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Prediction of Preeclampsia Using the sFlt-1:PLGF Ratio: A Prospective Cohort Study of Unselected Nulliparous Women

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Abstract

We sought to assess the ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PLGF) in maternal serum as a screening test for preeclampsia in unselected nulliparous women with a singleton pregnancy.

We studied 4,099 women recruited to the Pregnancy Outcome Prediction study (Cambridge, UK). The sFlt-1:PLGF ratio was measured using the Roche cobas e411 platform at ~20, ~28 and ~36 weeks of gestational age (wkGA). Screen positive was defined as an sFlt-1:PLGF ratio >38, but higher thresholds were also studied.

At 28wkGA, an sFlt-1:PLGF 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 36wkGA, an sFlt-1:PLGF ratio >38 had a PPV for severe preeclampsia of 20% in high risk women and 6.4% in low risk women. At 36wkGA, an sFlt-1:PLGF ratio >110 had a PPV of 30% for severe preeclampsia, and the PPV was similar comparing low and high risk women. Overall, at 36wkGA 195 (5.2%) women either had an sFlt-1:PLGF ratio of >110 or an sFlt-1:PLGF ratio >38 plus maternal risk factors: 43% of these women developed preeclampsia, about half with severe features. Among low risk women at 36wkGA, an sFlt-1:PLGF ratio >38 had a negative predictive value for severe preeclampsia of 99.2%.

The sFlt-1:PLGF ratio provided clinically useful prediction of the risk of the most important manifestations of preeclampsia in a cohort of unselected nulliparous women.

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Disclosures

Dr. Hund reports being an employee of Roche Diagnostics, holding stock in Roche, having a pending patent related to the sFlt-1:PLGF 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 syndrome or both (PCT/EP2012/072157) and the prediction of postpartum HELLP syndrome, postpartum eclampsia, or postpartum preeclampsia (PCT/EP2015/051457). Dr. Smith reports equipment loans and consumable support from Roche Diagnostics. Dr. Sovio, Dr. Gaccioli and Dr. Charnock-Jones report no conflicts of interest.

Keywords

preeclampsia; placenta growth factor; sFlt-1 protein; angiogenesis; cohort studies; pregnancy; circulating biomarkers

Introduction

Preeclampsia is one of the most common adverse outcomes of pregnancy.¹ The condition consists of new onset hypertension and proteinuria in the second half of pregnancy, but can also be "super-imposed" on pre-existing 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 occurs as a consequence of preterm birth due to preeclampsia, and a substantial proportion of adverse maternal outcomes occurs in severe preeclampsia.¹

Preeclampsia is associated with an altered maternal pattern of circulating, placentally-derived proteins regulating angiogenesis,^{2, 3} such as soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF). One of the simplest methods to quantify the pattern is to calculate the ratio between these two angiogenic factors in maternal serum. A recent study of women with clinically suspected disease demonstrated that a sFlt-1:PlGF ratio cut-off of 38 provided clinically useful prediction of the risk of preeclampsia.⁴ Higher sFlt-1:PlGF ratios, namely >85 at 28wkGA and >110 at 36wkGA, have been shown to be more strongly associated with the risk of preeclampsia.⁵ 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.⁶

The aim of the present analysis was to evaluate the effectiveness of the sFlt-1:PlGF ratio as a screening test for preeclampsia in unselected nulliparous women recruited to the Pregnancy Outcome Prediction (POP) study.^{7, 8} Most of the participants were healthy, as the cohort selection was solely based on nulliparity, singleton pregnancy and the study catchment area. We analyzed the sFlt-1:PlGF ratio measured repeatedly at 20, 28 and 36 weeks of gestational age (wkGA) using the Roche Cobas e411 Elecsys immunoassay system, which has been certified by the Conformité Européenne (CE) mark for use as an *in vitro* medical device. Screen positive was defined on the basis of the previously described and validated cut off of >38.⁴ 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 (UK), as previously described.^{7, 8} 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 socio-economic deprivation. Therefore, the cohort can be considered a population with low prior risk of disease and homogeneous from a socio-economic perspective. Blood was obtained at the time of recruitment (not analyzed in the present study). Study participants attended the NIHR Cambridge Clinical Research Facility at ~20wkGA, ~28wkGA and ~36wkGA 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, UK), biochemical tests (Meditech, Westwood MA, USA) and neonatal intensive care (Badgernet, Clevermed Ltd, Edinburgh, UK). 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 (i.e. with or without severe features) on the basis of the objective criteria of the 2013 ACOG Guideline (Supplemental Material).⁹ Super-imposed preeclampsia was defined as preeclampsia in women with pre-existing renal disease or hypertension. Socio-economic status was quantified using the Index of Multiple Deprivation¹⁰ and birth weight percentiles were calculated using a population-based UK reference.¹¹

Analysis plan and reporting

The definition of exposures, primary outcomes, secondary outcomes and sensitivity analyses were agreed in an analysis plan (Supplemental Material) prior to performing any analysis of the sFlt-1 and PlGF data. Analyses which were not pre-defined are identified as such. The primary outcome for the 20wkGA measurement was a composite of (i) preeclampsia with delivery prior to 28wkGA or (ii) preeclampsia with delivery prior to 37wkGA where the onset of hypertension was prior to 28wkGA. The primary outcome for the 28wkGA measurement was preeclampsia with delivery prior to 37wkGA. The primary outcome for the 36wkGA measurement was preeclampsia with severe features (i.e. preeclampsia with either severe hypertension or evidence of hepatic, renal, hematological, cerebral or pulmonary complications: see Supplemental Material). Secondary outcomes are defined in the Supplemental Material. High risk of preeclampsia was defined as either (i) maternal characteristics, using the UK's National Institute for Health and Care Excellence (NICE) Guideline (Supplemental Material)¹² or (ii) elevated 20wkGA uterine artery Doppler, defined as a mean pulsatility index in the highest decile, as previously described.⁸ Family history of preeclampsia was not included in the definition of risk status as 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 reported⁶ and stored at -80°C. All samples used in the current analysis had not previously been thawed prior to 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 electro-chemiluminescence 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 inter-assay 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 >38.4. We also studied more severe elevation of the ratio, namely, >85 at 28wkGA and >110 at 36wkGA.⁵

Statistical analysis

Full details of the statistical analysis are described in the Analysis plan (Supplemental Material). In brief, standard screening summary statistics were calculated from 2x2 tables. In addition, sFlt-1:PIGF ratio was analyzed as a continuous variable using the area under the receiver operating characteristic curve (AUROCC). Time to event analysis was performed where delivery with the given outcome was the event and delivery without 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 4,512 women recruited, 67 (1.5%) women withdrew and 233 (5.2%) delivered elsewhere, leaving 4,212 eligible women.⁸ 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 28wkGA or 36wkGA visits.

Results

Description of the study cohort

The study group consisted of 4,099 women, of whom 3,953 had sFlt-1:PIGF measurement available from the 20wkGA visit, 3,989 from the 28wkGA visit and 3,776 from the 36wkGA visit. The overall incidence of preeclampsia was 6.5% (265/4,099). The incidence of preterm preeclampsia with onset prior to 28wkGA following the 20wkGA measurement was 0.10% (4/3,953). The incidence of preeclampsia leading to preterm delivery following the 28wkGA measurement was 0.65% (26/3,989). The incidence of delivery with severe preeclampsia following the 36wkGA measurement was 2.8% (106/3,776). 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 36wkGA, respectively. The median ratio at 28wkGA was 6.5 (interquartile range [IQR] 3.4-22.5) in women who developed preterm preeclampsia, and the median ratio at 36wkGA was 42.3 (IQR 22.6-88.2) in women who had severe preeclampsia.

Screening performance at 20wkGA

None of the four women who experienced the primary outcome following the 20wkGA measurement had an sFlt-1:PIGF ratio >38. The AUROCC for the sFlt-1:PIGF ratio was 0.70 (95% CI 0.43-0.97) (Figure 1A). Ten women had a ratio >38 and one woman had a

ratio >85 at 20wkGA. Further analysis was not performed due to the very small number of events.

Screening performance at 28wkGA

The AUROCC for the sFlt-1:PIGF ratio was 0.80 (95% CI 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% vs 31%, respectively, P=0.91).

Screening performance at 36wkGA

The AUROCC for the sFlt-1:PIGF ratio was 0.81 (95% CI 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 >38 had a high negative predictive value for subsequent development of severe preeclampsia (>99%).

Analysis of severe elevation of the sFlt1:PIGF ratio

We studied more severe elevation of the ratio, using pre-defined thresholds, namely, 85 at 28wkGA and 110 at 36wkGA (Table 3). Only 7 women had an sFlt1:PIGF ratio >85 at 28wkGA. However, 4 out of 7 delivered preterm with a diagnosis of preeclampsia (PPV=57%). At 36wkGA, 70 women had an sFlt1: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 36wkGA using the sFlt1:PIGF ratio combined with maternal risk factors

We then analyzed a composite definition of screen positive at 36wkGA, 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 pre-eclampsia with severe features and 43 (PPV=22%) developed preeclampsia without severe features. The characteristics and outcomes for the 195 women are summarized (Supplemental Material).

Time to event analysis

We plotted the cumulative incidence of the primary outcomes following the 28wkGA and 36wkGA measurements of the sFlt-1:PIGF ratio using a competing risks model (Figure 2A & B). The 36wkGA 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 7-8 weeks following the test. We also plotted the cumulative incidence of preeclampsia for women at 36wkGA with the composite definition of screen positive (Figure 2C), i.e. 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 three analyses. In all three plots, it is evident that more than 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 the present 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 of experiencing the clinically most important manifestations of preeclampsia. At 28wkGA, an sFlt-1:PIGF 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 36wkGA, about 5% of women were identified as *high risk* on the basis of either an sFlt-1:PIGF ratio of >110 or an sFlt-1:PIGF 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 36wkGA, i.e. they had no maternal risk factors and an sFlt-1:PIGF ratio \leq 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 (36wkGA), the rationale being that delivery is the main intervention to prevent preeclampsia. This can more easily and more safely be performed at term.¹³ Moreover, a randomized controlled trial has shown improved outcome following immediate induction of labor compared with expectant management in women with gestational hypertension or mild preeclampsia near term.¹⁴ In the current study we evaluated the diagnostic effectiveness of the sFlt-1:PIGF 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 36wkGA using maternal risk factors and sFlt-1:PIGF ratio, monitor screen positive women closely and perform induction of labor prior to 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 39wkGA does not increase the risk of caesarean delivery or perinatal morbidity in another high risk population of nulliparous women, namely, those aged 35 or more.¹⁵

The sFlt-1:PIGF ratio was also informative for the risk of women experiencing preterm preeclampsia. Women with a ratio >38 at 28wkGA had a 32% risk of preterm delivery with preeclampsia, and the ratio >38 had a very high positive likelihood ratio (~70). This may reflect the fact that this threshold represents much more significant elevation at 28wkGA (99.5th percentile) than 36wkGA (85th percentile). However, the sensitivity of the sFlt-1:PIGF ratio >38 at 28wkGA was only 23%. Although the POP study included mostly healthy women, this is consistent with findings in women clinically suspected to have preeclampsia, where an elevated sFlt-1:PIGF ratio between 24wkGA and 37wkGA was also associated with an increased risk of developing the disorder within 4 weeks after the

measurement.¹⁶ Hence, while 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 28wkGA. However, the AUROC for the sFlt-1:PIGF ratio at 28wkGA 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 28wkGA 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 36wkGA assessment identified them as being at intermediate risk (5% to 6%) of severe preeclampsia, namely, women with an sFlt-1:PIGF 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:PIGF ratio after 36wkGA.

The present study had a number of 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:PIGF ratio can be calculated without modelling 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 the present study were planned and specified in advance.

A large scale study of screening women using the sFlt-1:PIGF ratio has recently been reported.¹⁷ However, their findings are difficult to compare to the present study as they pooled the results from a wide range of gestational ages (30 to 37wkGA). 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 multi-ethnic 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.¹⁸ 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 the present study on nulliparous women was purposeful. One of the strongest clinical predictors of the risk of preeclampsia is whether a woman has a prior 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 the present study was measurement of biomarkers throughout gestation. Results reported in the current and previous studies¹³ suggest that screening tests for pregnancy complications have a better predictive value when performed close to disease onset.

A number of studies have evaluated trying to predict preeclampsia solely using measurements made in the first trimester. Statistical models are used to determine prior risk. Further modelling 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 11wkGA and 14wkGA.¹⁹ A study-derived threshold (10% false positive rate) yielded positive predictive values of 5-12%, 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 (i) the definition of screen positive was externally defined, (ii) the positive predictive values were higher, (iii) the outcome was confined to the clinically most significant cases, and (iv) the handling of clinical and biochemical predictors is simpler.

Perspectives

We conclude that measurement of the sFlt-1:PIGF ratio at 36wkGA, combined with maternal risk factors, provides clinically useful prediction of the risk of preeclampsia at term for about three 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/or induction of labor, and this would be an appropriate focus for future randomized controlled trials. Women with extreme elevation of the ratio at 28wkGA have high absolute risks of preterm disease and the test may be useful to identify women for evaluation of candidate disease-modifying therapies as they become available.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What is New?

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

- An sFlt-1:PIGF 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.

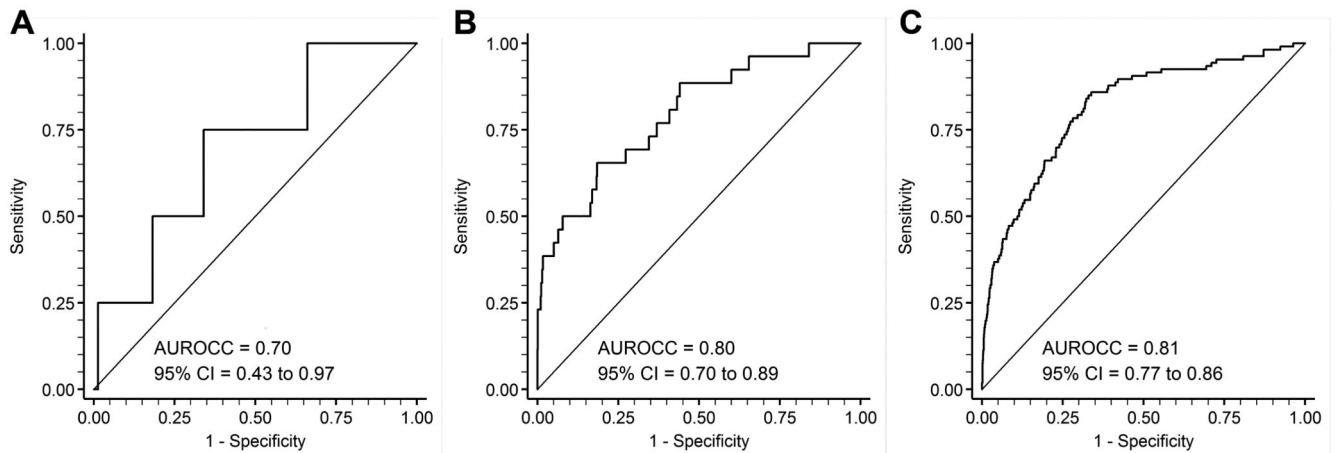


Figure 1.

Receiver operating characteristic curve analysis of the relationship between **A.** sFlt-1:PIGF ratio at 20wkGA and (i) preeclampsia with delivery prior to 28wkGA or (ii) preeclampsia with delivery prior to 37 weeks where the onset of hypertension was prior to 28wkGA (n=4), **B.** sFlt-1:PIGF at 28wkGA and preeclampsia leading to preterm birth (n=26), and **C.** sFlt-1:PIGF at 36wkGA 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.

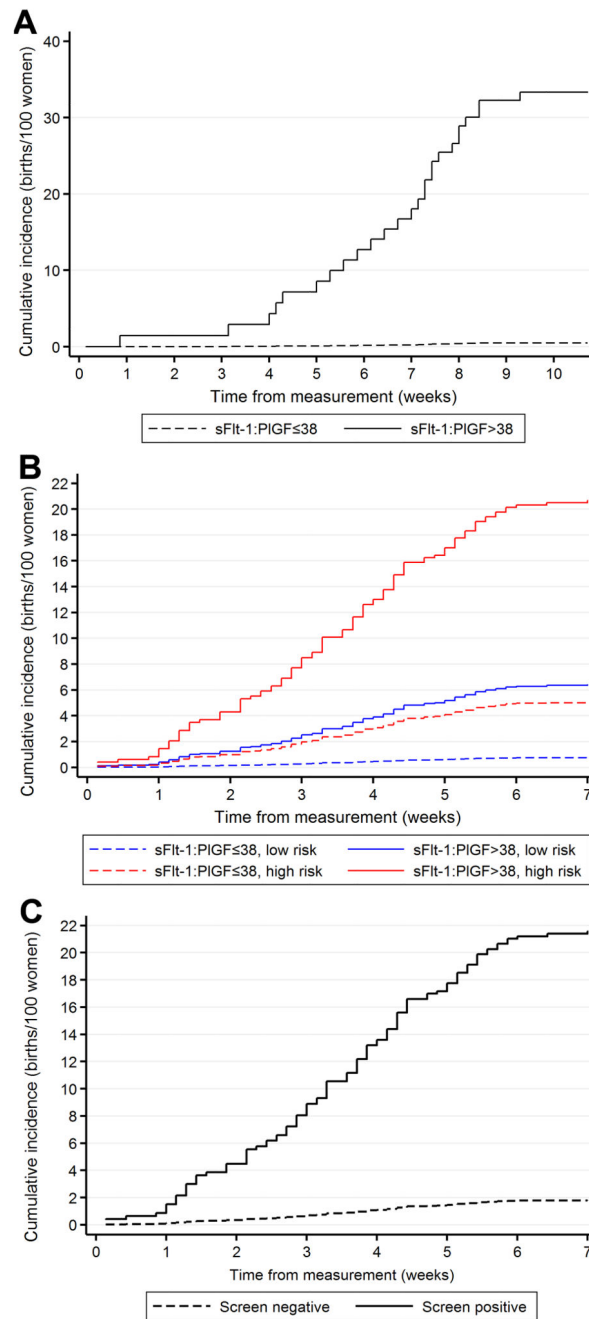


Figure 2.

Cumulative incidence of the primary outcomes (see methods) by the sFlt-1:PIGF ratio: **A.** sFlt-1:PIGF at 28wkGA and preeclampsia leading to preterm birth, **B.** sFlt-1:PIGF at 36wkGA and severe preeclampsia, stratified by maternal risk. High risk was defined on the basis of maternal risk factors or 20wkGA uterine artery Doppler (see Methods for details), and **C.** Composite risk status at 36wkGA. Screen positive was defined as (i) sFlt-1:PIGF ratio of >38 AND maternal risk factors OR (ii) sFlt-1:PIGF 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 three 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.

Table 1

Characteristics of the study cohort (N=4,099).

Characteristic	No hypertensive disorder	Preterm preeclampsia	Preeclampsia with severe features*	Preeclampsia without severe features*	Gestational hypertension
n (%)	3,751 (91.5%)	26 (0.6%)	111 (2.7%)	128 (3.1%)	83 (2.0%)
Maternal characteristics					
Age, years	30 (27 to 33)	29 (25 to 34)	30 (26 to 34)	29 (26 to 33)	29 (25 to 33)
Age stopped FTE [‡] , years	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)
Deprivation quartile					
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)
Ethnicity					
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					
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/m²					
Body mass index, kg/m ²	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[†]					
Type 1 or type 2 DM [†]	7 (0.2)	3 (12)	0 (0.0)	2 (1.6)	2 (2.4)
Chronic hypertension					
Chronic hypertension	95 (2.5)	9 (35)	34 (31)	74 (58)	0 (0.0)
Renal disease					
Renal disease	28 (0.8)	1 (3.9)	3 (2.7)	8 (6.3)	0 (0.0)

Characteristic	No hypertensive disorder	Preterm preeclampsia	Preeclampsia with severe features*	Preeclampsia without severe features*	Gestational hypertension
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)
Birth outcomes					
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, weeks	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)
Mode of delivery					
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 caesarean	616 (16)	3 (12)	43 (39)	31 (24)	21 (25)
Pre-labor 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)
sFlt-1:PlGF ratio					
At 20wkGA	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 28wkGA	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 36wkGA	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)

* Confined to women delivering 37wkGA.

[†] Abbreviations: FTE denotes full time education, DM denotes diabetes mellitus, Uta denotes uterine artery and PI denotes pulsatility index. Data are expressed as median (IQR) or n (column %) as appropriate. For fields where there is no category labelled "missing", data were 100% complete.

Maternal age was defined as age at recruitment. All other maternal characteristics were defined by self-report at the 20wkGA 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 2007/10, 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. 11 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. See Supplemental Material for details.

Table 2

Screening statistics for the primary outcomes by maternal risk status using the threshold of sFlt-1:PIGF ratio of >38 at 28wkGA and 36wkGA.

Screening statistic	28wkGA *			36wkGA *		
	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)

* wkGA denotes 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 non-cases $([S+|D+]/[S+|D-])$.

‡ Negative likelihood ratio was defined as the ratio between the proportions of screen negatives among cases and screen negatives among non-cases $([S-|D+]/[S-|D-])$.

See Supplemental Tables for raw data from 2x2 tables.

Screening statistics for the primary outcomes by maternal risk status using the threshold of sFlt-1:PIGF ratio of >85 at 28wkGA and >110 at 36wkGA.

Table 3

Screening statistic	28wkGA *		36wkGA *	
	Preeclampsia with preterm delivery		Preeclampsia with severe features	
	All	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)

* wkGA denotes weeks of gestational age (of measurement of the sFlt-1:PIGF ratio).

See Methods for definition of risk status and Supplemental Tables for raw data from 2x2 tables.