sup>th</sup> percentile).

1.3.4.3 At each time point (20, 28 or 36 weeks), we will also analyse associations based on the previous measurement (i.e. 12, 20 or 28 weeks, respectively). We will then determine whether the associations between the outcomes and the sFlt-1/PIGF ratio differ according to the trend of increasing or decreasing value. Low will be defined as <38, moderate as 38-85/110 (depending on GA), and high as >85 at 20 or 28 weeks, or >110 at 36 weeks.

## 1.4 Analytic approach

Analysis will be performed using 2x2 tables and calculation of standard screening summary statistics (sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood ratios). We will also describe the association between pre-eclampsia and the sFlt-1/PIGF ratio as a continuous variable by plotting the receiver operating characteristic (ROC) curve and estimating the c statistic (= the area under the ROC curve). We will perform analyses for a ratio of >38 ruling in pre-eclampsia, and a ratio of <38 for ruling out pre-eclampsia. We will repeat the analysis for severe elevation of the ratio (>85 at 20 or 28 weeks, or >110 at 36 weeks).

Finally, we will analyse by time to event methods, i.e. a Kaplan-Meier plot of cumulative incidence of pre-eclampsia from time of measurement, and use a Cox model to generate HR for subsequent diagnosis with pre-eclampsia. We may also use time to event methods that account for competing risks (Akolekar et al, 2013, PMID 22906914). We will use these methods to perform secondary analyses of screening performance to rule in/out pre-eclampsia for 1, 2, 4 and 6 weeks.

## 1.5 Exploratory analyses

We will compare the predictive ability of the ratio using ROC curve analyses for different phenotypes of pre-eclampsia, specifically, associated with (i) maternal renal dysfunction, (ii) HELLP syndrome, or (iii) fetal growth restriction.

We will also determine the relationship between the risk of pre-eclampsia and the trend of the level of the sFlt-1/PlGF ratio (i.e. increasing or decreasing) treating the ratio as a continuous variable and modelling the change across gestation.

#### 1.6 Presentation of results.

In the publication, primary and secondary outcomes will be defined as above. Any analyses not described above will be clearly identified as not part of the original analysis plan.

#### 1.7 Statement

The above plan was drawn up prior to any analysis of the relationship between sFlt-1, PlGF, or the ratio of sFlt-1/PlGF and the maternal risk of pre-eclampsia in the Pregnancy Outcome Prediction study.

#### *Missing data*

Of 4,512 women recruited to the study, we excluded 300 (6.6%) women who were not eligible, 5 (0.1%) who did not have preeclampsia status available and 108 (2.6%) who did not have any sFlt-1:PlGF measurements available from the 28 or 36 week visits.

#### *Trend analyses*

Change in the sFlt-1:PlGF ratio was defined as the change in the measurement between the 28 and 36 week visits divided by the time difference between the measurements in weeks. The association between the change in the sFlt-1:PlGF ratio was quantified using the c statistic. We then determined whether the associations between the primary outcome and the sFlt-1:PlGF ratio differed according to the trend of increasing value, which was analysed in tertiles to ensure an adequate number of cases in each stratum.

### **Supplemental Results**

#### *Missing data*

Among the 4,099 women included in the study population, 3,953 had sFlt-1:PlGF measurement available from the 20 week visit, 3,989 from the 28 week visit and 3,776 from the 36 week visit. Among the variables used for defining risk status based on maternal characteristics, the proportion of missing values was low (105/4,099 = 2.6% in total, including 104 missing values in UtA PI from the 20 week visit and one missing value in BMI from the 12 week visit). We replaced them with the most common value for the purpose of defining high risk of preeclampsia in the entire study population, i.e. UtA PI  $\leq 90^{\text{th}}$  percentile and BMI < 35 kg/m<sup>2</sup>.

#### *Descriptive statistics*

Overall, 81% of the women were classified into the low risk and 19% were classified into the high risk category, based on maternal characteristics without considering the sFlt-1:PlGF measurement. Raw

data on screening for the primary outcomes by maternal risk status using different thresholds is given in **Tables S1 and S2**.

A total of 195 women were screen positive using a composite definition at 36wkGA (sFlt-1:PIGF ratio >110 irrespective of maternal risk factors or sFlt-1:PIGF ratio >38 with maternal risk factors). Their characteristics and outcomes are summarized in **Table S3**.

In total, 21 cases of severe preeclampsia had sFlt-1:PIGF ratio >110 at 36wkGA, and 32 cases had sFlt-1:PIGF ratio >38 and maternal risk factors. Twelve of these overlap (20 women had maternal risk factors and sFlt-1/PIGF ratio between 38 and 110, and 9 women had sFlt-1/PIGF ratio >110 but no maternal risk factors).

A total of 2,660 women were screen negative using a definition of sFlt-1:PIGF ratio of  $\leq 38$  and the absence of maternal risk factors. Their characteristics and outcomes are summarized in **Table S4**.

Of the 106 women who had pre-eclampsia with severe features, 72 had proteinuria, 69 had abnormal blood results (including 43 elevated creatinine, 30 elevated ALT and 18 low platelets), 67 had severe hypertension, ten had severe cerebral or visual disturbances and two had pulmonary oedema. The overlap of five of these features including blood abnormalities, hypertension and severe cerebral/visual disturbances has been described using a Venn diagram (**Figure S1**).

#### *Model performance*

For the 28wkGA and 36wkGA measurements, the area under the ROC curve (AUROCC) was similar regardless of the maternal risk status (**Figures S2 and S3**). The model performance was generally weaker for the secondary outcomes (**Figures S4 and S5**). When the primary outcomes were compared according to whether the preeclampsia was superimposed on prior hypertension or renal disease, the AUROCC was  $\sim 0.8$  in all analyses (**Figures S6 and S7**). No further analysis was performed using the 20wkGA measurement due to small number of events.

Of the secondary outcomes, gestational hypertension leading to preterm birth was strongly associated with the continuous sFlt-1:PIGF ratio at 28wkGA (**Table S5**). The association between the ratio measured at 36wkGA and any gestational hypertension was much weaker (AUROCC = 0.88 vs. 0.62, respectively).

Finally, the associations were analysed by phenotype of preeclampsia. The number of cases was small in the analysis of the sFlt-1:PIGF ratio and preeclampsia with renal dysfunction (n=1) or HELLP syndrome (n=2) leading to preterm birth (**Figure S8**). The sFlt-1:PIGF ratio at 36wkGA was strongly associated with preeclampsia combined with HELLP syndrome but not with preeclampsia combined with renal dysfunction (**Figure S9**).

#### *Trend analyses*

The primary outcome at 36wkGA was additionally analysed in relation to change in the sFlt-1:PIGF ratio between 28 and 36 week visit. A total of 3,666 women had both measurements available. The median change was 1.11 (IQR 0.35-2.67) per wkGA. The change in the sFlt-1:PIGF ratio was highly correlated with the sFlt-1:PIGF ratio at 36wkGA (spearman correlation coefficient = 0.98). The variation in the ratio at 36wkGA was much larger than at 28wkGA, and the change in the ratio was

driven by the 36wkGA measurement. Therefore, its association with the primary outcome at 36wkGA was very similar: the c statistic was 0.82 (95% CI 0.77–0.86). We did not observe substantial differences in the associations between the primary outcome and the sFlt-1:PIGF ratio according to the trend of increasing value although the c statistic was slightly higher in the highest tertile (**Table S6**).

#### *Proximity of measurement and the risk of preeclampsia*

We performed an analysis not described in the analysis plan to determine the relationship between the timing of screening and the strength of association with preeclampsia. In order to allow comparison of measurements made at different gestational ages, we studied the top decile of sFlt-1:PIGF ratio using the distribution of values observed in this study. The risk of preeclampsia resulting in preterm birth was increased in women with values of the ratio in the top decile at both 20wkGA and 28wkGA (**Figure S10**). However, the association was stronger for measurement at 28wkGA. Top decile of the sFlt-1:PIGF ratio at 20wkGA was not associated with the risk of preeclampsia with severe features at or near term but there were associations with both the 28wkGA and 36wkGA measurements. However, the association was stronger at 36wkGA. Hence, the ratio was most strongly predictive when it was measured closest to the timing of the given event.

#### *Cut offs within the study cohort*

We generated data derived cut offs for the prediction of preeclampsia based on the ROC curve using the sFlt-1:PIGF ratio at 28 weeks (Figure 1B). The cut off of sFlt-1:PIGF ratio of 14.6 resulted in 38% sensitivity and 98% specificity. We repeated the primary analysis with the new cut offs (**Table S7**). The positive predictive values were 12.7% for all women and 20.6% and 6.7% for high risk and low risk women, respectively.



**Table S1.** Raw data on screening for the primary outcomes by maternal risk status using the threshold of sFlt-1:PIGF ratio of >38 at 28 and 36wkGA.

<b>Screening result</b>	<b>28 wkGA, PE delivery&lt;37</b>			<b>36 wkGA, PE severe</b>		
	<i>All</i>	<i>High risk</i>	<i>Low risk</i>	<i>All</i>	<i>High risk</i>	<i>Low risk</i>
True positive (n)	6	4	2	58	32	26
False positive (n)	13	9	4	508	126	382
False negative (n)	20	14	6	48	28	20
True negative (n)	3950	745	3205	3162	522	2640

**Table S2.** Raw data on screening for the primary outcomes using the threshold of sFlt-1:PIGF ratio of >85 at 28wkGA and >110 at 36wkGA (by maternal risk status).

<b>Screening result</b>	<b>28 wkGA, PE delivery&lt;37</b>		<b>36 wkGA, PE severe</b>	
	<i>All</i>	<i>All</i>	<i>High risk</i>	<i>Low risk</i>
True positive (n)	4	21	12	9
False positive (n)	3	49	21	28
False negative (n)	22	85	48	37
True negative (n)	3960	3621	627	2994

**Table S3.** Characteristics of composite high risk group\* at 36 weeks of gestational age (n=195).

<b>Maternal characteristic or birth outcome</b>	<b>High risk 36wkGA†</b>
sFlt-1:PIGF ratio >110	70 (36)
Age≥40 years	6 (3.1)
Body mass index ≥35 kg/m <sup>2</sup>	25 (13)
Type 1 or type 2 diabetes mellitus	5 (2.6)
Chronic hypertension	56 (29)
Renal disease	7 (3.6)
Uterine artery mean PI <sub>z</sub> (Doppler) highest decile	90 (46)
Preeclampsia with severe features	41 (21)
Preeclampsia without severe features	43 (22)
Gestational hypertension	9 (4.6)
Time from sampling to first onset of hypertension, weeks	0.86 (-2.1 to 2.6)
Time from sampling to delivery, weeks	3.1 (2.0 to 4.1)
sFlt-1:PIGF ratio by time from sampling to delivery	
1 <sup>st</sup> quartile (≤2.0 weeks)	110 (61 to 172)
2 <sup>nd</sup> quartile (2.1 to 3.1 weeks)	95 (58 to 151)
3 <sup>rd</sup> quartile (3.2 to 4.1 weeks)	67 (50 to 116)
4 <sup>th</sup> quartile (≥4.2 weeks)	64 (46 to 85)
Gestational age, weeks	39.4 (38.3 to 40.4)
Birth weight, g	3140 (2820 to 3430)
Birth weight, centile	33 (13 to 57)
Small for gestational age	41 (21)
Induction of labor	91 (47)
Mode of delivery	
Spontaneous vaginal	95 (49)
Assisted vaginal	37 (19)
Intrapartum caesarean	39 (20)
Pre-labor caesarean	24 (12)

Data are expressed as median (IQR) or n (%) as appropriate.

\*Composite risk status at 36wkGA: high risk was defined as sFlt-1:PIGF ratio of >38 with maternal risk factors, or sFlt-1:PIGF ratio>110 irrespective of maternal risk factors. Time from sampling to first onset of hypertension is given for the 93 (48%) women who had preeclampsia or gestational hypertension. Among the 41 women who had severe preeclampsia, 30 (73%) had severe hypertension, 30 (73%) had proteinuria, 10 (24%) had low platelets (at median GA [IQR] = 39.4 [39.0 to 40.1] weeks), 13 (32%) had elevated ALT (at median GA [IQR] = 39.3 [38.6 to 40.0] weeks), 14 (34%) had elevated creatinine (at median GA [IQR] = 39.3 [37.6 to 40.4] weeks), one (2.4%) had

pulmonary edema, and one (2.4%) had severe cerebral or visual symptoms. In 14 (34%) of 41 cases severe preeclampsia was superimposed. Sex and gestational age corrected birth weight percentiles were calculated using a population-based UK reference. Small for gestational age was defined as birth weight <10<sup>th</sup> percentile. Data for the variables included in the table were 100% complete within the composite high risk group. †wkGA denotes weeks of gestational age. ‡PI denotes pulsatility index.

**Table S4.** Characteristics of low risk women\* at 36 weeks of gestational age (n=2,660).

<b>Maternal characteristic or birth outcome</b>	<b>Low risk 36wkGA†</b>
Age, years	30.1 (26.8 to 33.0)
Body mass index, kg/m <sup>2</sup>	23.7 (21.7 to 26.6)
Uterine artery mean PI‡ (Doppler), Z score	-0.20 (-0.76 to 0.39)
Preeclampsia with severe features	20 (0.8)
Preeclampsia without severe features	20 (0.8)
Gestational hypertension	47 (1.8)
Time from sampling to first onset of hypertension, weeks	3.3 (1.3 to 4.4)
Time from sampling to delivery, weeks	4.3 (3.3 to 5.0)
Gestational age, weeks	40.6 (39.6 to 41.3)
Birth weight, g	3490 (3195 to 3780)
Birth weight, centile	45 (26 to 68)
Small for gestational age	192 (7.2)
Induction of labor	799 (30)
Mode of delivery	
Spontaneous vaginal	1323 (50)
Assisted vaginal	681 (26)
Intrapartum caesarean	453 (17)
Pre-labor caesarean	196 (7.4)
Missing	7 (0.3)

Data are expressed as median (IQR) or n (%) as appropriate.

\*Low risk women at 36wkGA: defined as sFlt-1:PIGF ratio of  $\leq 38$  without maternal risk factors. Time from sampling to first onset of hypertension is given for the 87 (3.3%) women who had preeclampsia or gestational hypertension. Among the 20 women who had severe preeclampsia, 12 (60%) had severe hypertension, 12 (60%) had proteinuria, 3 (15%) had low platelets (at median GA [IQR] = 41.4 [39.9 to 41.4] weeks), 5 (25%) had elevated ALT (at median GA [IQR] = 40.6 [38.9 to 41.1] weeks), 9 (45%) had elevated creatinine (at median GA [IQR] = 41.1 [39.9 to 41.4] weeks), none had pulmonary edema, and 3 (15%) had severe cerebral or visual symptoms. Sex and gestational age corrected birth weight percentiles were calculated using a population-based UK reference. Small for gestational age was defined as birth weight <10<sup>th</sup> percentile. Data for the variables included in the table were 100% complete within the composite high risk group. †wkGA denotes weeks of gestational age. ‡PI denotes pulsatility index.

**Table S5.** Area under the ROC curve for the secondary outcomes and sub-types of preeclampsia.

<b>Outcome</b>	<b>c statistic (95% CI)</b>
<b>Measurement of sFlt-1:PIGF ratio at 28wkGA</b>	
Preeclampsia, onset of hypertension <37wkGA	0.70 (0.65 to 0.75)
Gestational hypertension, delivery <37wkGA	0.88 (0.85 to 0.92)
Gestational hypertension, onset of hypertension <37wkGA	0.49 (0.38 to 0.60)
Preeclampsia or gestational hypertension, onset of hypertension <37wkGA	0.66 (0.61 to 0.71)
De novo preeclampsia, delivery <37wkGA	0.80 (0.68 to 0.92)
Superimposed preeclampsia, delivery <37wkGA	0.78 (0.63 to 0.94)
Preeclampsia with renal dysfunction, delivery <37wkGA	0.92 (N/A to 1.00)
Preeclampsia with HELLP syndrome, delivery <37wkGA	0.83 (0.49 to 1.00)
<b>Measurement of sFlt-1:PIGF ratio at 36wkGA</b>	
Preeclampsia without severe features	0.76 (0.72 to 0.80)
Severe gestational hypertension	0.70 (0.59 to 0.81)
Any gestational hypertension	0.62 (0.56 to 0.68)
Any preeclampsia or gestational hypertension	0.76 (0.73 to 0.79)
De novo preeclampsia with severe features	0.83 (0.77 to 0.88)
Superimposed preeclampsia with severe features	0.78 (0.70 to 0.85)
Preeclampsia with renal dysfunction	0.66 (0.47 to 0.84)
Preeclampsia with HELLP syndrome	0.89 (0.81 to 0.97)

HELLP denotes hemolysis, elevated liver enzymes and low platelets, wkGA denotes weeks of gestational age, ROC denotes receiver operating characteristic, CI denotes confidence interval.

**Table S6.** Area under the ROC curve for the analysis of preeclampsia with severe features and sFlt-1:PIGF ratio at 36wkGA, stratified by the tertile of per week increase in the sFlt1-PIGF ratio between the 28wkGA and 36wkGA measurements.

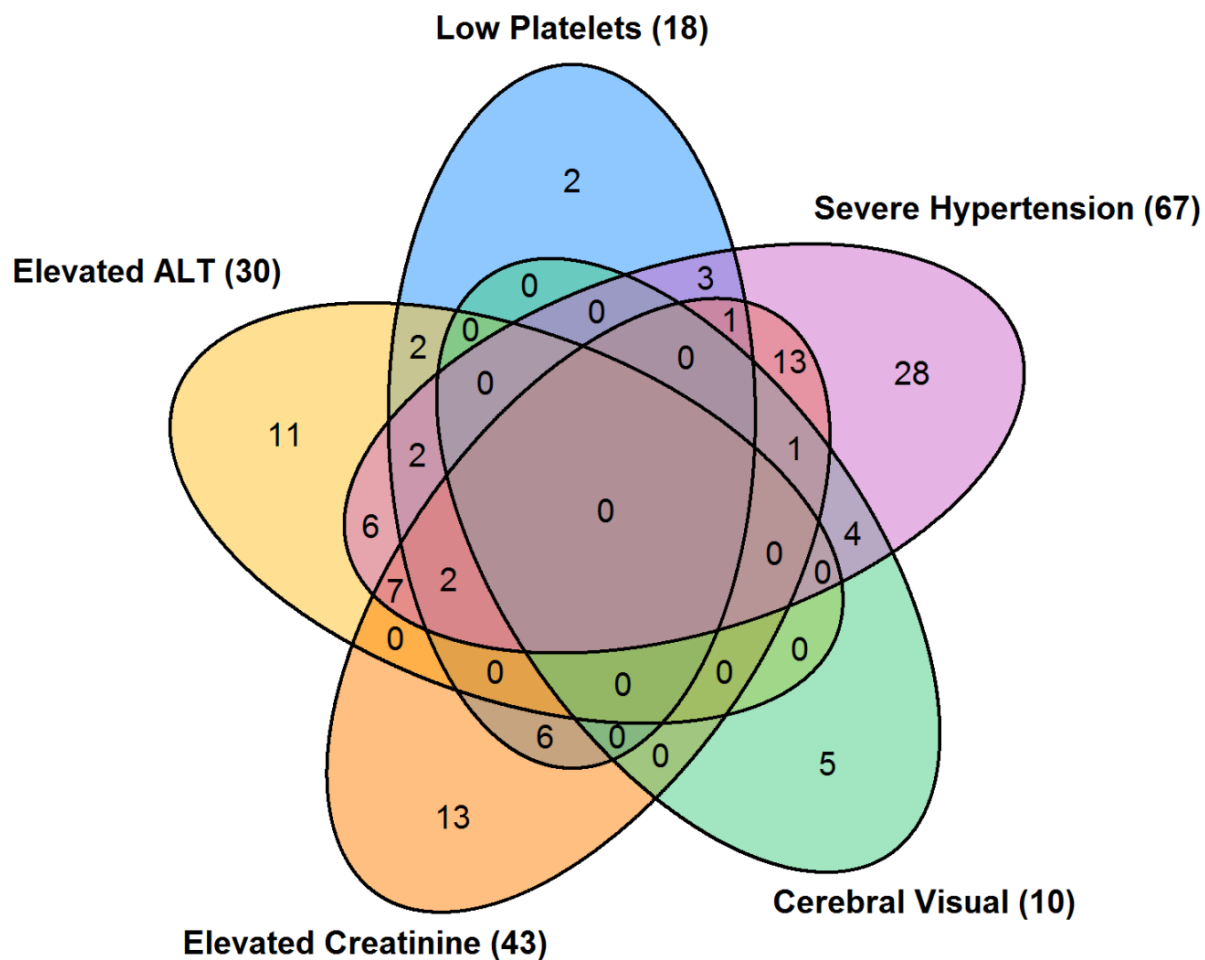
<b>Tertile</b>	<b>Total N</b>	<b>c statistic (95% CI)</b>
1	1223	0.54 (0.32 to 0.77)
2	1222	0.58 (0.33 to 0.83)
3	1221	0.66 (0.59 to 0.73)
Total	3666	0.82 (0.77 to 0.86)

In total, 3,666 women had sFlt-1 and PIGF measurements available at 28wkGA and 36wkGA. ROC denotes receiver operating characteristic, CI denotes confidence interval, wkGA denotes weeks of gestational age. P-value from the Chi-square test for the equality of the three c statistics = 0.52.

**Table S7.** Screening statistics for the primary outcomes by maternal risk status using the threshold of sFlt-1:PIGF ratio of >14.6 at 28 wkGA.

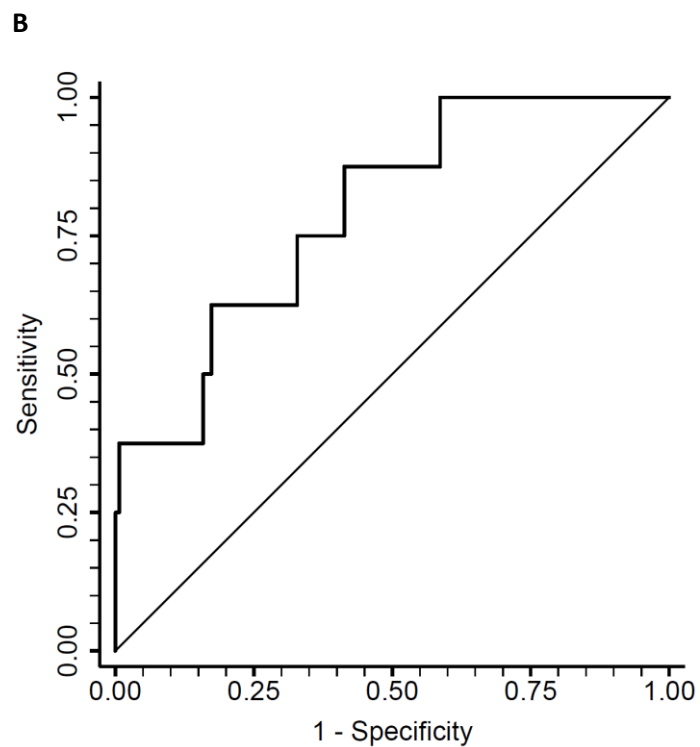
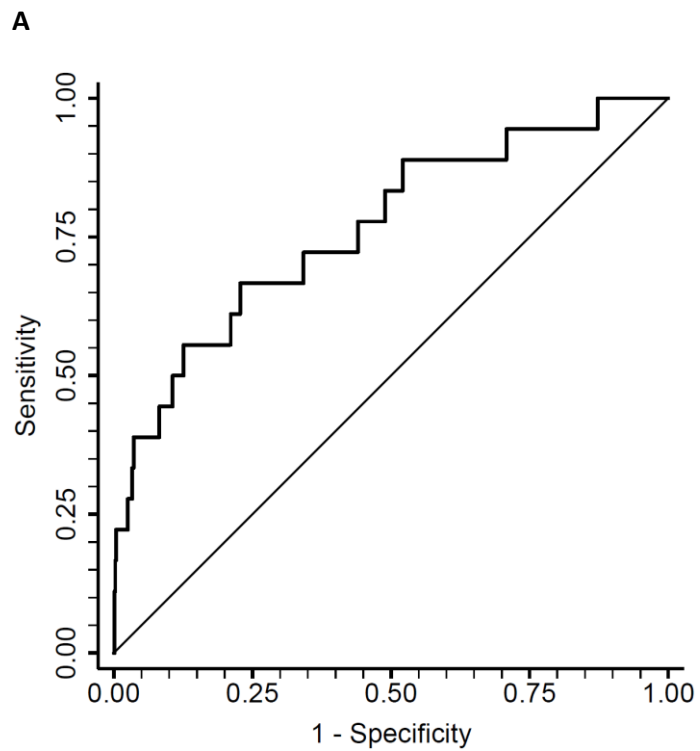
Screening statistic (95% CI)	28 wkGA, PE delivery<37		
	<i>All</i>	<i>High risk</i>	<i>Low risk</i>
Sensitivity (%)	38.5 (19.8-57.2)	38.9 (16.4-61.4)	37.5 (4.0-71.0)
Specificity (%)	98.3 (97.9-98.7)	96.4 (95.1-97.7)	98.7 (98.3-99.1)
Positive predictive value (%)	12.7 (5.3-20.0)	20.6 (7.0-34.2)	6.7 (0.0-14.0)
Negative predictive value (%)	99.6 (99.4-99.8)	98.5 (97.6-99.4)	99.8 (99.7-100.0)
False positive rate (%)	1.7 (1.3-2.1)	3.6 (2.3-4.9)	1.3 (0.92-1.7)
False negative rate (%)	61.5 (42.8-80.2)	61.1 (38.6-83.6)	62.5 (29.0-96.0)
Positive likelihood ratio	22.1 (12.9-37.9)	10.9 (5.5-21.6)	28.7 (11.2-73.6)
Negative likelihood ratio	0.63 (0.46-0.85)	0.63 (0.44-0.92)	0.63 (0.37-1.08)



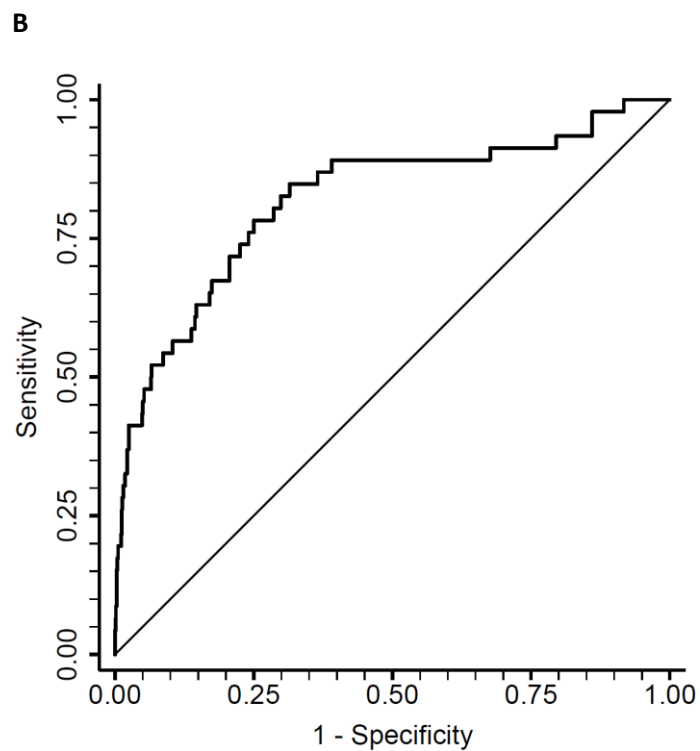
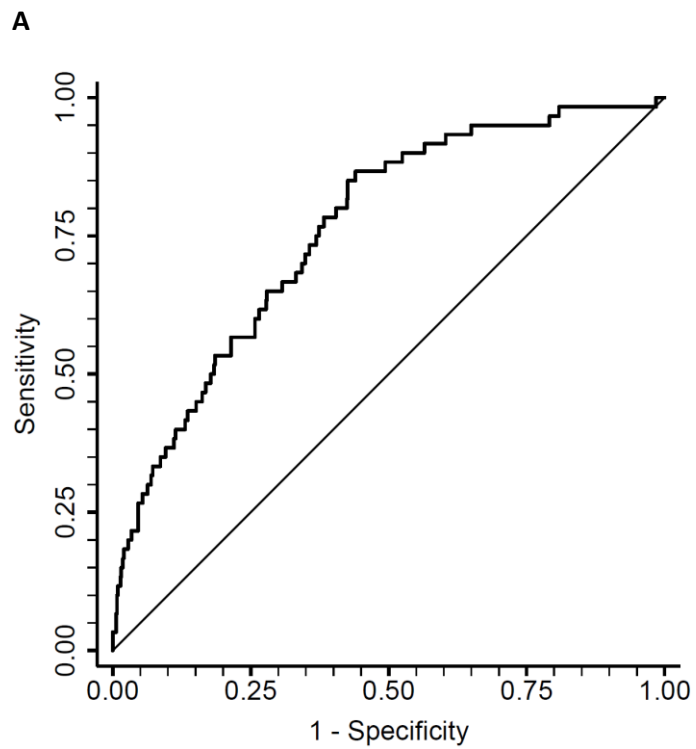


**Figure S1.** Venn diagram describing the condition of women who had pre-eclampsia with severe features (n=106).

The total number of women having each severe feature of preeclampsia is given in brackets. Cerebral Visual denotes severe cerebral or visual disturbances. Proteinuria (n=72) and pulmonary oedema (n=2) are omitted from the diagram and both of these overlap with at least one other feature.

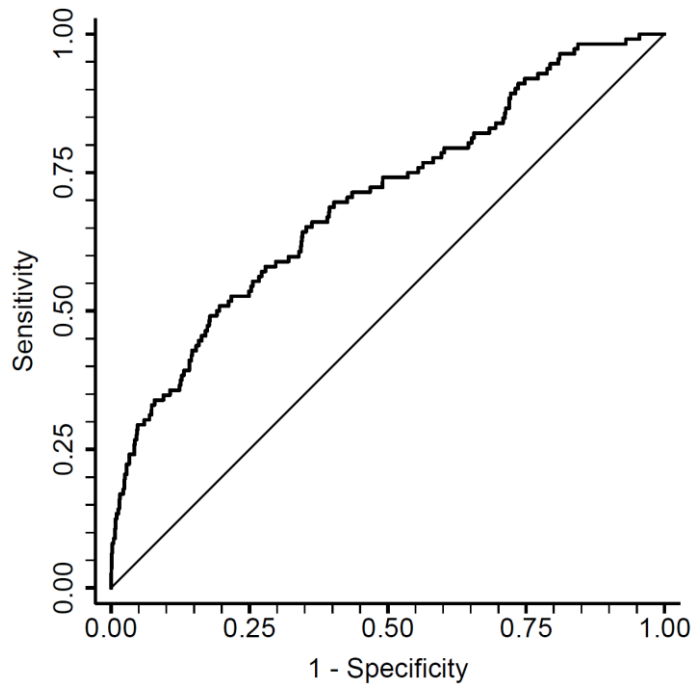


**Figure S2.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 28wkGA and primary outcome, stratified by **A.** high risk of preeclampsia, area under the ROC curve (95% CI) is 0.77 (0.64-0.89), and **B.** low risk, area under the ROC curve (95% CI) is 0.79 (0.64-0.94).

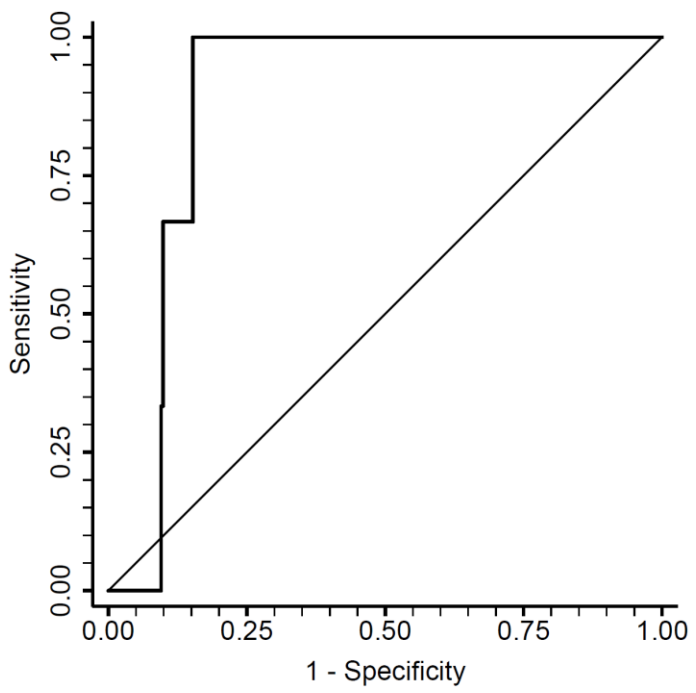


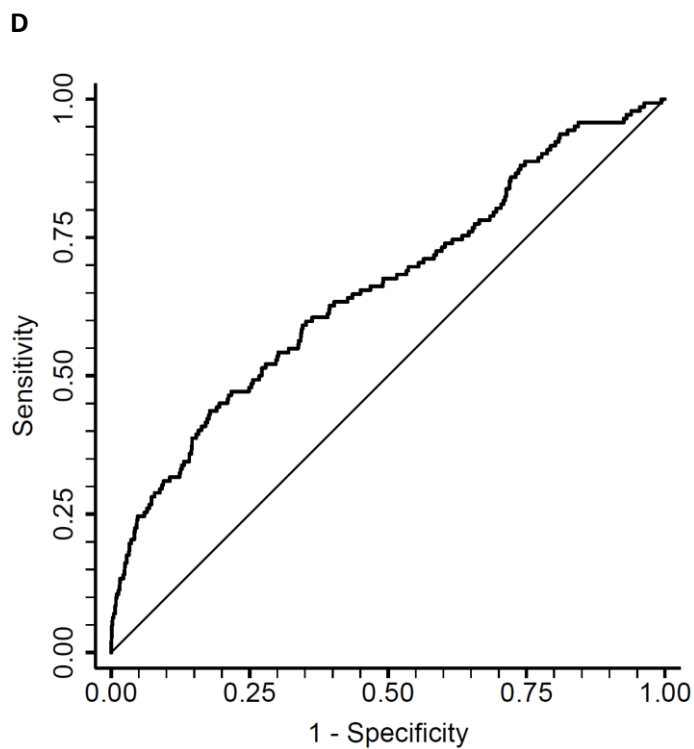
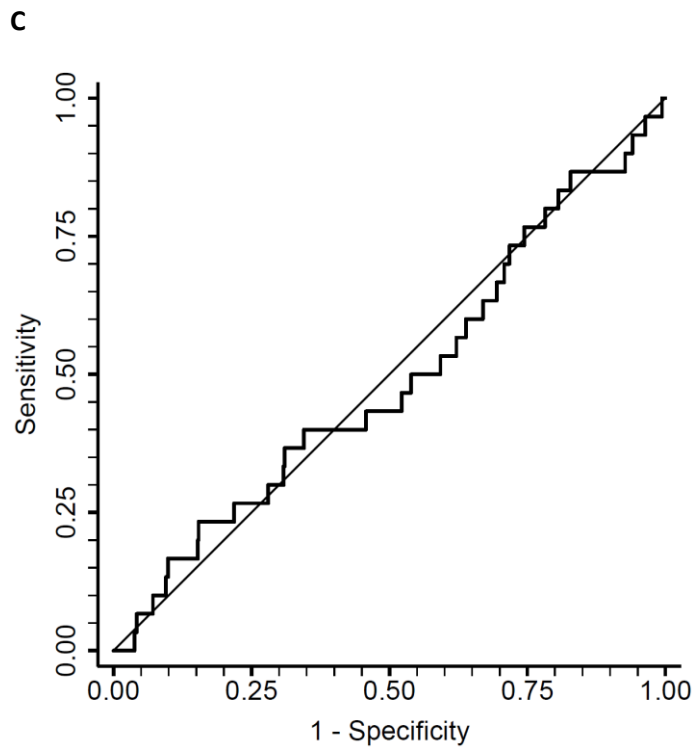
**Figure S3.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 36wkGA and primary outcome, stratified by **A.** high risk of preeclampsia, area under the ROC curve (95% CI) is 0.76 (0.70-0.82), and **B.** low risk of preeclampsia, area under the ROC curve (95% CI) is 0.82 (0.75-0.89).

**A**



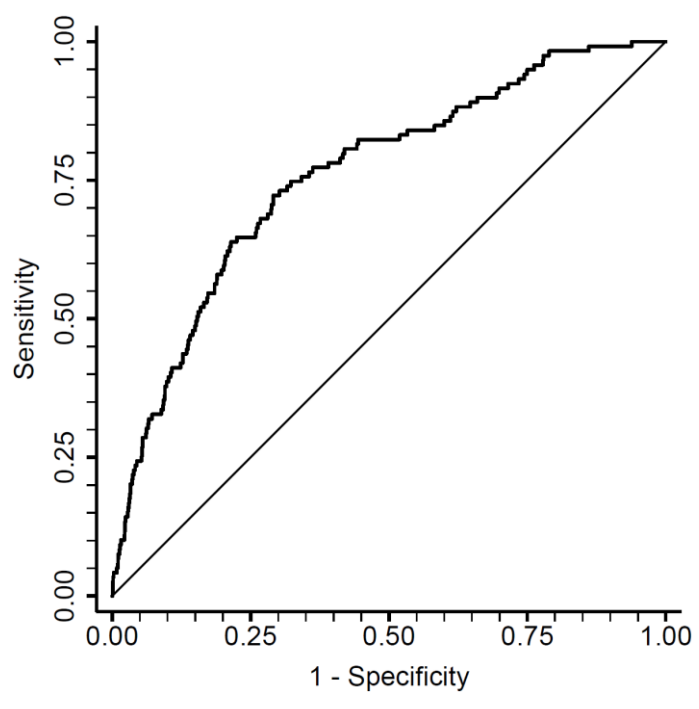
**B**



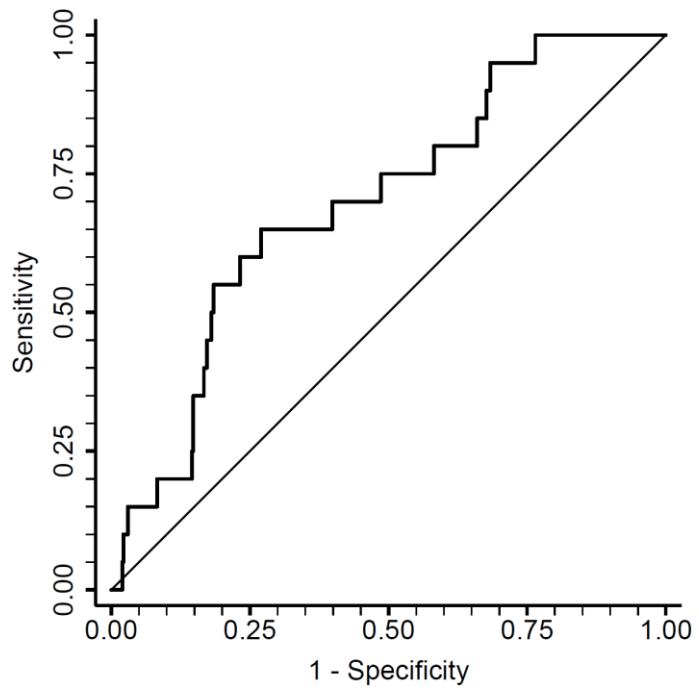


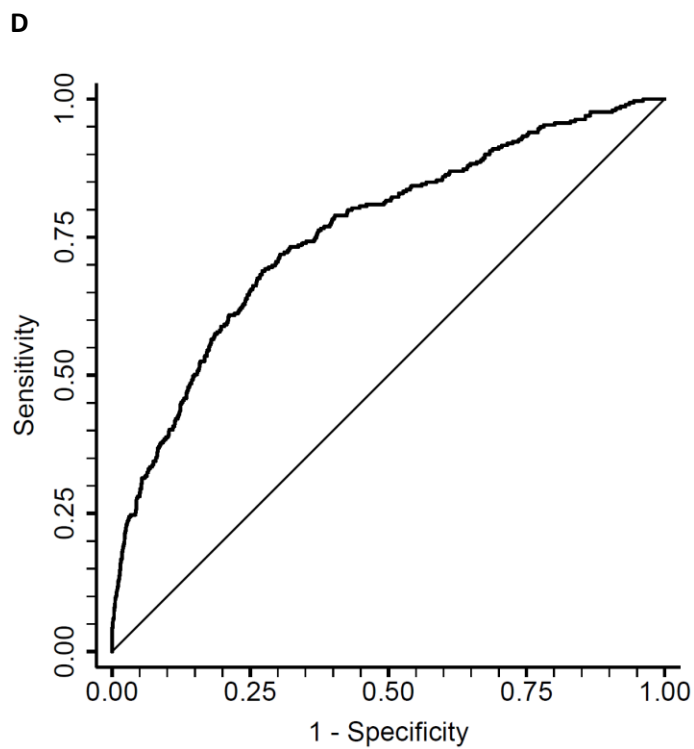
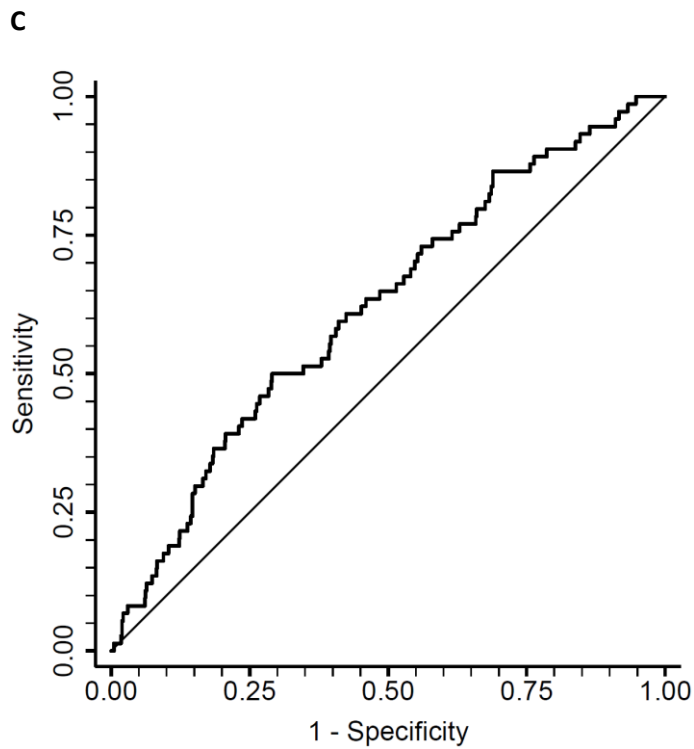
**Figure S4.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 28wkGA and secondary outcomes. Area under the ROC curve (95% CI) is given after the outcome. **A.** Preeclampsia, onset of hypertension <37wkGA, 0.70 (0.65-0.75). **B.** Gestational hypertension, delivery <37wkGA, 0.88 (0.85-0.92). **C.** Gestational hypertension, onset of hypertension <37wkGA, 0.49 (0.38-0.60). **D.** Preeclampsia or gestational hypertension, onset of hypertension <37wkGA, 0.66 (0.61-0.71).

**A**

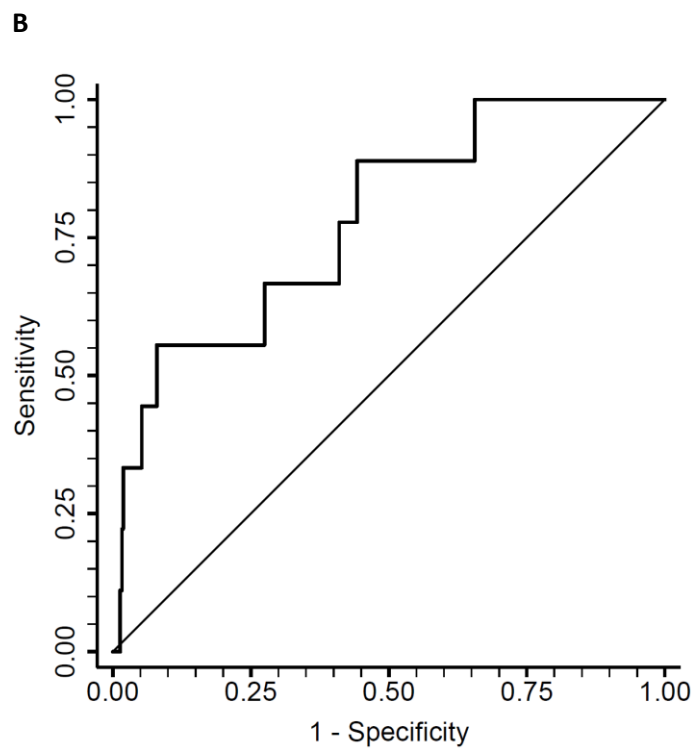
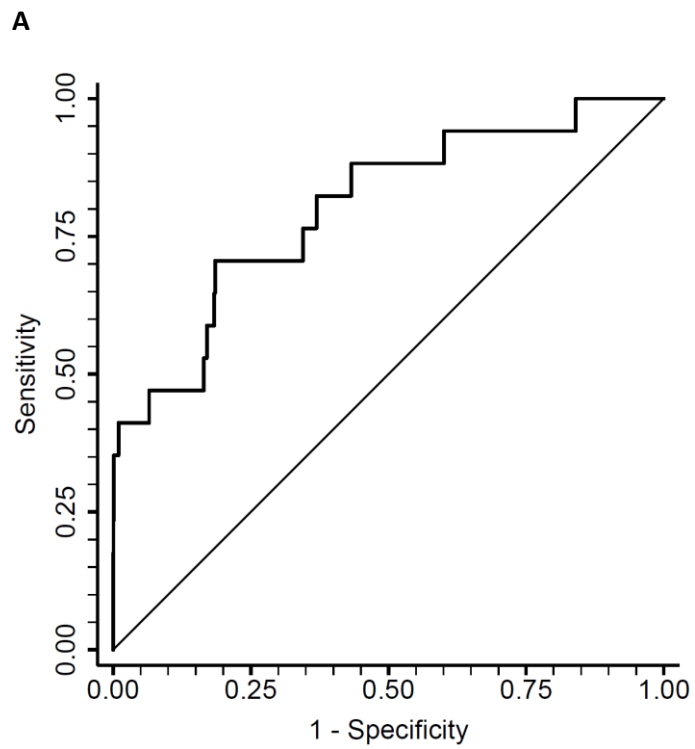


**B**



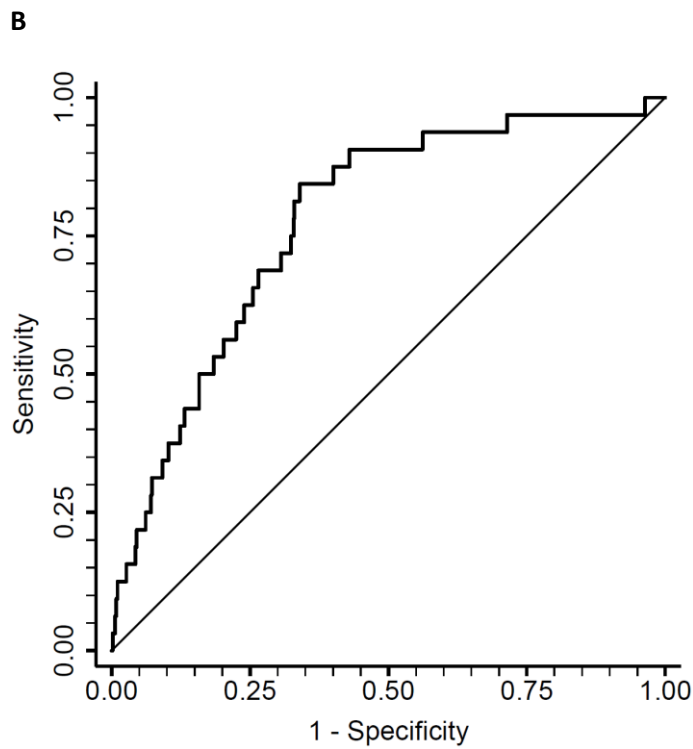
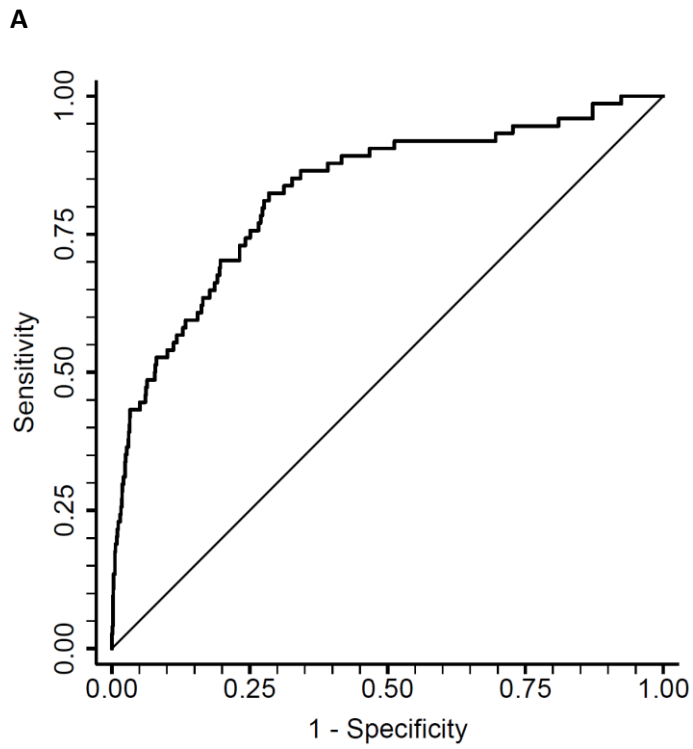


**Figure S5.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 36wkGA and secondary outcomes. Area under the ROC curve (95% CI) is given after the outcome. **A.** Preeclampsia without severe features, 0.76 (0.72-0.80). **B.** Severe gestational hypertension, 0.70 (0.59-0.81). **C.** Any gestational hypertension, 0.62 (0.56-0.68). **D.** Any preeclampsia or gestational hypertension, 0.76 (0.73-0.79).

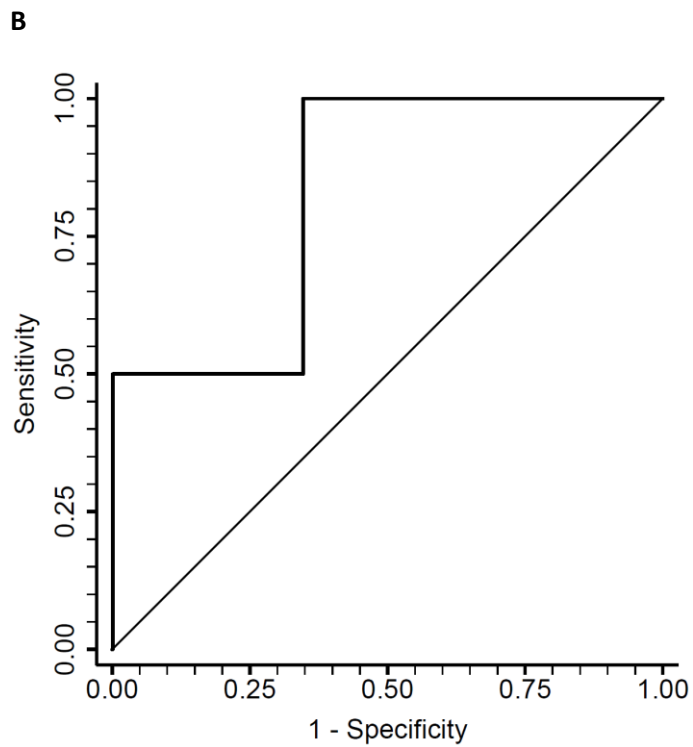
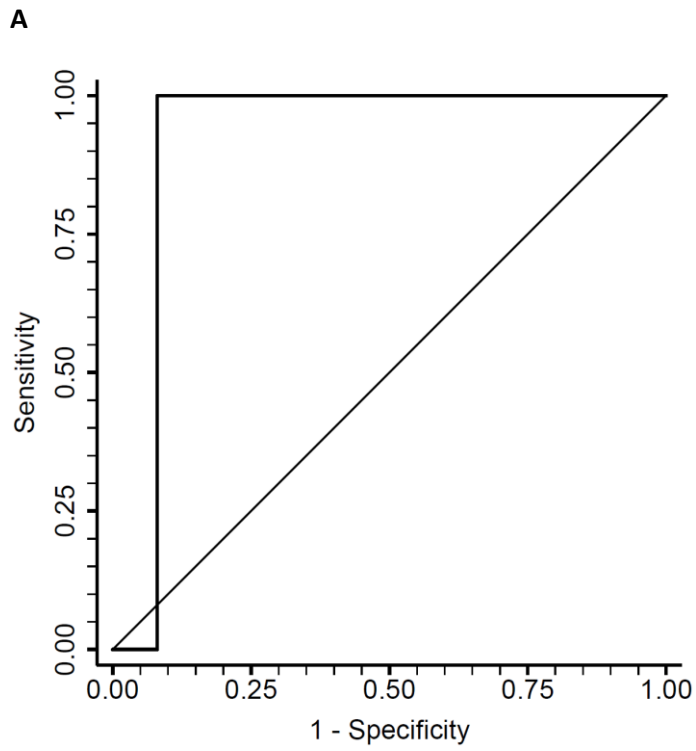


**Figure S6.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 28wkGA and **A.** de novo preeclampsia with delivery <37wkGA, area under the ROC curve (95% CI) is 0.80 (0.68-0.92), and **B.** superimposed preeclampsia with delivery <37wkGA, area under the ROC curve (95% CI) is 0.78 (0.63-0.94).

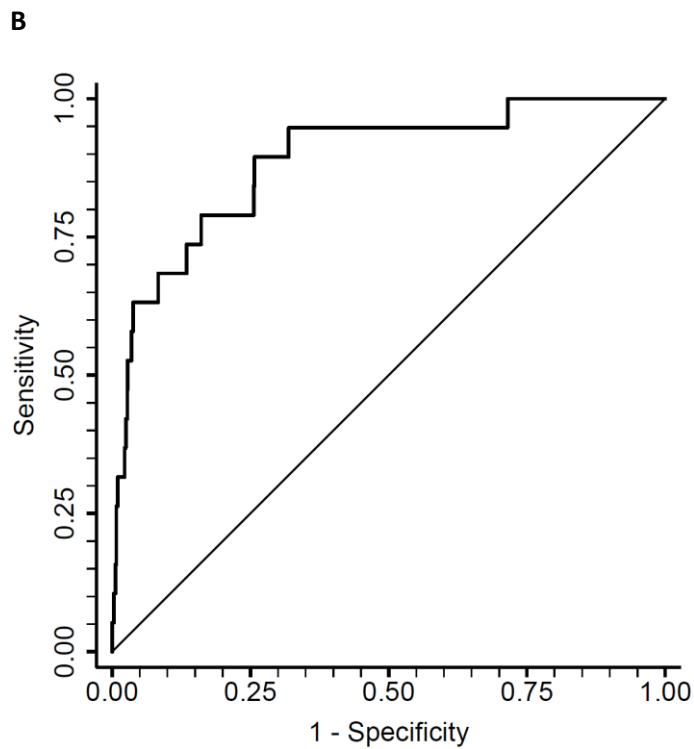
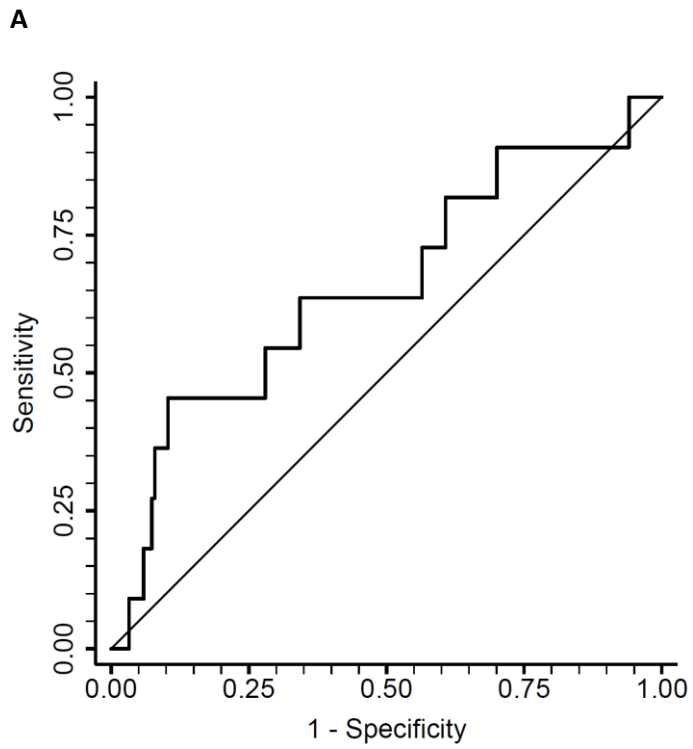




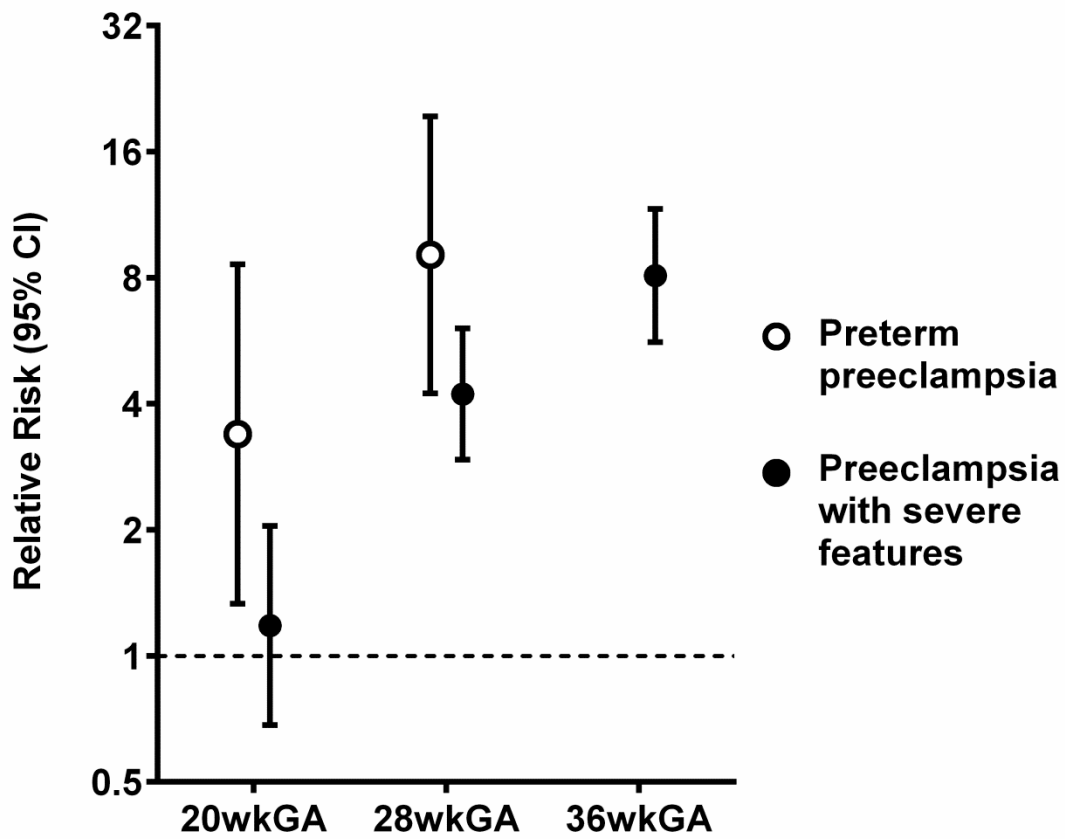
**Figure S7.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 36wkGA and **A.** de novo preeclampsia with severe features, area under the ROC curve (95% CI) is 0.83 (0.77-0.88), and **B.** superimposed preeclampsia with severe features, area under the ROC curve (95% CI) is 0.78 (0.70-0.85).



**Figure S8.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 28wkGA and primary outcome with **A.** renal dysfunction, area under the ROC curve (95% CI) is 0.92 (N/A-1.00), **B.** HELLP syndrome, 0.83 (0.49-1.00).



**Figure S9.** Receiver operating characteristic (ROC) curve analysis of the continuous sFlt-1:PIGF ratio at 36wkGA and preeclampsia with **A.** renal dysfunction, area under the ROC curve (95% CI) is 0.66 (0.47-0.84), **B.** HELLP syndrome, 0.89 (0.81-0.97).



**Figure S10.** Relative risk of (i) preeclampsia leading to preterm birth and (ii) preeclampsia with severe features, comparing women in the top decile of sFlt1:PIGF ratio against all other women. Cut-off points for the top decile of sFlt1:PIGF ratio are 13.30 at 20wkGA, 6.90 at 28wkGA and 49.82 at 36wkGA. The 36wkGA measurement was not analyzed in relation to preterm preeclampsia.