*Ultrasound Obstet Gynecol* 2016; **47**: 472–477
Published online 7 March 2016 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.15816

# Maternal serum placental growth factor at 12, 22, 32 and 36 weeks' gestation in screening for pre-eclampsia

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**KEYWORDS:** Bayes' theorem; impaired placentation; placental growth factor; pre-eclampsia; pyramid of pregnancy care; screening

## ABSTRACT

***Objective*** *To examine the distribution of maternal serum placental growth factor (PlGF) at 12, 22, 32 and 36 weeks' gestation in singleton pregnancies which develop pre-eclampsia (PE) and examine the performance of this biomarker in screening for PE.*

***Methods*** *Serum PlGF was measured in 40 212 cases at 11–13 weeks, in 10 282 cases at 19–24 weeks, in 10 400 at 30–34 weeks and 4043 at 35–37 weeks. Bayes' theorem was used to combine the* a-priori *risk from maternal characteristics and medical history with serum PlGF. The performance of screening for PE requiring delivery $< 32$, at $32 + 0$ to $36 + 6$ and $\geq 37$ weeks' gestation was estimated.*

***Results*** *In pregnancies that developed PE, serum PlGF was decreased and the separation in multiples of the median (MoM) values from normal was greater with earlier, compared to later, gestational age at which delivery for PE became necessary. Additionally, the slope of the regression lines of PlGF MoM with gestational age at delivery in pregnancies that developed PE increased with advancing gestational age at screening. The detection rates (DRs), at a false-positive rate (FPR) of 10%, for PE delivering $< 32$ weeks were 79% and 97% with screening at 12 and 22 weeks, respectively. The DRs for PE delivering at $32 + 0$ to $36 + 6$ weeks were 57%, 65% and 90% with screening at 12, 22 and 32 weeks. The DRs for PE delivering $\geq 37$ weeks were 40%, 37%, 54% and 64% with screening at 12, 22, 32 and 36 weeks, respectively.*

***Conclusions*** *The performance of combined screening with maternal factors, medical history and PlGF is superior in screening for early, compared to late, PE and improves with advancing gestational age at screening.* 

## INTRODUCTION

Pre-eclampsia (PE) is a major cause of maternal and perinatal morbidity and mortality affecting 2–3% of all pregnancies[1–3]. In the last decade extensive research has been devoted to screening for PE with the aims of first, reducing the prevalence of the disease through pharmacological intervention in those at high risk[4,5] and second, minimizing adverse perinatal events for those that develop PE by determining the appropriate time and place for delivery[6].

Our approach to risk assessment and screening for PE is to apply Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the results of various combinations of biophysical and biochemical measurements made at different times during pregnancy[7–9]. However, in the application of Bayes' theorem in combined screening for PE, it is essential to standardize the measured values of biomarkers for any variables included in the prior model. A useful biochemical marker in screening for PE is serum placental growth factor (PlGF). This is a member of the vascular endothelial growth factor family and is implicated in angiogenesis and trophoblastic invasion of the maternal spiral arteries[10–12].

The objectives of this study were to present the distribution of serum PlGF values at 11–13, 19–24, 30–34 and 35–37 weeks' gestation in pregnancies that develop PE and examine the performance of screening for PE by serum PlGF at these stages in pregnancy.

## METHODS

### Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending three routine hospital visits at King's College Hospital,

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*Accepted: 13 November 2015*

 

**Table 1** Maternal and pregnancy characteristics in women with singleton pregnancy screened at different weeks for prediction of pre-eclampsia (PE)

| | *11–13 weeks* | | *19–24 weeks* | | *30–34 weeks* | | *35–37 weeks* | |
|---|---|---|---|---|---|---|---|---|
| *Characteristic* | *Normal (n = 39 009)* | *PE (n = 1203)* | *Normal (n = 9947)* | *PE (n = 335)* | *Normal (n = 10 104)* | *PE (n = 296)* | *Normal (n = 3978)* | *PE (n = 65)* |
| Maternal age (years) | 31.1 (26.6–34.9) | 31.4 (26.8–35.6)* | 31.1 (26.6–34.7) | 31.5 (27.0–35.7) | 31.1 (26.7–34.8) | 31.4 (27.0–34.8) | 31.6 (26.8–35.3) | 33.1 (28.8–35.7)* |
| Maternal weight (kg) | 66.5 (59.0–77.0) | 72.6 (63.0–87.0)* | 71.0 (63.1–81.8) | 78.0 (68.7–92.5)* | 76.8 (68.7–87.0) | 83.5 (72.0–97.8)* | 78.8 (70.7–89.4) | 84.4 (76.0–98.3)* |
| Maternal height (cm) | 165 (160–169) | 164 (159–168)* | 165 (160–169) | 164 (160–168)* | 165 (160–169) | 164 (159–168)* | 164 (160–169) | 165 (161–171) |
| BMI (kg/m$^2$) | 24.5 (21.9–28.4) | 27.1 (23.5–32.3)* | 26.1 (23.5–29.9) | 28.8 (25.6–33.3)* | 28.2 (25.4–32.0) | 31.2 (27.5–35.5)* | 29.2 (26.3–33.0) | 31.0 (28.0–34.3)* |
| GA (weeks) | 12.7 (12.3–13.1) | 12.7 (12.3–13.1) | 21.9 (21.2–22.1) | 22.0 (21.1–22.2) | 32.1 (32.0–32.5) | 32.1 (32.0–32.4) | 36.1 (36.0–36.4) | 36.1 (35.9–36.4) |
| Racial origin | | * | | * | | * | | |
| Caucasian | 28 768 (73.8) | 635 (52.8) | 7321 (73.6) | 202 (60.3) | 7369 (72.9) | 171 (57.8) | 2939 (73.9) | 47 (72.3) |
| Afro-Caribbean | 6649 (17.0) | 455 (37.8) | 1786 (18.0) | 110 (32.8) | 1924 (19.0) | 104 (35.1) | 685 (17.2) | 11 (16.9) |
| South Asian | 1740 (4.5) | 64 (5.3) | 425 (4.3) | 10 (3.0) | 381 (3.8) | 11 (3.7) | 146 (3.7) | 3 (4.6) |
| East Asian | 899 (2.3) | 19 (1.6) | 196 (2.0) | 7 (2.1) | 194 (1.9) | 6 (2.0) | 87 (2.2) | 1 (1.5) |
| Mixed | 953 (2.4) | 30 (2.5) | 219 (2.2) | 6 (1.8) | 236 (2.3) | 4 (1.4) | 121 (3.0) | 3 (4.6) |
| Medical history | | | | | | | | |
| CH | 467 (1.2) | 160 (13.3)* | 104 (1.1) | 41 (12.2)* | 109 (1.1) | 40 (13.5)* | 49 (1.2) | 5 (7.7)* |
| Diabetes mellitus | 336 (0.9) | 24 (2.0)* | 100 (1.0) | 9 (2.7)* | 101 (1.0) | 4 (1.4) | 34 (0.9) | 65 (100) |
| SLE/APS | 57 (0.2) | 8 (0.7)* | 18 (0.2) | 0 (0.0) | 16 (0.2) | 0 (0.0) | 10 (0.3) | 0 (0.0) |
| Cigarette smoker | 3853 (1.0) | 78 (6.5)* | 1015 (10.2) | 27 (8.1) | 1004 (9.9) | 16 (5.4)* | 367 (9.2) | 3 (4.6) |
| Family history of PE | 1611 (4.1) | 99 (8.2)* | 322 (3.2) | 20 (6.0)* | 329 (3.3) | 16 (5.4) | 137 (3.4) | 8 (12.3)* |
| Obstetric history | | * | | * | | * | | * |
| Parous | | | | | | | | |
| No previous PE | 19 257 (49.4) | 323 (26.9) | 4898 (49.2) | 83 (24.8) | 4828 (47.8) | 84 (28.4) | 2037 (51.2) | 10 (15.4) |
| Previous PE | 1258 (3.2) | 178 (14.8) | 329 (3.3) | 47 (14.0) | 349 (3.5) | 40 (13.5) | 90 (2.3) | 8 (12.3) |
| Nulliparous | 18 494 (47.4) | 702 (58.4) | 4720 (47.5) | 205 (61.2) | 4927 (48.8) | 172 (58.1) | 1851 (46.5) | 47 (72.3) |
| Interpregnancy interval (years) | 3.0 (2.0–5.0) | 4.1 (2.3–7.2)* | 3.0 (1.9–4.9) | 4.4 (2.6–6.7)* | 3.1 (2.1–5.2) | 3.8 (2.4–6.2)* | 3.1 (2.1–5.1) | 4.5 (2.3–8.7) |

Data are given as median (interquartile range) or *n* (%). APS, antiphospholipid syndrome; BMI, body mass index; CH, chronic hypertension; GA, gestational age at screening; SLE, systemic lupus erythematosus. Comparisons with normal group: chi-square or Fisher's exact tests for categorical variables and Mann–Whitney *U*-test for continuous variables: $^*P < 0.05$.

University College London Hospital and Medway Maritime Hospital, UK, between March 2006 and December 2014. In the first visit, at 11 + 0 to 13 + 6 weeks' gestation, we recorded maternal characteristics and medical history and performed combined screening for aneuploidies. The second visit, at 19 + 0 to 24 + 6 weeks' gestation, and third visit, initially at 30 + 0 to 34 + 6 weeks and subsequently at 35 + 0 to 37 + 6 weeks, included ultrasound examination of the fetal anatomy and estimation of fetal size from measurement of fetal head circumference, abdominal circumference and femur length. Gestational age was determined by the measurement of fetal crown–rump length at 11–13 weeks or fetal head circumference at 19–24 weeks[13,14].

Written informed consent was obtained from the women agreeing to participate in a study on adverse pregnancy outcome, which was approved by the ethics committee of each participating hospital. The inclusion criteria for this study were singleton pregnancy delivering phenotypically normal live birth or stillbirth at or after 24 weeks' gestation. Pregnancies with aneuploidy or major fetal abnormalities, and those ending in termination, miscarriage or fetal death before 24 weeks' gestation were excluded.

### Patient characteristics

Patient characteristics included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous/assisted conception requiring the use of ovulation drugs/*in-vitro* fertilization), cigarette smoking during pregnancy, medical history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS), family history of PE in the mother of the patient and obstetric history including parity (parous/nulliparous if no previous pregnancy at or after 24 weeks), previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interval in years between birth of the last child and estimated date of conception of the current pregnancy. Maternal height was measured in the first visit and maternal weight in each visit.

### Measurement of maternal serum placental growth factor

In the patients included in this study, maternal serum PlGF was measured at each visit by automated biochemical

 

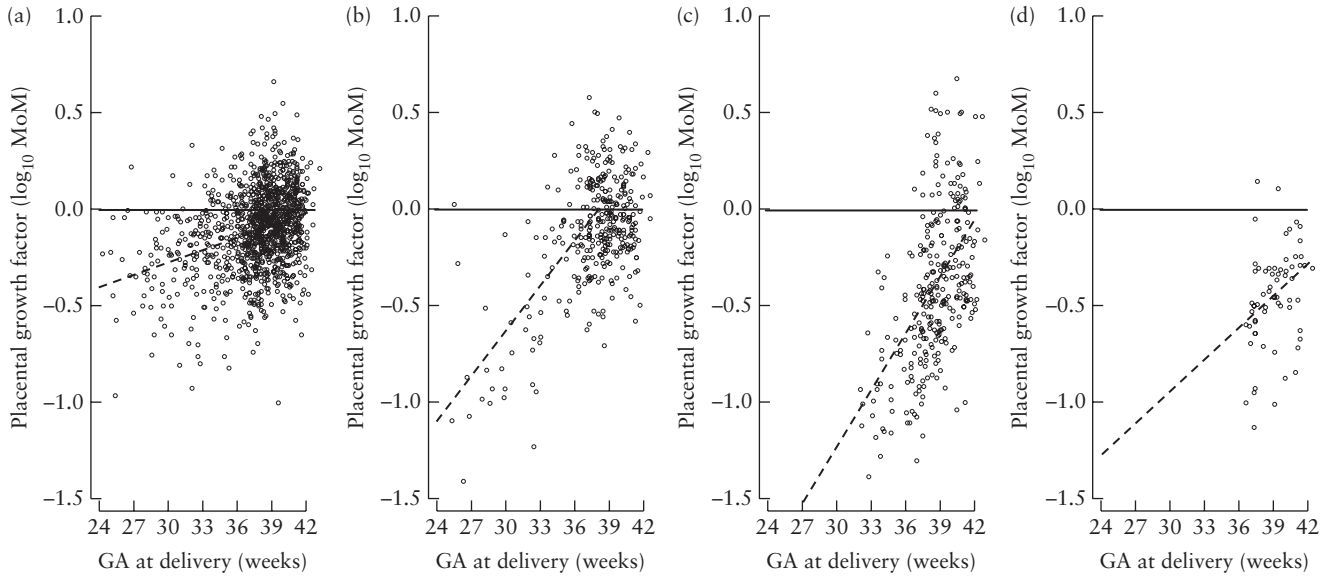


**Figure 1** Relationship between serum placental growth factor multiples of the median (MoM) and gestational age (GA) at delivery in pregnancies with pre-eclampsia, with screening at: (a) 11–13, (b) 19–24, (c) 30–34 and (d) 35–37 weeks' gestation. Regression lines (– – –) are shown.

analyzers within 10 min of blood sampling. In 37 279 cases the sample was analyzed using the DELFIA Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, USA) and in 27 658 cases the analysis was by the Cobas e411 system (Roche Diagnostics Ltd., Penzberg, Germany).

### Outcome measures

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[15]. The outcome measures for this study were PE delivering $< 32$, at $32 + 0$ to $36 + 6$, $< 37$ and $\geq 37$ weeks' gestation.

### Statistical analysis

#### *Competing-risks model*

The distribution of gestational age at delivery with PE was defined by two components: first, the prior distribution based on maternal characteristics[7] and second, the distribution of PlGF multiples of the median (MoM) values with gestational age at delivery in pregnancies affected by PE. The values of PlGF were $\log_{10}$ transformed to achieve homogeneity of variance and approximate Gaussian distributional form. Each measured value in the unaffected and PE pregnancies was expressed as a MoM, adjusting for those characteristics found to provide a substantive contribution to the $\log_{10}$-transformed value; these included gestational age, maternal age, weight, racial origin, cigarette smoking, diabetes mellitus, machine used for the assay and gestational age at delivery and birth-weight *Z*-score of the neonate in the previous pregnancy[16]. In the PE group, regression analysis demonstrated that the $\log_{10}$ MoM PlGF changed linearly with gestational age at delivery and this linear relationship was assumed to continue until the mean $\log_{10}$ MoM reached zero, beyond which the mean was taken as zero. The point at which the mean $\log_{10}$ MoM reached zero was determined using the method of least squares. Standard errors were obtained using bootstrapping. Risks of PE were obtained by applying Bayes' theorem to derive the posterior distribution of gestational age at delivery with PE from the maternal factors specific prior distribution[7] and the likelihood function of serum PlGF. The likelihood function comprises the regression of $\log_{10}$ MoM PlGF on gestational age at delivery with PE.

#### *Model-based estimates of screening performance using Bayes' theorem*

To provide model-based estimates of screening performance, the following procedure was adopted. First, we obtained the dataset of 123 406 singleton pregnancies, including 2748 (2.2%) with PE, that was previously used to develop a model for PE based on maternal demographic characteristics and medical history[7,17]. Second, for each of the records, PlGF MoM values were simulated from the fitted multivariate Gaussian distribution for log-transformed MoM values. Third, risks were obtained using the competing-risks model from the simulated MoM values and the pregnancy characteristics. These three steps were applied to the pregnancies within the normal group with no restriction on the time of delivery. Fourth, for a given false-positive rate, risks from the normal group were used to define a risk cut-off. The proportion of PE risks was then used to obtain an estimate of

 

**Table 2** Empirical and model-based detection rates of screening for pre-eclampsia (PE) by maternal factors and a combination of maternal factors and serum placental growth factor at 11–13, 19–24, 30–34 and 35–37 weeks' gestation

| | *Detection rate of PE delivering:* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *< 32 weeks* | | *32 + 0 to 36 + 6 weeks* | | *< 37 weeks* | | *≥ 37 weeks* | |
| *Screening* | *Empirical (95% CI) (%) (n/N)* | *Model (%)* | *Empirical (95% CI) (%) (n/N)* | *Model (%)* | *Empirical (95% CI) (%) (n/N)* | *Model (%)* | *Empirical (95% CI) (%) (n/N)* | *Model (%)* |
| *Maternal factors* | | | | | | | | |
| FPR = 5% | | | | | | | | |
| 11–13 weeks | 42 (31–53) 34/81 | 41 | 33 (27–39) 85/256 | 31 | 35 (30–41) 119/337 | 34 | 28 (25–31) 243/866 | 26 |
| 19–24 weeks | 47 (24–71) 9/19 | 41 | 31 (20–44) 19/62 | 31 | 35 (24–46) 28/81 | 34 | 25 (20–31)64/254 | 26 |
| 30–34 weeks | | | 25 (14–38) 14/56 | 31 | 25 (14–38) 14/56 | 31 | 25 (20–31) 60/240 | 26 |
| 35–37 weeks | | | | | | | 23 (13–35) 14/62 | 26 |
| FPR = 10% | | | | | | | | |
| 11–13 weeks | 51 (39–62) 41/81 | 52 | 47 (41–53) 120/256 | 45 | 48 (42–53) 161/337 | 47 | 38 (35–42) 332/866 | 37 |
| 19–24 weeks | 58 (33–80) 11/19 | 52 | 47 (34–60) 29/62 | 45 | 49 (38–61) 40/81 | 47 | 36 (30–42) 92/254 | 37 |
| 30–34 weeks | | | 34 (22–48) 19/56 | 45 | 34 (22–48) 19/56 | 45 | 36 (30–43) 87/240 | 37 |
| 35–37 weeks | | | | | | | 35 (24–49) 22/62 | 37 |
| *Combined* | | | | | | | | |
| FPR = 5% | | | | | | | | |
| 11–13 weeks | 62 (50–72) 50/81 | 68 | 46 (39–52) 117/256 | 44 | 50 (44–55) 167/337 | 50 | 30 (27–33) 258/866 | 28 |
| 19–24 weeks | 89 (67–99) 17/19 | 92 | 45 (32–58) 28/62 | 53 | 56 (44–67) 45/81 | 64 | 28 (22–33) 70/254 | 27 |
| 30–34 weeks | | | 79 (66–88) 44/56 | 82 | 79 (66–88) 44/56 | 82 | 40 (33–46) 95/240 | 41 |
| 35–37 weeks | | | | | | | 39 (27–52) 24/62 | 50 |
| FPR = 10% | | | | | | | | |
| 11–13 weeks | 74 (63–83) 60/81 | 79 | 61 (55–67) 157/256 | 57 | 64 (59–70) 217/337 | 63 | 42 (39–46) 365/866 | 40 |
| 19–24 weeks | 89 (67–99) 17/19 | 97 | 65 (51–76) 40/62 | 65 | 70 (59–80) 57/81 | 73 | 37 (31–43) 94/254 | 37 |
| 30–34 weeks | | | 88 (76–95) 49/56 | 90 | 88 (76–95) 49/56 | 90 | 51 (44–57) 122/240 | 54 |
| 35–37 weeks | | | | | | | 55 (42–68) 34/62 | 64 |

FPR, false-positive rate.

the associated DR. The area under the receiver–operating characteristics curve (AUC) was also calculated. The simulations were repeated 10 times to reduce variability due to the simulation process and provide suitably precise model-based estimates of performance.

### *Empirical performance of screening*

Five-fold cross-validation was used to assess the performance of screening for subgroups of PE according to gestational age at delivery, by models combining maternal factors with PlGF. The data were divided into five equal subgroups, the model was then fitted five times to different combinations of four of the five subgroups and used to predict risk of PE in the remaining fifth of the data. In each case, the maternal-factor model and the regression models were fitted to the training dataset comprising four-fifths of the data and used to produce risks for the hold-out sample comprising the remaining fifth of the data.

The statistical software package R was used for data analyses[18] and the survival package[19] was used for fitting the maternal factors model.

## RESULTS

The characteristics of the study population of singleton pregnancies with measurements of serum PlGF are summarized in Table 1. In the first phase of the study, PlGF was measured only in the first-trimester visit but this was subsequently extended to the second- and then the third-trimester visits.

At each stage of screening, PlGF MoM in pregnancies that developed PE was related to gestational age at delivery (Figure 1). The regression equations are given in Table S1. The standard deviation for $\log_{10}$ PlGF MoM in unaffected pregnancies and in those that developed PE are given in Table S2.

Empirical and model-based performance of screening for PE by maternal factors and PlGF at 11–13, 19–24, 30–34 and 35–37 weeks' gestation are shown in Tables 2 and S3 and Figure 2. In general, there was good agreement between empirical and model-based results. On the basis of the results from combined screening, the following conclusions can be drawn concerning performance of screening; first, this was superior for early compared to late PE and second, it increased with increasing gestational age at screening.

## DISCUSSION

### Principal findings of this study

The finding of this study demonstrates that serum PlGF improves the prediction of PE provided by maternal factors alone. In pregnancies that develop PE, serum PlGF is

 

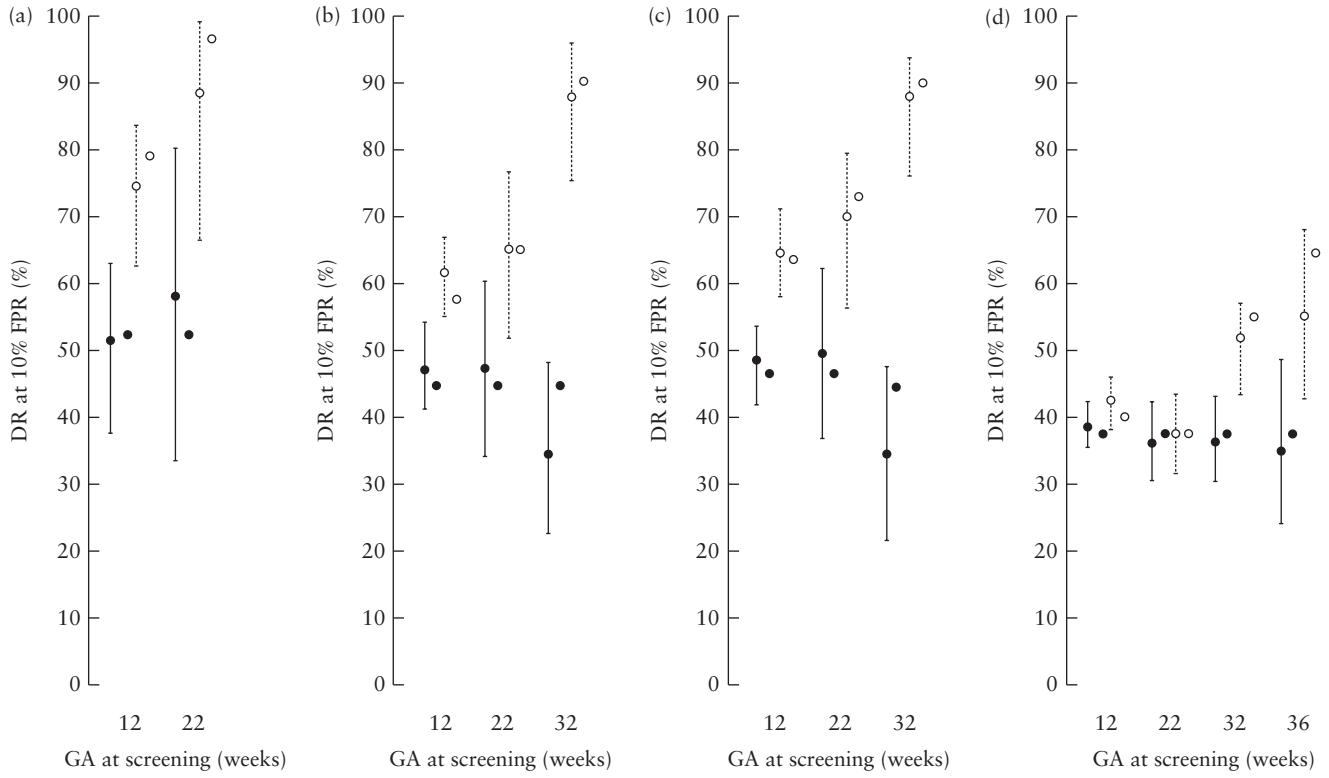


**Figure 2** Empirical detection rate (DR) of pre-eclampsia delivering: (a) < 32; (b) at 32 + 0 to 36 + 6; (c) < 37; and (d) ≥ 37 weeks' gestation, when screening by maternal factors (●) and by a combination of maternal factors with serum placental growth factor (○) at 11–13, 19–24, 30–34 and 35–37 weeks' gestation. Vertical lines represent 95% CIs. Adjacent circles without 95% CI represent model-based DR. FPR, false-positive rate; GA, gestational age.

decreased and the separation in MoM values from normal is greater with earlier compared to later gestational age at which delivery for PE becomes necessary; consequently, the performance of screening is superior for PE delivering < 37 weeks than PE delivering ≥ 37 weeks. The slope of the regression lines of PlGF MoM with gestational age at delivery in pregnancies that develop PE increases with advancing gestational age at screening; consequently, the performance of screening for PE delivering < 32 weeks is superior with screening at 22 than at 12 weeks, the performance of screening for PE delivering at 32 + 0 to 36 + 6 weeks is superior with screening at 32 than at 22 or 12 weeks and the performance of screening for PE delivering ≥ 37 weeks is superior with screening at 36 weeks than at earlier gestations.

## Strengths and limitations

The strengths of this screening study for PE in the three trimesters of pregnancy are first, examination of a large population of pregnant women attending for routine care, second, recording of data on maternal characteristics and medical history to identify known risk factors associated with PE, third, measurement of serum PlGF by automated machines that provide reproducible results within 40 min of sampling so that complete assessment and counseling can potentially be undertaken at the same hospital visit, fourth, expression of the values of serum PlGF as MoMs after adjustment for factors that affect the measurements, and fifth, use of Bayes' theorem to combine the prior risk from maternal factors with PlGF to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy.

A potential limitation of the study is that the performance of screening by a model, derived and tested using the same dataset, is overestimated. We have used cross-validation to reduce this effect and demonstrated that, in general, there was good agreement between the modeled and empirical performance.

## Comparison with previous studies

Several studies have documented that development of PE, especially preterm PE, is associated with decrease in serum PlGF during the first and third trimesters of pregnancy[9,20–22]. In this study we examined the performance of screening by a combination of maternal factors and serum PlGF, compared to screening with maternal factors alone, in the prediction of early, intermediate and late PE and documented the relationship between gestational age at screening and performance of the test.

## Clinical implications of the study

In a proposed new pyramid of pregnancy care[23], assessment at 11–13 weeks aims to identify those at high risk of developing preterm PE and through

 

pharmacological intervention, with such medications as low-dose aspirin, reduce the prevalence of the disease[4,5]. Measurement of mean arterial pressure is an essential component of such assessment, which also includes measurement of uterine artery pulsatility index and serum placental growth factor[9].

Assessment in the second and third trimesters aims to estimate the patient-specific risk of developing PE and, on the basis of such risk, define the subsequent management of pregnancy, including the timing and content of subsequent visits and decide on appropriate time, method and place for delivery[17]. We found that, during the second and third trimesters, the performance of screening for early, intermediate and late PE achieved by maternal factors was improved by the addition of serum PlGF. The performance of such screening for preterm PE is good and the approach we use is the basis for further improvement through the use of additional biomarkers.

## ACKNOWLEDGMENTS

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116) and by the European Union 7th Framework Programme -FP7-HEALTH-2013-INNOVATION-2 (ASPRE Project # 601852). The reagents and equipment for the measurement of serum placental growth factor were provided by PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland and Roche Diagnostics Limited, Penzberg, Germany.

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## SUPPORTING INFORMATION ON THE INTERNET

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

**Table S1** Regression equations of serum placental growth factor multiples of the median in pregnancies that developed pre-eclampsia

**Table S2** Standard deviation (SD) for $\log_{10}$ serum placental growth factor multiples of the median in unaffected pregnancies and those that developed pre-eclampsia

**Table S3** Modelled and empirical areas under the receiver–operating characteristics curve (AUC) in screening for pre-eclampsia (PE) delivering $< 32$, $< 37$ and $\geq 37$ weeks' gestation by maternal factors and a combination of maternal factors and serum placental growth factor at 11–13, 19–24, 30–34 and 35–37 weeks' gestation

 