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# Comparison of screening for pre-eclampsia at 31–34 weeks' gestation by sFlt-1/PlGF ratio and a method combining maternal factors with sFlt-1 and PlGF

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**KEYWORDS:** placental growth factor; pre-eclampsia; pyramid of antenatal care; soluble fms-like tyrosine kinase-1

## ABSTRACT

***Objective*** *To estimate the patient-specific risk of pre-eclampsia (PE) at 31–34 weeks' gestation by a combination of maternal characteristics and medical history with multiples of the median (MoM) values of serum placental growth factor (PlGF) and serum soluble fms-like tyrosine kinase-1 (sFlt-1) and to compare the performance of screening to that achieved by the sFlt-1/PlGF ratio.*

***Methods*** *This was a prospective observational study in women attending a third-trimester ultrasound scan at 31–34 weeks as part of routine pregnancy care. We estimated the performance of screening for PE with delivery within 4 weeks of assessment and PE with delivery from 4 weeks after assessment up to 40 weeks' gestation by the sFlt-1/PlGF ratio and by a method utilizing Bayes' theorem that combines maternal factors and MoM values of sFlt-1 and PlGF. The significance of the difference in screening performance between the two methods was assessed by comparison of the areas under the receiver–operating characteristics curves (AUC).*

***Results*** *The study population of 8063 singleton pregnancies included 231 (2.9%) that subsequently developed PE. In the prediction of delivery with PE at < 4 weeks from assessment, the performance of the method utilizing Bayes' theorem was similar to that using the sFlt-1/PlGF ratio (AUC, 0.987 (95% CI, 0.979–0.995)* vs *0.988 (95% CI, 0.981–0.994);* $P = 0.961$*). In contrast, the performance of screening for delivery with PE at ≥ 4 weeks after assessment up to 40 weeks' gestation was better with the method utilizing Bayes' theorem than that with the sFlt-1/PlGF ratio (AUC, 0.884 (95% CI, 0.854–0.914)* vs *0.818 (95% CI, 0.775–0.860);* $P < 0.0001$*).*

***Conclusion*** *At 31–34 weeks' gestation the performance of screening for PE delivering at < 4 weeks from assessment by the method utilizing Bayes' theorem is similar to that using the sFlt-1/PlGF ratio, but the former is superior to the latter in prediction of PE delivering ≥ 4 weeks from assessment.* *Copyright © 2016 ISUOG. Published by John Wiley & Sons Ltd.*

## INTRODUCTION

In women with pre-eclampsia (PE), the maternal serum concentration of angiogenic placental growth factor (PlGF) is decreased and the level of antiangiogenic soluble fms-like tyrosine kinase 1 (sFlt-1) is increased[1,2]. There is also evidence that the altered levels of PlGF and sFlt-1 precede the clinical onset of the disease and measurement of these biomarkers can be used for the prediction of PE[2–9]. Our approach to screening for PE is to use Bayes' theorem to derive the posterior risk by combining the prior risk from maternal characteristics and medical history with multiples of the median (MoM) values of biomarkers[8,10–15]. Others advocate the use of the simpler sFlt-1/PlGF ratio[4,9].

We have proposed recently an approach for stratification of pregnancies into high-, intermediate- and low-risk management groups based on the results of a risk assessment for PE at 32 weeks' gestation (Figure 1)[16]. The high-risk group would require intensive monitoring from the time of the initial assessment and up to 40 weeks' gestation, the intermediate-risk group would require reassessment 4 weeks after the initial assessment or intensive monitoring starting from 4 weeks and up to 40 weeks' gestation and the low-risk group would be reassessed only at 40 weeks' gestation. The performance of screening at 32 weeks is poor for prediction of PE delivering at > 40 weeks' gestation[8] and it would therefore be necessary to reassess all remaining pregnancies at 40 weeks to decide the best time and method of delivery.

The objective of this study was to compare the performance of screening by the method utilizing Bayes'

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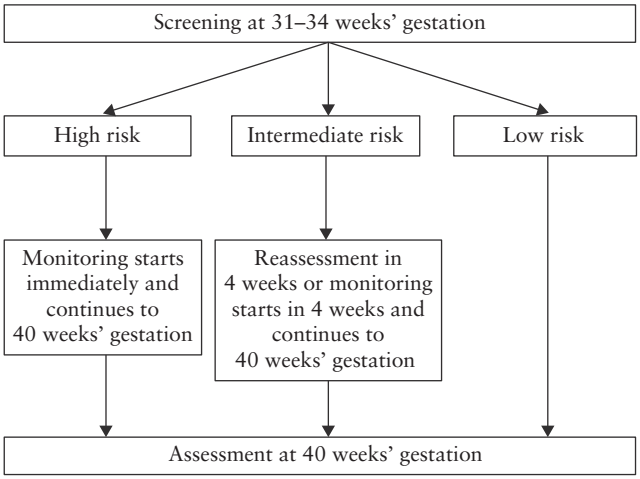


**Figure 1** Stratification of pregnancies into high-, intermediate- and low-risk management groups based on screening for pre-eclampsia at 31–34 weeks' gestation. High-risk group would require intensive monitoring from time of initial assessment and up to 40 weeks' gestation, intermediate-risk group would require intensive monitoring from 4 weeks after initial assessment and up to 40 weeks' gestation and low-risk group would be reassessed only at 40 weeks' gestation.

theorem to that of the sFlt-1/PlGF ratio in the prediction of delivery with PE at < 4 weeks from assessment and delivery with PE at 4 weeks from assessment and up to 40 weeks' gestation.

## METHODS

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending a 32-week routine hospital visit at King's College Hospital, London, or Medway Maritime Hospital, Gillingham, UK between March 2012 and January 2014. This visit included recording of maternal demographic characteristics and medical history, ultrasound examination of fetal anatomy and growth, and measurement of serum concentrations of PlGF and sFlt-1 in pg/mL by an automated biochemical analyzer (Cobas e411 system, Roche Diagnostics, Penzberg, Germany) within 10 min of blood sampling and results being available 30 min later. Gestational age was determined by measurement of fetal crown–rump length at 11–13 weeks or fetal head circumference at 19–24 weeks[17,18].

The women gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee. The inclusion criteria for this study were singleton pregnancy examined at 31 + 0 to 33 + 6 weeks' gestation and delivering a non-malformed live birth or stillbirth at ≥ 31 weeks' gestation. We excluded pregnancies with aneuploidy or major fetal abnormality. The study population was included in our previous report[8].

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[19].

### Statistical analysis

Patient-specific risks of delivery with PE at < 4 weeks from assessment and at < 40 weeks' gestation were calculated using the competing-risks model to combine the prior risk for PE from maternal characteristics and medical history with MoM values of PlGF and sFlt-1[8,10–15]. Pregnancies were allocated to the high-risk group if their risk for PE with delivery at < 4 weeks from assessment was above a specific high-risk threshold and they were allocated to the low-risk group if their risk for PE with delivery at < 40 weeks' gestation was below a specified low-risk threshold. Otherwise, they were allocated to the intermediate-risk group[16]. Different risk cut-offs were used to vary the proportion of the population stratified into each risk category and performance was assessed in terms of the distribution of pregnancy outcomes by risk group. In order to compare stratification based on risks utilizing Bayes' theorem with that based on sFlt-1/PlGF ratios we computed the cut-offs for stratification on the basis of ratios that would give the same proportions in the high-, intermediate- and low-risk groups as those obtained by the risks. We also examined the performance of sFlt-1/PlGF ratio > 38, because this ratio was reported previously as being useful in the prediction of PE with delivery at < 4 weeks after assessment in high-risk pregnancies[9].

The significance of the difference in performance between the method utilizing Bayes' theorem and that of the sFlt-1/PlGF ratio was assessed by comparison of the areas under the receiver–operating characteristics (ROC) curves (AUC)[20]. The statistical software package R was used for data analyses[21].

## RESULTS

The study population of 8063 singleton pregnancies included 231 (2.9%) that subsequently developed PE. Maternal and pregnancy characteristics of the study population are summarized in Table 1.

### Comparison of screening performance

The ROC curves for the performance of screening for PE with delivery < 4 weeks from assessment and PE with delivery ≥ 4 weeks after assessment and up to 40 weeks' gestation by the method utilizing Bayes' theorem and that of the sFlt-1/PlGF ratio are shown in Figure 2. There was no significant difference in performance of screening for PE with delivery at < 4 weeks between the method utilizing Bayes' theorem and that of the sFlt-1/PlGF ratio (AUC, 0.987 (95% CI, 0.979–0.995) *vs* 0.988 (95% CI, 0.981–0.994); $P = 0.961$). In contrast, the performance of screening for PE with delivery ≥ 4 weeks after assessment

**Table 1** Maternal and pregnancy characteristics in pregnancies that developed pre-eclampsia (PE) within 4 weeks from assessment, ≥ 4 weeks from assessment and up to 40 weeks' gestation and at > 40 weeks' gestation, compared with pregnancies that remained normotensive

| | | *Pre-eclampsia at:* | | |
|---|---|---|---|---|
| *Characteristic* | *Normotensive* (n = *7832*) | *< 4 weeks* (n = *29*) | *≥ 4 weeks to 40 GW* (n = *141*) | *> 40 GW* (n = *61*) |
| Age (years) | 31.0 (26.7–34.7) | 31.0 (26.4–34.0) | 31.7 (27.5–35.2) | 31.0 (24.9–34.8) |
| Weight (kg) | 67.3 (59.5–78.0) | 70.4 (60.0–86.0) | 76.0 (64.5–89.9) | 69.0 (61.0–84.8) |
| Height (m) | 1.65 (1.60–1.69) | 1.60 (1.58–1.65) | 1.65 (1.60–1.69) | 1.64 (1.60–1.69) |
| Racial origin | | | | |
| Caucasian | 5880 (75.1) | 19 (65.5) | 80 (56.7) | 41 (67.2) |
| Afro-Caribbean | 1339 (17.1) | 8 (27.6) | 50 (35.5) | 17 (27.9) |
| South Asian | 295 (3.8) | 2 (6.9) | 6 (4.3) | 2 (3.3) |
| East Asian | 145 (1.9) | 0 (0) | 3 (2.1) | 1 (1.6) |
| Mixed | 173 (2.2) | 0 (0) | 2 (1.4) | 0 (0.0) |
| Mode of conception | | | | |
| Spontaneous | 7570 (96.7) | 27 (93.1) | 136 (96.5) | 59 (96.7) |
| Assisted | 262 (3.3) | 2 (6.9) | 5 (3.5) | 2 (3.3) |
| Cigarette smoker | 790 (10.1) | 1 (3.4) | 8 (5.7) | 4 (6.6) |
| Chronic hypertension | 87 (1.1) | 6 (20.7) | 23 (16.3) | 3 (4.9) |
| APS/SLE | 14 (0.2) | 0 (0) | 0 (0) | 0 (0) |
| Diabetes mellitus | 76 (1.0) | 0 (0) | 3 (2.1) | 0 (0) |
| Parity | | | | |
| Nulliparous | 3839 (49.0) | 18 (62.1) | 68 (48.2) | 48 (78.7) |
| Parous no previous PE | 3726 (47.6) | 8 (27.6) | 42 (29.8) | 12 (19.7) |
| Parous previous PE | 267 (3.4) | 3 (10.3) | 31 (22.0) | 1 (1.6) |
| Family history of PE | 234 (3.0) | 1 (3.4) | 6 (4.3) | 2 (3.3) |
| Interpregnancy interval (years)* | 3.1 (2.1–5.1) | 7.1 (4.2–7.9) | 3.6 (2.4–5.3) | 6.5 (2.9–8.2) |

Data are given as median (interquartile range) or *n* (%). *Interpregnancy interval reported for parous women. APS, antiphospholipid syndrome; GW, gestational weeks; SLE, systemic lupus erythematosus.

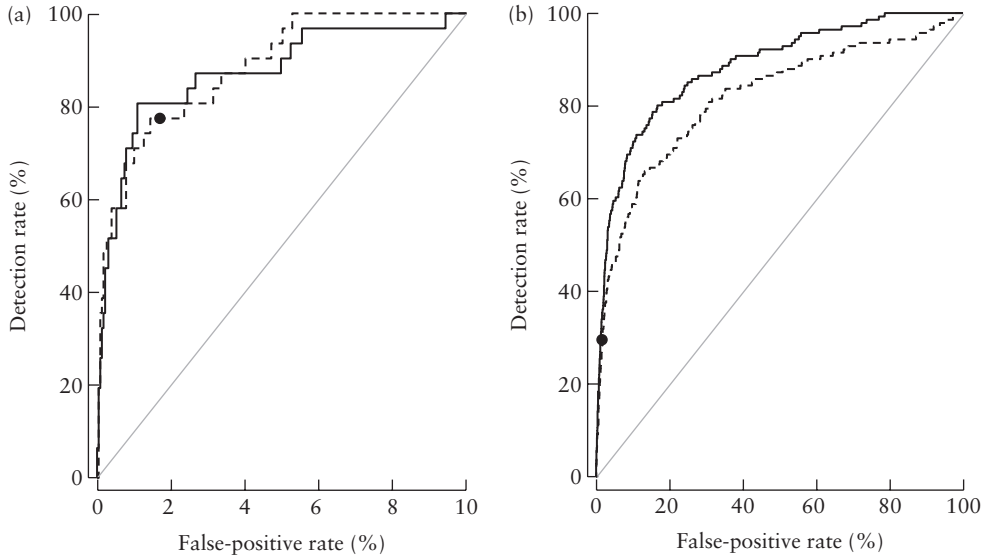


**Figure 2** Receiver–operating characteristics curves for prediction of pre-eclampsia: (a) within 4 weeks of assessment and (b) from 4 weeks after assessment and up to 40 weeks' gestation, by a method utilizing Bayes' theorem (———) and by the serum soluble fms-like tyrosine kinase-1 to placental growth factor (sFlt-1/PlGF) ratio (- - - - -). Circle represents performance of screening by sFlt-1/PlGF ratio > 38.

up to 40 week's gestation using the Bayes' theorem was significantly better than that of the sFlt-1/PlGF ratio (AUC, 0.884 (95% CI, 0.854–0.914) *vs* 0.818 (95% CI, 0.775–0.860); $P < 0.0001$).

### Performance of sFlt-1/PlGF ratio > 38

The sFlt-1/PlGF ratio was > 38 in 1.9% of the population and this group contained 75.9% of pregnancies with PE delivering at < 4 weeks and 24.1% of those with PE delivering at ≥ 4 weeks after assessment to 40 weeks' gestation. In the method utilizing Bayes' theorem, the risk cut-off for PE at < 4 weeks, allocating 1.9% of pregnancies to the high-risk group, was 1 in 8.3, and the 1.9% of pregnancies selected by this method comprised 79.3% of the pregnancies with PE at < 4 weeks and 29.1% of those with PE at ≥ 4 weeks from assessment to 40 weeks' gestation.

 

### Stratification of population into high-, intermediate- and low-risk groups

Allocation of pregnancies to risk group by pregnancy outcome is given in Table 2.

In the study population, there were 29 pregnancies that delivered with PE at < 4 weeks from assessment. At a risk cut-off of 1 in 3 for PE at < 4 weeks, 72.4% of pregnancies with PE at < 4 weeks were allocated to the high-risk group which comprised 1.2% of all pregnancies. The proportion of all pregnancies and those with PE at < 4 weeks allocated to the high-risk group increased from 1.2% and 72.4%, respectively, if the risk cut-off was 1 in 3, to 5.8% and 93.1% if the risk cut-off was 1 in 150.

The cut-off of sFlt-1/PlGF ratio that would be equivalent to a risk cut-off of 1 in 3, allocating 1.2% of pregnancies to the high-risk group, was 56.88; at this cut-off, 65.5% of pregnancies with PE at < 4 weeks were allocated to the high-risk group. The cut-off of sFlt-1/PlGF ratio that would be equivalent to a risk cut-off of 1 in 150, allocating 5.8% of pregnancies to the high-risk group, was 16.74; at this cut-off, 100% of pregnancies with PE at < 4 weeks were allocated to the high-risk group. For the same proportion of all pregnancies allocated to the high-risk group by the method utilizing Bayes' theorem and the one by the sFlt-1/PlGF ratio, the proportion of pregnancies with PE at < 4 weeks contained within this group was similar (proportion of the population: 1.2%, proportion of PE at < 4 weeks: 72.4% *vs* 65.5%; population: 2.1%, PE: 79.3% *vs* 75.9%; population: 3.8%, PE: 86.2% for both methods; population: 5.0%, PE: 86.2% *vs* 89.7%; population: 5.8%, PE: 93.1% *vs* 100%).

In the study population, there were 141 pregnancies that delivered with PE at ≥ 4 weeks after assessment and up to 40 weeks' gestation. The allocation of these cases into the high-, intermediate- and low-risk groups is shown in Table 2. For example, the high-risk group defined by a risk cut-off of 1 in 50 for PE at < 4 weeks constituted 3.8% of the population and contained 44.0% (62/141) of pregnancies with PE at ≥ 4 weeks to 40 weeks' gestation. Using this risk cut-off of 1 in 50 for PE at < 4 weeks and a risk cut-off of 1 in 150 for PE at < 40 weeks' gestation, 29.0% of pregnancies were allocated to the intermediate-risk group which contained 43.3% (61/141) of pregnancies with PE at ≥ 4 weeks to 40 weeks' gestation. Consequently, for these particular risk cut-offs, 32.8% of pregnancies were allocated to the high- or intermediate-risk group and the combination of these groups contained a total of 87.2% (123/141) of pregnancies with PE at ≥ 4 weeks to 40 weeks' gestation.

The cut-off of sFlt-1/PlGF ratio that would be equivalent to a risk cut-off of 1 in 50 for PE at < 4 weeks, allocating 3.8% of pregnancies to the high-risk group, was 22.44; at this cut-off, 40.4% of pregnancies with PE at ≥ 4 weeks to 40 weeks' gestation were allocated to the high-risk group, compared to 44.0% when the group of 3.8% of pregnancies was selected by the method utilizing Bayes' theorem. The combination of a risk cut-off of 1 in 50 for PE at < 4 weeks (sFlt-1/PlGF ratio 22.44) and a risk cut-off of 1 in 150 for PE at < 40 weeks' gestation (sFlt-1/PlGF ratio 4.35) allocated 29.0% of pregnancies to the intermediate-risk group; this group contained 41.1% of pregnancies with PE delivering at ≥ 4 weeks to 40 weeks' gestation when selection of the group was by the sFlt-1/PlGF ratio, compared to 43.3% with selection by the method utilizing Bayes' theorem. Consequently, for these particular risk or ratio cut-offs, 32.8% of pregnancies were allocated to the high- or intermediate-risk group and the combination of these groups contained a total of 81.6% of pregnancies with PE at ≥ 4 weeks to 40 weeks' gestation when the groups were selected by sFlt-1/PlGF ratio, compared to 87.2% with selection by the method utilizing Bayes' theorem.

## DISCUSSION

### Main findings

The study has demonstrated how assessment of risk for PE at 31–34 weeks' gestation can be used to stratify the population into high-, intermediate- and low-risk groups. Two approaches were applied to achieve such stratification and their performance was compared. The first approach utilized Bayes' theorem to combine maternal factors with MoM values of sFlt-1 and PlGF and derive patient-specific risks and the second approach used a simple division of the measured concentration of sFlt-1 by that of PlGF. The advantage of using the ratio is its simplicity in clinical practice. However, such approach does not take into account the prior risk of the individual patient in the study population and ignores the effects of maternal characteristics on the measured serum concentrations and their interrelations in both normal and pathological pregnancies[10,14,15].

We found that the performance of screening for PE at < 4 weeks from assessment was similar by the two methods, but the method utilizing Bayes' theorem was superior to that of the sFlt-1/PlGF ratio in predicting PE at ≥ 4 weeks from assessment. These findings confirm that the sFlt-1/PlGF ratio is a very strong predictor of imminent PE and the contribution of the prior risk from maternal factors in identifying the high-risk group is relatively small. With an increasing interval between sampling and development of PE, the contribution of maternal factors in prediction of PE becomes more apparent.

The proportion of the population stratified into high-, intermediate- and low-risk groups and the proportion of each stratum developing PE with delivery at < 4 weeks, at ≥ 4 weeks up to 40 weeks' gestation and at > 40 weeks' gestation would inevitably depend on the risk cut-offs used for defining the groups. In order to compare stratification based on risks utilizing Bayes' theorem with that based on sFlt-1/PlGF ratio, we computed the cut-offs for stratification on the basis of ratios that would give the same proportions in the high-, intermediate- and low-risk groups as those obtained by the risks. The cut-offs of sFlt-1/PlGF ratio for identifying the high-risk group for delivery with PE at < 4 weeks ranged from 56.88

**Table 2** Stratification of risk for pre-eclampsia (PE) delivering < 4 weeks from assessment, ≥ 4 weeks from assessment and up to 40 weeks' gestation and > 40 weeks' gestation, with cut-offs given as risks estimated with Bayes' theorem, combining maternal characteristics with multiples of the median values of serum soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), or as sFlt-1/PlGF ratio

| *Combined test: risk cut-off* | *sFlt-1/PlGF ratio cut-off* | *Risk group* | *All pregnancies (n = 8063)* | *PE at < 4 w (n = 29)* | | *PE at ≥ 4 w to 40 GW (n = 141)* | | *PE at > 40 GW (n = 61)* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Combined test* | *sFlt-1/PlGF ratio* | *Combined test* | *sFlt-1/PlGF ratio* | *Combined test* | *sFlt-1/PlGF ratio* |
| 1 in 3 for PE < 4 w and 1 in 50 for PE < 40 GW | 56.88 for PE < 4 w and 7.41 for PE < 40 GW | High | 97 (1.2; 1.0–1.5) | 21 (72.4; 52.8–87.3) | 19 (65.5; 45.7–82.1) | 22 (15.6; 10.0–22.7) | 23 (16.3; 10.6–23.5) | 1 (1.6; 0–8.8) | 1 (1.6; 0–8.8) |
| | | Inter | 1255 (15.6; 14.8–16.4) | 8 (27.6; 12.7–47.2) | 10 (34.5; 17.9–54.3) | 89 (63.1; 54.6–71.1) | 71 (50.4; 41.8–58.9) | 26 (42.6; 30.0–55.9) | 21 (34.4; 22.7–47.7) |
| | | Low | 6711 (83.2; 82.4–84.0) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 30 (21.3; 14.8–29.0) | 47 (33.3; 25.6–41.8) | 34 (55.7; 42.4–68.5) | 39 (63.9; 50.6–75.8) |
| 1 in 3 for PE < 4 w and 1 in 100 for PE < 40 GW | 56.88 for PE < 4 w and 5.35 for PE < 40 GW | High | 97 (1.2; 1.0–1.5) | 21 (72.4; 52.8–87.3) | 19 (65.5; 45.7–82.1) | 22 (15.6; 10.0–22.7) | 23 (16.3; 10.6–23.5) | 1 (1.6; 0–8.8) | 1 (1.6; 0–8.8) |
| | | Inter | 1970 (24.4; 23.5–25.4) | 8 (27.6; 12.7–47.2) | 10 (34.5; 17.9–54.3) | 97 (68.8; 60.5–76.3) | 80 (56.7; 48.1–65.0) | 34 (55.7; 42.4–68.5) | 32 (52.5; 39.3–65.4) |
| | | Low | 5996 (74.4; 73.4–75.3) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 22 (15.6; 10.0–22.7) | 38 (27.0; 19.8–35.1) | 26 (42.6; 30.0–55.9) | 28 (45.9; 33.1–59.2) |
| 1 in 3 for PE < 4 w and 1 in 150 for PE < 40 GW | 56.88 for PE < 4 w and 4.35 for PE < 40 GW | High | 97 (1.2; 1.0–1.5) | 21 (72.4; 52.8–87.3) | 19 (65.5; 45.7–82.1) | 22 (15.6; 10.0–22.7) | 23 (16.3; 10.6–23.5) | 1 (1.6; 0–8.8) | 1 (1.6; 0–8.8) |
| | | Inter | 2552 (31.7; 30.6–32.7) | 8 (27.6; 12.7–47.2) | 10 (34.5; 17.9–54.3) | 101 (71.6; 63.4–78.9) | 92 (65.2; 56.8–73.1) | 41 (67.2; 54.0–78.7) | 35 (57.4; 44.1–70.0) |
| | | Low | 5414 (67.1; 66.1–68.2) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 18 (12.8; 7.7–19.4) | 26 (18.4; 12.4–25.8) | 19 (31.1; 19.9–44.3) | 25 (41.0; 28.6–54.3) |
| 1 in 10 for PE < 4 w and 1 in 50 for PE < 40 GW | 35.63 for PE < 4 w and 7.41 for PE < 40 GW | High | 167 (2.1; 1.8–2.4) | 23 (79.3; 60.3–92) | 22 (75.9; 56.5–89.7) | 42 (29.8; 22.4–38.1) | 36 (25.5; 18.6–33.6) | 1 (1.6; 0–8.8) | 3 (4.9; 1.0–13.7) |
| | | Inter | 1185 (14.7; 13.9–15.5) | 6 (20.7; 8.0–39.7) | 7 (24.1; 10.3–43.5) | 69 (48.9; 40.4–57.5) | 58 (41.1; 32.9–49.7) | 26 (42.6; 30–55.9) | 19 (31.1; 19.9–44.3) |
| | | Low | 6711 (83.2; 82.4–84.0) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 30 (21.3; 14.8–29.0) | 47 (33.3; 25.6–41.8) | 34 (55.7; 42.4–68.5) | 39 (63.9; 50.6–75.8) |
| 1 in 10 for PE < 4 w and 1 in 100 for PE < 40 GW | 35.63 for PE < 4 w and 5.35 for PE < 40 GW | High | 167 (2.1; 1.8–2.4) | 23 (79.3; 60.3–92.0) | 22 (75.9; 56.5–89.7) | 42 (29.8; 22.4–38.1) | 36 (25.5; 18.6–33.6) | 1 (1.6; 0–8.8) | 3 (4.9; 1.0–13.7) |
| | | Inter | 1900 (23.6; 22.6–24.5) | 6 (20.7; 8.0–39.7) | 7 (24.1; 10.3–43.5) | 77 (54.6; 46.0–63.0) | 67 (47.5; 39.1–56.1) | 34 (55.7; 42.4–68.5) | 30 (49.2; 36.1–62.3) |
| | | Low | 5996 (74.4; 73.4–75.3) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 22 (15.6; 10.0–22.7) | 38 (27.0; 19.8–35.1) | 26 (42.6; 30.0–55.9) | 28 (45.9; 33.1–59.2) |
| 1 in 10 for PE < 4 w and 1 in 150 for PE < 40 GW | 35.63 for PE < 4 w and 4.35 for PE < 40 GW | High | 167 (2.1; 1.8–2.4) | 23 (79.3; 60.3–92.0) | 22 (75.9; 56.5–89.7) | 42 (29.8; 22.4–38.1) | 36 (25.5; 18.6–33.6) | 1 (1.6; 0–8.8) | 3 (4.9; 1.0–13.7) |
| | | Inter | 2482 (30.8; 29.8–31.8) | 6 (20.7; 8.0–39.7) | 7 (24.1; 10.3–43.5) | 81 (57.4; 48.8–65.7) | 79 (56.0; 47.4–64.4) | 41 (67.2; 54.0–78.7) | 33 (54.1; 40.8–66.9) |
| | | Low | 5414 (67.1; 66.1–68.2) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 18 (12.8; 7.7–19.4) | 26 (18.4; 12.4–25.8) | 19 (31.1; 19.9–44.3) | 25 (41.0; 28.6–54.3) |
| 1 in 50 for PE < 4 w and 1 in 50 for PE < 40 GW | 22.44 for PE < 4 w and 7.41 for PE < 40 GW | High | 309 (3.8; 3.4–4.3) | 25 (86.2; 68.3–96.1) | 25 (86.2; 68.3–96.1) | 62 (44.0; 35.6–52.6) | 57 (40.4; 32.3–49.0) | 5 (8.2; 2.7–18.1) | 5 (8.2; 2.7–18.1) |
| | | Inter | 1043 (12.9; 12.2–13.7) | 4 (13.8; 3.9–31.7) | 4 (13.8; 3.9–31.7) | 49 (34.8; 26.9–43.2) | 37 (26.2; 19.2–34.3) | 22 (36.1; 24.2–49.4) | 17 (27.9; 17.1–40.8) |
| | | Low | 6711 (83.2; 82.4–84.0) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 30 (21.3; 14.8–29.0) | 47 (33.3; 25.6–41.8) | 34 (55.7; 42.4–68.5) | 39 (63.9; 50.6–75.8) |
| 1 in 50 for PE < 4 w and 1 in 100 for PE < 40 GW | 22.44 for PE < 4 w and 5.35 for PE < 40 GW | High | 309 (3.8; 3.4–4.3) | 25 (86.2; 68.3–96.1) | 25 (86.2; 68.3–96.1) | 62 (44.0; 35.6–52.6) | 57 (40.4; 32.3–49.0) | 5 (8.2; 2.7–18.1) | 5 (8.2; 2.7–18.1) |

**Table 2** *Continued*

| *Combined test: risk cut-off* | *sFlt-1/PlGF ratio cut-off* | *Risk group* | *All pregnancies (n = 8063)* | *PE at < 4 w (n = 29)* | | *PE at ≥ 4 w to 40 GW (n = 141)* | | *PE at > 40 GW (n = 61)* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Combined test* | *sFlt-1/PlGF ratio* | *Combined test* | *sFlt-1/PlGF ratio* | *Combined test* | *sFlt-1/PlGF ratio* |
| | | Inter | 1758 (21.8; 20.9–22.7) | 4 (13.8; 3.9–31.7) | 4 (13.8; 3.9–31.7) | 57 (40.4; 32.3–49.0) | 46 (32.6; 25.0–41.0) | 30 (49.2; 36.1–62.3) | 28 (45.9; 33.1–59.2) |
| | | Low | 5996 (74.4; 73.4–75.3) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 22 (15.6; 10.0–22.7) | 38 (27.0; 19.8–35.1) | 26 (42.6; 30.0–55.9) | 28 (45.9; 33.1–59.2) |
| 1 in 50 for PE < 4 w and 1 in 150 for PE < 40 GW | 22.44 for PE < 4 w and 4.35 for PE < 40 GW | High | 309 (3.8; 3.4–4.3) | 25 (86.2; 68.3–96.1) | 25 (86.2; 68.3–96.1) | 62 (44.0; 35.6–52.6) | 57 (40.4; 32.3–49.0) | 5 (8.2; 2.7–18.1) | 5 (8.2; 2.7–18.1) |
| | | Inter | 2340 (29.0; 28.0–30.0) | 4 (13.8; 3.9–31.7) | 4 (13.8; 3.9–31.7) | 61 (43.3; 35.0–51.9) | 58 (41.1; 32.9–49.7) | 37 (60.7; 47.3–72.9) | 31 (50.8; 37.7–63.9) |
| | | Low | 5414 (67.1; 66.1–68.2) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 18 (12.8; 7.7–19.4) | 26 (18.4; 12.4–25.8) | 19 (31.1; 19.9–44.3) | 25 (41.0; 28.6–54.3) |
| 1 in 100 for PE < 4 w and 1 in 50 for PE < 40 GW | 18.61 for PE < 4 w and 7.41 for PE < 40 GW | High | 400 (5.0; 4.5–5.5) | 25 (86.2; 68.3–96.1) | 26 (89.7; 72.6–97.8) | 73 (51.8; 43.2–60.3) | 63 (44.7; 36.3–53.3) | 9 (14.8; 7.0–26.2) | 6 (9.8; 3.7–20.2) |
| | | Inter | 952 (11.8; 11.1–12.5) | 4 (13.8; 3.9–31.7) | 3 (10.3; 2.2–27.4) | 38 (27.0; 19.8–35.1) | 31 (22.0; 15.5–29.7) | 18 (29.5; 18.5–42.6) | 16 (26.2; 15.8–39.1) |
| | | Low | 6711 (83.2; 82.4–84.0) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 30 (21.3; 14.8–29.0) | 47 (33.3; 25.6–41.8) | 34 (55.7; 42.4–68.5) | 39 (63.9; 50.6–75.8) |
| 1 in 100 for PE < 4 w and 1 in 100 for PE < 40 GW | 18.61 for PE < 4 w and 5.35 for PE < 40 GW | High | 400 (5.0; 4.5–5.5) | 25 (86.2; 68.3–96.1) | 26 (89.7; 72.6–97.8) | 73 (51.8; 43.2–60.3) | 63 (44.7; 36.3–53.3) | 9 (14.8; 7.0–26.2) | 6 (9.8; 3.7–20.2) |
| | | Inter | 1667 (20.7; 19.8–21.6) | 4 (13.8; 3.9–31.7) | 3 (10.3; 2.2–27.4) | 46 (32.6; 25.0–41.0) | 40 (28.4; 21.1–36.6) | 26 (42.6; 30.0–55.9) | 27 (44.3; 31.5–57.6) |
| | | Low | 5996 (74.4; 73.4–75.3) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 22 (15.6; 10.0–22.7) | 38 (27.0; 19.8–35.1) | 26 (42.6; 30.0–55.9) | 28 (45.9; 33.1–59.2) |
| 1 in 100 for PE < 4 w and 1 in 150 for PE < 40 GW | 18.61 for PE < 4 w and 4.35 for PE < 40 GW | High | 400 (5.0; 4.5–5.5) | 25 (86.2; 68.3–96.1) | 26 (89.7; 72.6–97.8) | 73 (51.8; 43.2–60.3) | 63 (44.7; 36.3–53.3) | 9 (14.8; 7.0–26.2) | 6 (9.8; 3.7–20.2) |
| | | Inter | 2249 (27.9; 26.9–28.9) | 4 (13.8; 3.9–31.7) | 3 (10.3; 2.2–27.4) | 50 (35.5; 27.6–44.0) | 52 (36.9; 28.9–45.4) | 33 (54.1; 40.8–66.9) | 30 (49.2; 36.1–62.3) |
| | | Low | 5414 (67.1; 66.1–68.2) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 18 (12.8; 7.7–19.4) | 26 (18.4; 12.4–25.8) | 19 (31.1; 19.9–44.3) | 25 (41.0; 28.6–54.3) |
| 1 in 150 for PE < 4 w and 1 in 50 for PE < 40 GW | 16.74 for PE < 4 w and 7.41 for PE < 40 GW | High | 470 (5.8; 5.3–6.4) | 27 (93.1; 77.2–99.2) | 29 (100; 88.1–100) | 80 (56.7; 48.1–65.0) | 65 (46.1; 37.7–54.7) | 11 (18.0; 9.4–30.0) | 9 (14.8; 7.0–26.2) |
| | | Inter | 882 (10.9; 10.3–11.6) | 2 (6.9; 0.8–22.8) | 0 (0; 0–11.9) | 31 (22.0; 15.5–29.7) | 29 (20.6; 14.2–28.2) | 16 (26.2; 15.8–39.1) | 13 (21.3; 11.9–33.7) |
| | | Low | 6711 (83.2; 82.4–84.0) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 30 (21.3; 14.8–29.0) | 47 (33.3; 25.6–41.8) | 34 (55.7; 42.4–68.5) | 39 (63.9; 50.6–75.8) |
| 1 in 150 for PE < 4 w and 1 in 100 for PE < 40 GW | 16.74 for PE < 4 w and 5.35 for PE < 40 GW | High | 470 (5.8; 5.3–6.4) | 27 (93.1; 77.2–99.2) | 29 (100; 88.1–100) | 80 (56.7; 48.1–65.0) | 65 (46.1; 37.7–54.7) | 11 (18.0; 9.4–30.0) | 9 (14.8; 7.0–26.2) |
| | | Inter | 1597 (19.8; 18.9–20.7) | 2 (6.9; 0.8–22.8) | 0 (0; 0–11.9) | 39 (27.7; 20.5–35.8) | 38 (27.0; 19.8–35.1) | 24 (39.3; 27.1–52.7) | 24 (39.3; 27.1–52.7) |
| | | Low | 5996 (74.4; 73.4–75.3) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 22 (15.6; 10.0–22.7) | 38 (27.0; 19.8–35.1) | 26 (42.6; 30.0–55.9) | 28 (45.9; 33.1–59.2) |
| 1 in 150 for PE < 4 w and 1 in 150 for PE < 40 GW | 16.74 for PE < 4 w and 4.35 for PE < 40 GW | High | 470 (5.8; 5.3–6.4) | 27 (93.1; 77.2–99.2) | 29 (100; 88.1–100) | 80 (56.7; 48.1–65.0) | 65 (46.1; 37.7–54.7) | 11 (18.0; 9.4–30.0) | 9 (14.8; 7.0–26.2) |
| | | Inter | 2179 (27.0; 26.1–28.0) | 2 (6.9; 0.8–22.8) | 0 (0; 0–11.9) | 43 (30.5; 23.0–38.8) | 50 (35.5; 27.6–44.0) | 31 (50.8; 37.7–63.9) | 27 (44.3; 31.5–57.6) |
| | | Low | 5414 (67.1; 66.1–68.2) | 0 (0; 0–11.9) | 0 (0; 0–11.9) | 18 (12.8; 7.7–19.4) | 26 (18.4; 12.4–25.8) | 19 (31.1; 19.9–44.3) | 25 (41.0; 28.6–54.3) |

Data are given as *n* (%; 95% CI). GW, gestational weeks; Inter, intermediate; w, weeks.

to 16.74, with respective proportions of the population allocated to the high-risk group ranging from 1.2–5.8% and the proportion of cases with PE at < 4 weeks in this group varying from 65.5–100%. A previous study advocated the use of the specific ratio cut-off of > 38 to identify a group at high risk of developing PE within the subsequent 4 weeks[9]; in this study, 1.9% of the population fulfilled this criterion and this group contained 75.9% of pregnancies with PE at < 4 weeks and 24.1% of those with PE at ≥ 4 weeks to 40 weeks' gestation.

### Strengths and limitations

The strengths of this study are first, examination of a large population of pregnant women attending for routine care in a gestational age range which is widely used for assessment of fetal growth and wellbeing, second, recording of data on maternal characteristics and medical history to define the prior risk, third, use of automated machines to provide accurate measurement within 40 min of sampling of maternal serum concentration of PlGF and sFlt-1, fourth, expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements and use of Bayes' theorem to combine the prior risk from maternal factors with biomarkers to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy and fifth, direct comparison of the performance of screening for PE by a method utilizing Bayes' theorem to that of the sFlt-1/PlGF ratio.

A limitation of the study is that fitting of the risk model[8] and development and assessment of risk stratification were done with the same data, which introduces a degree of optimistic bias into the results. However, our risk model[4] is a parsimonious one with just two parameters for the mean log MoM value for each of the markers and a pooled estimate of an assumed common covariance matrix which limits the degree of bias. Nevertheless, prospective evaluation using an independent test data set is needed to validate the results.

### Comparison with previous studies

Our findings are comparable with those of a previous screening study for PE at 30–34 weeks' gestation, which included 118 cases of PE and 3734 unaffected pregnancies; in the cases of PE the sFlt-1 MoM to PlGF MoM ratio was increased and the deviation from normal was inversely related to the interval between sampling and the gestational age at delivery[7]. Our findings are also comparable with those of previous studies investigating high-risk pregnancies which reported that the sFlt-1/PlGF ratio is highly accurate in identifying the subgroup that will develop severe PE requiring delivery within the subsequent few weeks[3–6,9].

A study investigating serum PlGF at 11–13, 19–25, 30–34 and 35–37 weeks' gestation demonstrated that, in pregnancies that develop PE, serum PlGF was decreased in all four gestational-age groups, but the separation in MoM values from normal was greater when the interval between sampling and the development of PE was smaller; the performance of screening for PE < 37 weeks' gestation was superior with screening at 32 than at 22 or 12 weeks and the performance of screening for PE at ≥ 37 weeks was superior with screening at 36 weeks than at earlier gestations[22]. A similar study demonstrated that, in pregnancies that develop PE, serum sFlt-1 is increased and the separation in MoM values from normal was greater when the interval between sampling and the development of PE was smaller; however, unlike PlGF, sFlt-1 at 11–13 weeks was not a useful marker of PE[23].

### Clinical implications of the study

In the traditional approach to prenatal care, screening and diagnosis of PE is based on the demonstration of elevated blood pressure and proteinuria during a routine clinical visit in the late second or third trimester of pregnancy. In a proposed new pyramid of pregnancy care, the timing and content of clinical visits should be defined by the patient-specific risk of developing PE[24]. This study provides the framework for subsequent management of pregnancies based on the results of screening by maternal factors and the measurements of serum sFlt-1 and PlGF at 30–34 weeks' gestation. A small high-risk group can be monitored by measurement of blood pressure and urinalysis at least on a weekly basis, a larger intermediate-risk group would either be reassessed in 4 weeks or would undergo intensive monitoring from 4 weeks after the initial assessment, whereas patients in the large low-risk group can be reassured that development of PE before 40 weeks is very unlikely.

The best approach for stratification of risks for development of PE is the one that takes into account the prior risk of the individual patient based on maternal characteristics and medical history and defines the posterior risk by adjusting the measured serum concentrations of sFlt-1 and PlGF for those maternal characteristics that affect these measurements[10,14,15]. This approach also allows incorporation into the risk algorithm of additional potentially useful biomarkers, such as uterine artery pulsatility index and mean arterial pressure[8]. The alternative approach for allocation of patients into management groups is by the simple ratio of the measured concentrations of sFlt-1 and PlGF; this appears to be equally good as that utilizing Bayes' theorem in identifying the group at high risk of developing PE at < 4 weeks from assessment. However, in this respect, the best ratio is not > 38[9], but varies according to the desired proportion of the population allocated into the different management groups. The method utilizing Bayes' theorem is superior to the sFlt-1/PlGF ratio in identifying pregnancies at high risk of developing PE at ≥ 4 weeks from assessment.

The cut-offs in risks or sFlt-1/PlGF ratios to define the proportion of the population stratified into each of the three management groups and the protocols for such management will inevitably vary according to local

preferences and health economic considerations. Future studies will examine whether the implementation of such protocols could improve perinatal outcome.

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