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Maternal serum soluble fms-like tyrosine kinase-1 at 12, 22, 32 and 36 weeks' gestation in screening for pre-eclampsia

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KEYWORDS: Bayes' theorem; impaired placentation; pre-eclampsia; pyramid of pregnancy care; screening; sFlt-1

ABSTRACT

Objective *To examine the distribution of maternal serum soluble fms-like tyrosine kinase-1 (sFlt-1) at 12, 22, 32 and 36 weeks' gestation in singleton pregnancies that develop pre-eclampsia (PE) and examine the performance of this biomarker in screening for PE.*

Methods *Serum sFlt-1 was measured in 7066 cases at 11–13 weeks, 8079 cases at 19–24 weeks, 8472 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 levels of sFlt-1. The performance of screening for PE in women requiring delivery < 32, between 32 + 0 and 36 + 6 and ≥ 37 weeks' gestation was estimated.*

Results *In pregnancies that developed PE, serum sFlt-1 was increased 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. In pregnancies that developed PE, the slope of the regression lines of sFlt-1 MoM with gestational age at delivery increased with advancing gestational age at screening. Measurement of sFlt-1 at 11–13 weeks did not improve the prediction of PE achieved by maternal factors alone, sFlt-1 at 19–24 weeks improved the prediction of PE delivering < 37 weeks but not for PE delivering ≥ 37 weeks, sFlt-1 at 30–34 weeks improved the prediction of PE delivering < 37 and PE delivering ≥ 37 weeks and sFlt-1 at 35–37 weeks improved the prediction of PE delivering ≥ 37 weeks. The detection rates (DRs), at a false-positive rate (FPR) of 10%, of PE delivering < 32 weeks were 52% and 65% with screening at 12 and 22 weeks, respectively. The DRs for PE delivering between 32 + 0 and 36 + 6 weeks were 44%, 44% and 93% with screening at 12, 22 and 32 weeks. The DR for PE delivering ≥ 37 weeks were 37%, 37%, 52% and 69% with screening at 12, 22, 32 and 36 weeks, respectively.*

Conclusions *The performance of combined screening with maternal factors, medical history and serum sFlt-1 is superior for detection of 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 the high-risk group[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, for 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 soluble fms-like tyrosine kinase-1 (sFlt-1). This is a circulating anti-angiogenic protein and its serum concentration is increased in the few weeks preceding the clinical onset of PE[10–14]. Additionally, exogenous sFlt-1 administered to pregnant rats induces hypertension, proteinuria and glomerular endotheliosis[15].

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The objectives of this study were first, to present the distribution of serum sFlt-1 values at 11–13, 19–24, 30–34 and 35–37 weeks' gestation in pregnancies that develop PE, and second, examine the performance of screening for PE by serum sFlt-1 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, University College London Hospital and Medway Maritime Hospital, UK, between November 2011 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 the fetal head circumference at 19–24 weeks[16,17].

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 a phenotypically normal live birth or stillbirth at ≥ 24 weeks' gestation. Pregnancies with aneuploidies or major fetal abnormalities, and those ending in termination, miscarriage or fetal death before 24 weeks were excluded.

Patient characteristics

Patient characteristics that were recorded 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 or antiphospholipid syndrome, 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 at the first visit and weight at each visit.

Measurement of maternal serum sFlt-1

Of the patients included in the study, maternal serum sFlt-1 was measured at each visit by an automated biochemical analyzer within 10 min of blood sampling (Cobas e411 system, Roche Diagnostics, 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[18]. The outcome measures for this study were PE delivering < 32, between $32+0$ and $36+6$, < 37 and ≥ 37 weeks' gestation.

Statistical analysis

Competing-risks model

The distribution of gestational age at delivery with PE was defined by two components: the prior distribution based on maternal characteristics[7] and the distribution of sFlt-1 multiples of the median (MoM) values with gestational age at delivery in pregnancies affected by PE. The values of sFlt-1 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 weight, racial origin, cigarette smoking, birth-weight *Z*-score of the neonate in the previous pregnancy and interpregnancy interval[19]. In the PE group, regression analysis demonstrated that the $\log_{10}$ MoM sFlt-1 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 by the method of least squares. Standard errors were obtained by bootstrapping. Risks of PE were obtained by applying Bayes' theorem to derive the posterior distribution of gestational age at delivery with PE from maternal factors' specific prior distribution[7] and the likelihood function of serum sFlt-1. The likelihood function comprises the regression of $\log_{10}$ MoM sFlt-1 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, which were previously used to develop a model for PE based on maternal demographic characteristics and medical history[7,20]. Second, for each of the records, sFlt-1 MoM values were simulated from the fitted multivariate Gaussian distribution

for $\log_{10}$ transformed MoM values. Third, risks were obtained using the competing-risks model from the simulated MoM values and pregnancy characteristics. These three steps were applied to 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 the associated detection rate. 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 that combined maternal factors with sFlt-1. 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 on 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[21] and the survival package[22] was used for fitting the maternal-factors model.

RESULTS

The characteristics of the study population of singleton pregnancies with measurements of serum sFlt-1 are summarized in Table 1. At each stage of screening, sFlt-1 MoM in pregnancies that developed PE was inversely related to gestational age at delivery (Figure 1). The regression equations are given in Table S1. The standard deviation for $\log_{10}$ sFlt-1 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 sFlt-1 at 11–13, 19–24, 30–34 and 35–37 weeks' gestation are shown

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 = 6909)*	*PE (n = 157)*	*Normal (n = 7797)*	*PE (n = 282)*	*Normal (n = 8229)*	*PE (n = 243)*	*Normal (n = 3978)*	*PE (n = 65)*
Maternal age (years)	31.0 (26.4–34.7)	31.3 (26.7–34.8)	31.0 (26.5–34.7)	31.6 (26.5–35.7)	30.9 (26.6–34.7)	31.5 (27.0–35.0)	31.6 (26.8–35.3)	33.1 (28.8–35.7)*
Maternal weight (kg)	67.8 (59.6–78.8)	71.0 (63.0–86.9)*	71.0 (63.2–82.0)	78.0 (68.5–92.0)*	77.0 (68.6–87.5)	84.5 (72.9–98.5)*	78.8 (70.7–89.4)	84.4 (76.0–98.3)*
Maternal height (cm)	165 (160–169)	164 (160–168)	165 (160–169)	164 (160–168)	165 (160–169)	164 (159–168)*	164 (160–169)	165 (161–171)
BMI (kg/m^2)	24.8 (22.1–28.8)	26.5 (23.3–32.1)*	26.1 (23.5–30.0)	28.7 (25.4–33.2)*	28.2 (25.4–32.0)	31.3 (27.9–35.7)*	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)	32.2 (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	5161 (74.7)	86 (54.8)	5948 (76.3)	175 (62.1)	6170 (75.0)	148 (60.9)	2939 (73.9)	47 (72.3)
Afro-Caribbean	1181 (17.1)	63 (40.1)	1227 (15.7)	87 (30.9)	1419 (17.2)	78 (32.1)	685 (17.2)	11 (16.9)
South Asian	286 (4.1)	6 (3.8)	330 (4.2)	9 (3.2)	305 (3.7)	11 (4.5)	146 (3.7)	3 (4.6)
East Asian	121 (1.8)	1 (0.6)	141 (1.8)	6 (2.1)	150 (1.8)	4 (1.7)	87 (2.2)	1 (1.5)
Mixed	160 (2.3)	1 (0.6)	151 (1.9)	5 (1.8)	185 (2.3)	2 (0.8)	121 (3.0)	3 (4.6)
Medical history								
Chronic hypertension	80 (1.2)	21 (13.4)*	85 (1.1)	32 (11.3)*	94 (1.1)	34 (14.0)*	49 (1.2)	5 (7.7)*
Diabetes mellitus	63 (0.9)	5 (3.2)*	77 (1.0)	8 (2.8)*	79 (1.0)	3 (1.2)	34 (0.9)	0 (0.0)
SLE/APS	9 (0.1)	0 (0.0)	11 (0.1)	0 (0.0)	15 (0.2)	0 (0.0)	10 (0.3)	0 (0.0)
Cigarette smoker	686 (9.9)	11 (7.0)	795 (10.2)	21 (7.5)	836 (10.2)	13 (5.3)*	367 (9.2)	3 (4.6)
Family history of PE	204 (3.0)	10 (6.4)*	233 (3.0)	17 (6.0)*	242 (2.9)	10 (4.1)	137 (3.4)	8 (12.3)*
Obstetric history		*		*		*		*
Parous								
No previous PE	3500 (50.7)	46 (29.3)	3817 (49.0)	63 (22.3)	3919 (47.6)	64 (26.3)	2037 (51.2)	10 (15.4)
Previous PE	255 (3.7)	24 (15.3)	263 (3.4)	41 (14.5)	284 (3.5)	37 (15.2)	90 (2.3)	8 (12.3)
Nulliparous	3154 (45.7)	87 (55.4)	3717 (47.7)	178 (63.1)	4026 (48.9)	142 (58.4)	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; 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: $^*P < 0.05$.

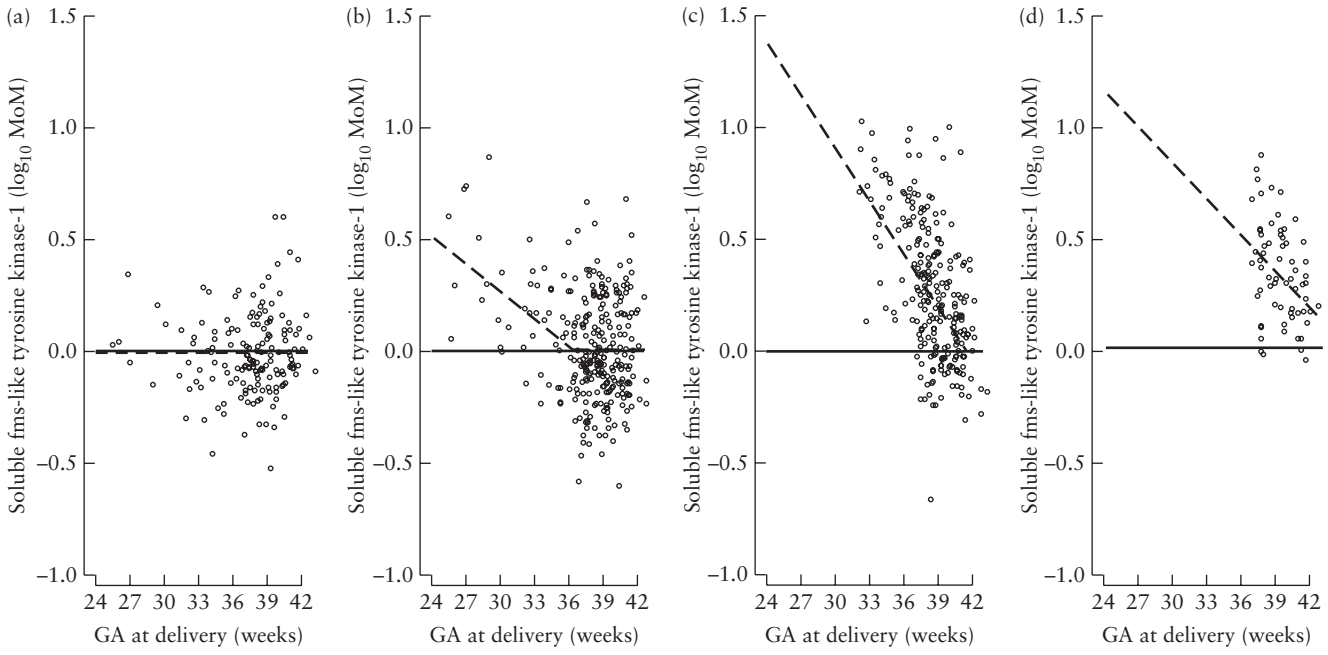

Figure 1 Relationship between serum soluble fms-like tyrosine kinase-1 multiples of the median (MoM) and gestational age (GA) at delivery in pregnancies with pre-eclampsia, with screening at: (a) 11–13, (b) 19–24, (c) 30–34 and (d) 35–37 weeks' gestation. Regression lines (— — -) are shown.

Table 2 Empirical and model-based detection rates of pre-eclampsia (PE) by screening with maternal factors and a combination of maternal factors and serum soluble fms-like tyrosine kinase-1 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	40 (12–74) 4/10	41	35 (21–52) 14/40	31	36 (23–51) 18/50	34	26 (18–36) 28/107	26
19–24 weeks	40 (16–68) 6/15	41	28 (16–42) 15/54	31	30 (20–43) 21/69	34	24 (19–31) 52/213	26
30–34 weeks			26 (14–40) 12/47	31	26 (14–40) 12/47	31	28 (22–35) 55/196	26
35–37 weeks							19 (10–31) 12/62	26
FPR = 10%								
11–13 weeks	60 (26–88) 6/10	52	53 (36–68) 21/40	45	54 (39–68) 27/50	47	35 (26–44) 37/107	37
19–24 weeks	53 (27–79) 8/15	52	41 (28–55) 22/54	45	43 (32–56) 30/69	47	34 (27–41) 72/213	37
30–34 weeks			36 (23–51) 17/47	45	36 (23–51) 17/47	45	37 (30–44) 73/196	37
35–37 weeks							29 (18–42) 18/62	37
Combined								
FPR = 5%								
11–13 weeks	40 (12–74) 4/10	41	35 (21–52) 14/40	31	36 (23–51) 18/50	34	26 (18–36) 28/107	27
19–24 weeks	53 (27–79) 8/15	54	26 (15–40) 14/54	32	32 (21–44) 22/69	38	28 (22–34) 59/213	26
30–34 weeks			81 (67–91) 38/47	87	81 (67–91) 38/47	91	37 (30–44) 72/196	40
35–37 weeks							58 (45–70) 36/62	56
FPR = 10%								
11–13 weeks	60 (26–88) 6/10	52	53 (36–68) 21/40	44	54 (39–68) 27/50	46	35 (26–44) 37/107	37
19–24 weeks	73 (45–92) 11/15	65	48 (34–62) 26/54	44	54 (41–66) 37/69	50	37 (31–44) 79/213	37
30–34 weeks			91 (80–98) 43/47	93	91 (80–98) 43/47	95	49 (42–57) 97/196	52
35–37 weeks							69 (56–80) 43/62	69

FPR, false-positive rate.

in Tables 2 and S3 and Figure 2. In general, there was good agreement between empirical and model-based results. Measurement of sFlt-1 at 11–13 weeks did not improve the prediction of PE achieved by maternal factors alone, sFlt-1 at 19–24 weeks improved the prediction of PE delivering < 37 weeks but not for those delivering ≥ 37 weeks, sFlt-1 at 30–34 weeks improved the prediction of PE delivering < 37 and ≥ 37 weeks and sFlt-1 at 35–37 weeks improved the prediction of PE delivering ≥ 37 weeks.

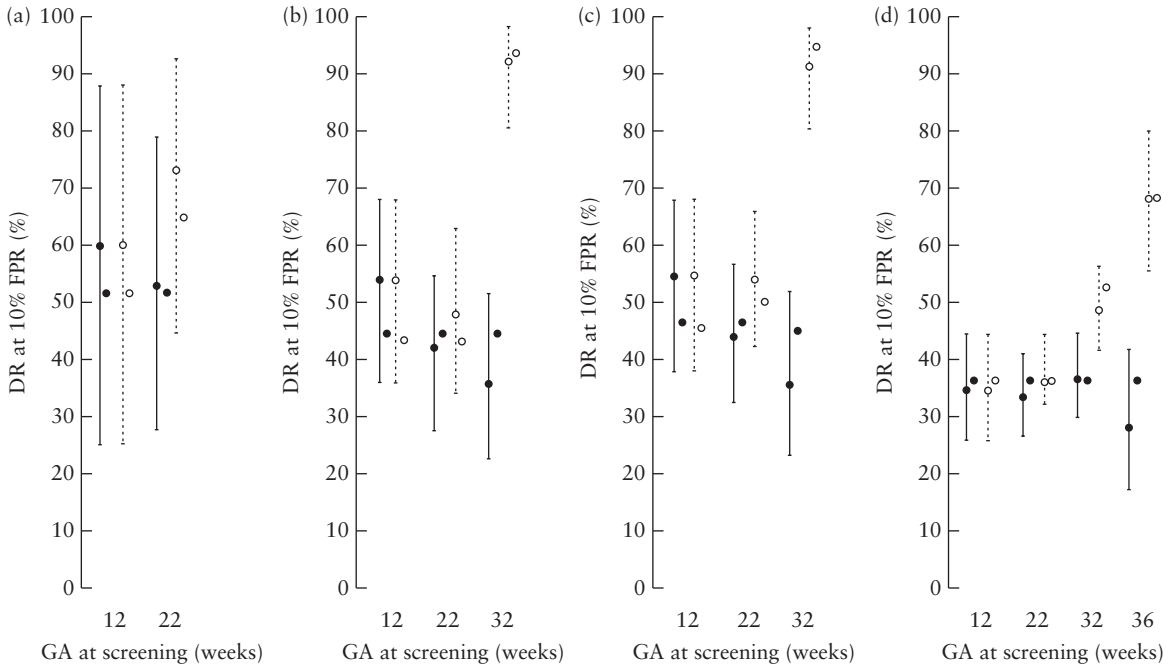

Figure 2 Empirical detection rate (DR) of pre-eclampsia delivering: (a) < 32; (b) at 32 + 0 to 36 + 6; (c) < 37; and (d) ≥ 37 weeks' gestation, when screening by maternal factors (•) and by a combination of maternal factors with serum soluble fms-like tyrosine kinase-1 (○) at 11–13, 19–24, 30–34 and 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.

DISCUSSION

Principal findings of the study

The findings of this study demonstrate that serum sFlt-1, measured in the second and third trimesters of pregnancy, improves the prediction of PE provided by maternal factors alone. In pregnancies that develop PE, serum sFlt-1 is increased and the separation in MoM values from normal is greater with earlier than 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 regression lines of sFlt-1 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 at 32 + 0 to 36 + 6 weeks is superior with screening at 32 than at 22 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 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 sFlt-1 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 sFlt-1 as MoM after adjustment for factors that affect the measurements, and fifth, use of Bayes' theorem to combine the prior risk from maternal factors with sFlt-1 to estimate patient-specific risks and the performance of screening for PE delivering at different stages in 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 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 serum sFlt-1 concentration is increased in the few weeks preceding the clinical onset of PE[10–14]. Case–control studies have reported that, in pregnancies that develop either early or late PE, serum sFlt-1 is increased from the first trimester and that inclusion of this measurement improves the prediction of screening with multiple biophysical and biochemical markers[23,24].

In this study, we examined the performance of screening by a combination of maternal factors and serum sFlt-1, 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. Measurement of sFlt-1 at 11–13 weeks did not improve the prediction of PE achieved by maternal factors alone.

Clinical implications of the study

In a proposed new pyramid of pregnancy care[25], assessment at 11–13 weeks aims to identify those at high risk of developing preterm PE and, through pharmacological intervention such as low-dose aspirin, reduce the prevalence of the disease[4,5]. Effective screening in the first trimester is provided by a combination of maternal factors, uterine artery pulsatility index, mean arterial pressure and serum placental growth factor[9,10]; the performance of screening is not improved by the addition of serum sFlt-1.

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 decisions concerning the time, method and place for delivery. 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 sFlt-1. Further improvement in performance is anticipated through the use of additional biomarkers.

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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 soluble fms-like tyrosine kinase-1 multiples of the median in singleton pregnancies that developed pre-eclampsia

Table S2 Standard deviation for $\log_{10}$ serum soluble fms-like tyrosine kinase-1 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 in screening for pre-eclampsia delivering < 32, < 37 and ≥ 37 weeks' gestation by maternal factors and a combination of maternal factors and serum soluble fms-like tyrosine kinase-1 at 11–13, 19–24, 30–34 and 35–37 weeks

