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Maternal serum soluble fms-like tyrosine kinase-1 at 22 and 32 weeks in the prediction of pre-eclampsia

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KEYWORDS: pre-eclampsia; repeat measurements; soluble fms-like tyrosine kinase-1; third-trimester screening

ABSTRACT

Objective *To investigate the potential value of repeat measurements of maternal serum concentration of soluble fms-like tyrosine kinase-1 (sFlt-1) at 22 and 32 weeks' gestation in the prediction of pre-eclampsia (PE) in women delivering after 32 weeks.*

Methods *The data were derived from prospective screening for adverse obstetric outcomes in women attending their routine hospital visit at 19–24 and/or 30–34 weeks' gestation in one of two maternity hospitals in England. Serum sFlt-1 was measured in 7565 and 8264 singleton pregnancies at 19–24 and 30–34 weeks, respectively. Bayes' theorem was used to combine the* a-priori *risk from maternal factors with sFlt-1 multiples of the median (MoM) values. The performance of screening for PE developing after the 30–34-week visit by sFlt-1, measured at 19–24, 30–34 and at both 19–24 and 30–34 weeks was examined.*

Results *In pregnancies with PE, sFlt-1 in both the second and third trimesters was increased and the deviation from normal was inversely related to the gestational age at which delivery became necessary for maternal or fetal indications. Serum sFlt-1 at 19–24 weeks was not useful in predicting PE beyond the 30–34-week visit, but the addition of sFlt-1 at 19–24 weeks improved the prediction of PE provided by sFlt-1 at 30–34 weeks. Screening by maternal factors and sFlt-1 at 30–34 weeks predicted 94% of preterm PE and 54% of term PE, at a false-positive rate of 10%; this was improved to 99% and 64%, respectively, by the additional measurement of sFlt-1 at 19–24 weeks.*

Conclusions *Measurement of sFlt-1 in the second trimester improves the prediction of PE provided by screening in the early third-trimester.* Copyright © 2016 ISUOG. Published by John Wiley & Sons Ltd.

INTRODUCTION

Soluble fms-like tyrosine kinase-1 (sFlt-1) is a circulating anti-angiogenic protein implicated in the pathogenesis of pre-eclampsia (PE). The concentration of sFlt-1 is increased in the placenta and serum of women with PE, and exogenous sFlt-1 administered to pregnant rats induces hypertension, proteinuria and glomerular endotheliosis[1–3]. There is also evidence that the levels of serum sFlt-1 are increased in the few weeks preceding the clinical onset of PE and, consequently, sFlt-1 may be a useful biochemical marker in screening for PE[2–6]. A meta-analysis of studies investigating the value of serum sFlt-1 before 30 weeks' gestation reported that the prediction of PE by this protein was poor[7]. A screening study for PE at 19–24 weeks' gestation reported that, although serum sFlt-1 improved the prediction of PE delivering < 32 weeks, it did not improve the prediction provided by maternal factors alone for PE delivering at 32–36 weeks or ≥ 37 weeks[8]. In contrast, screening by sFlt-1 at 30–34 weeks was useful in screening for both PE delivering < 37 weeks and PE delivering ≥ 37 weeks[9,10].

Screening for pregnancy complications often involves the use of multiple markers. The prevailing view in multimarker screening tests is that first, the individual markers should have good discriminatory power and second, there should be low correlations between markers so that they provide independent information. However, this view has been challenged by the demonstration that certain combinations of highly correlated markers, some of which have poor discriminatory power individually, can improve the overall performance of screening[11,12]. For example, serum pregnancy-associated plasma protein-A (PAPP-A) in the second trimester is a very poor marker for trisomy 21, but the addition of second-trimester PAPP-A to first-trimester PAPP-A results in substantial improvement in the performance of screening of the latter[11,12]. This is the consequence of a high correlation

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between first- and second-trimester PAPP-A in both euploid and trisomic pregnancies. Thus, contrary to the intuition that highly correlated markers are unlikely to be useful, joint discrimination of this type is more likely to occur when the two marker values show high correlation.

The objective of this study is to investigate whether the performance of screening for PE by the measurement of serum sFlt-1 in the third trimester is improved by taking into account the measurements obtained in the second trimester. Such improvement can potentially be achieved if there is a high correlation between the measurements obtained in the two trimesters.

METHODS

Study design and participants

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending their routine second- and third-trimester hospital visit at King's College Hospital and Medway Maritime Hospital, UK. These visits, which were held at 19 + 0 to 24 + 6 and 30 + 0 to 34 + 6 weeks' gestation, included the recording of maternal characteristics and medical history[13], ultrasound assessment of fetal growth, anatomy and wellbeing and measurement of serum concentration of sFlt-1 by an automated biochemical analyzer within 10 min of blood sampling (Cobas e411 system, Roche Diagnostics Ltd., Penzberg, Germany). Gestational age was determined from measurement of fetal crown–rump length at 11–13 weeks or fetal head circumference at 19–24 weeks[14,15]. The women were screened between November 2011 and July 2014 and 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 a singleton pregnancy delivering a phenotypically normal live birth or stillbirth at any stage after the visit at 30–34 weeks; pregnancies with major fetal abnormalities were excluded. For the visit at 30–34 weeks, we selected all cases with a sFlt-1 measurement. For the visit at 19–24 weeks, we selected all patients with a sFlt-1 measurement at this visit who also attended for a visit at 30–34 weeks, irrespective of whether sFlt-1 was measured in this later visit or not.

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.

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[16]. Outcome measures were PE requiring delivery at any stage after the third-trimester assessment. The unaffected group contained all pregnancies without PE or PIH.

Statistical analysis

Performance of screening was assessed first, by examining the empirical results using all available data on sFlt-1 at 19–24 weeks' gestation and/or 30–34 weeks, second, empirical results in 4589 pregnancies with complete data on sFlt-1 at both 19–24 and 30–34 weeks and third, by modeling, whereby values on sFlt-1 were simulated for our 123 406 singleton pregnancies with available data on maternal factors[13].

Competing-risks model

This model assumes that if the pregnancy was to continue indefinitely all women would develop PE, and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE. For any woman with specific maternal factors and biomarker multiples of the normal median (MoM) values, the posterior distribution of the time to delivery with PE, assuming there is no other cause of delivery, is obtained from the application of Bayes' theorem. Gestational age at delivery with PE was defined by two components: first, the prior distribution based on maternal factors[13] and second, the conditional distribution of multiple MoM biomarker values given the gestational age with PE and maternal factors. Values of sFlt-1 were expressed as MoMs adjusting for those characteristics found to provide a substantive contribution to their values, including the maternal factors in the prior model[17]. Multivariable Gaussian distributions were fitted to the second- and third-trimester $\log_{10}$ MoM values of sFlt-1 and a common covariance matrix was assumed for these distributions. Analysis of residuals was used to check the adequacy of the model and assess the effects of maternal factors on $\log_{10}$ MoM values in pregnancies with PE.

Empirical performance of screening

Five-fold cross validation was used to assess the empirical performance of screening for PE by maternal factors and the combination of maternal factors with sFlt-1. The data

Table 1 Maternal and pregnancy characteristics in women with singleton pregnancy screened by measurement of serum soluble fms-like tyrosine kinase-1 in the second and/or third trimester for prediction of pre-eclampsia (PE)

	19–24 weeks		*30–34 weeks*	
Characteristic	*Normal (n = 7318)*	*PE (n = 247)*	*Normal (n = 8021)*	*PE (n = 243)*
Maternal age (years)	30.9 (26.4–34.6)	31.6 (26.5–35.8)	30.9 (26.6–34.7)	31.5 (27.0–35.0)
Maternal weight (kg)	71.0 (63.0–82.0)	77.6 (68.5–91.0)*	76.7 (68.5–87.2)	84.5 (72.9–98.5)*
Maternal height (cm)	165 (160–169)	164 (160–168)	165 (160–169)	164 (159–168)*
BMI (kg/m^2)	26.1 (23.4–29.9)	28.6 (25.6–33.0)*	28.2 (25.4–32.0)	31.3 (27.9–35.7)*
GA at screening (weeks)	21.9 (21.2–22.1)	22.0 (21.1–22.2)	32.2 (32.0–32.5)	32.1 (32.0–32.4)
Racial origin		*		*
Caucasian	5606 (76.6)	153 (61.9)	6044 (75.4)	148 (60.9)
Afro-Caribbean	1136 (15.5)	76 (30.8)	1357 (16.9)	78 (32.1)
South Asian	298 (4.1)	9 (3.6)	294 (3.7)	11 (4.5)
East Asian	135 (1.8)	5 (2.0)	147 (1.8)	4 (1.7)
Mixed	143 (2.0)	4 (1.6)	179 (2.2)	2 (0.8)
Medical history				
Chronic hypertension	83 (1.1)	30 (12.2)*	94 (1.2)	34 (14.0)*
Diabetes mellitus	66 (0.9)	6 (2.4)*	75 (0.9)	3 (1.2)
SLE/APS	10 (0.1)	0 (0.0)	15 (0.2)	0 (0.0)
Family history of PE	209 (2.9)	12 (4.9)	231 (2.9)	10 (4.1)
Mode of conception				
Natural	7079 (96.7)	233 (94.3)	7757 (96.7)	233 (95.9)
In-vitro fertilization	173 (2.4)	8 (3.2)	190 (2.4)	5 (2.1)
Ovulation induction drugs	66 (0.9)	6 (2.4)	74 (0.9)	5 (2.1)
Obstetric history		*		*
Nulliparous	3443 (47.1)	156 (63.2)	3899 (48.6)	142 (58.4)
Parous				
No previous PE	3639 (49.7)	56 (22.7)	3860 (48.1)	64 (26.3)
Previous PE	236 (3.2)	35 (14.2)	262 (3.3)	37 (15.2)
Interpregnancy interval (years)	3.1 (2.0–5.0)	4.1 (2.5–6.2)*	3.1 (2.1–5.1)	4.1 (2.6–6.2)*

Data are given as median (interquartile range) or *n* (%). Comparisons with normal group: chi-square or Fisher's exact tests for categorical variables and Mann–Whitney *U*-test or student's *t*-test for continuous variables: $*P < 0.05$. APS, antiphospholipid syndrome; BMI, body mass index; GA, gestational age; SLE, systemic lupus erythematosus.

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 regression models and the covariance matrix 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. Our fitted model[13] for maternal factors was assumed for the prior distribution of time to delivery with PE, assuming no other cause of delivery.

Model-based estimates of screening performance

To provide model-based estimates of screening performance, the following procedure was adopted. First, we obtained the dataset of 123 406 singleton pregnancies that was previously used to develop a model for PE based on maternal demographic characteristics and medical history[13]. Second, for each case of PE ($n = 2748$) and pregnancies unaffected by PE or PIH ($n = 117\,710$), the MoM values for second- and third-trimester sFlt-1 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 unaffected group with no restriction on the time of delivery. Fourth, for a given false-positive rate (FPR), risks from the unaffected 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 (DR). The area under the receiver–operating characteristics curve (AUC) was also calculated. The simulations were repeated 100 times to reduce variability due to the simulation process and provide suitably precise model-based estimates of performance.

The statistical software package R was used for data analyses[18]. The survival package[19] was used for fitting the maternal-factors model and the package pROC[20] was used for the receiver–operating characteristics curve analysis.

RESULTS

The characteristics of the 7565 pregnancies with available data on sFlt-1 at 19–24 weeks and 8264 with data on sFlt-1 at 30–34 weeks are given in Table 1, those of the total population of 123 406 pregnancies with data on maternal factors are given in Table S1 and the 4589 pregnancies with complete data on sFlt-1 at both 19–24

Table 2 Empirical and model-based performance of screening for pre-eclampsia (PE) by maternal factors and a combination of maternal factors and serum soluble fms-like tyrosine kinase-1 (sFlt-1) at 22 weeks and/or 32 weeks' gestation

	Detection rate of PE delivering:							
	< 4 weeks from 32-week assessment		*< 6 weeks from 32-week assessment*		*< 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 (%)*
FPR = 1%								
Maternal factors	15 (10–22) 24/156	15	13 (10–17) 47/353	13	15 (10–21) 27/179	15	11 (9–14) 63/555	11
sFlt-1								
22 weeks	12 (4–26) 5/41	16	14 (8–21) 15/111	13	10 (3–21) 5/51	16	14 (10–20) 28/196	11
32 weeks	76 (58–89) 25/33	74	50 (39–60) 50/101	53	77 (62–88) 36/47	73	16 (11–22) 32/196	22
22 and 32 weeks	76 (50–93) 13/17	89	60 (46–72) 37/62	71	85 (65–96) 22/26	90	31 (23–40) 41/131	32
FPR = 5%								
Maternal factors	35 (27–43) 54/156	35	35 (30–40) 122/353	35	34 (27–42) 61/179	34	30 (27–34) 169/555	29
sFlt-1								
22 weeks	34 (20–51) 14/41	36	31 (22–40) 34/111	34	33 (21–48) 17/51	35	30 (23–37) 58/196	29
32 weeks	79 (61–91) 26/33	88	71 (61–80) 72/101	73	81 (67–91) 38/47	88	38 (31–45) 74/196	42
22 and 32 weeks	88 (64–99) 15/17	96	85 (74–93) 53/62	86	92 (75–99) 24/26	97	50 (41–58) 65/131	53
FPR = 10%								
Maternal factors	46 (38–54) 71/156	46	46 (40–51) 161/353	46	45 (37–52) 80/179	45	41 (37–45) 228/555	41
sFlt-1								
22 weeks	56 (40–72) 23/41	46	47 (37–57) 52/111	46	53 (38–67) 27/51	46	39 (32–46) 77/196	41
32 weeks	88 (72–97) 29/33	93	84 (76–91) 85/101	83	94 (82–99) 44/47	94	51 (44–58) 100/196	54
22 and 32 weeks	94 (71–100) 16/17	98	89 (78–95) 55/62	91	96 (80–100) 25/26	99	63 (54–71) 82/131	64

FPR, false-positive rate.

Table 3 Areas under the receiver–operating characteristics curve (AUC) in screening for pre-eclampsia (PE) by maternal factors and by a combination of maternal factors and serum soluble fms-like tyrosine kinase-1 (sFlt-1) at 22 weeks and/or 32 weeks' gestation

	AUC for PE delivering:							
	< 4 weeks from 32-week assessment		*< 6 weeks from 32-week assessment*		*< 37 weeks*		*≥ 37 weeks*	
Screening	*Empirical*	*Model*	*Empirical*	*Model*	*Empirical*	*Model*	*Empirical*	*Model*
Maternal factors	0.7855 (0.7497–0.8212)	0.7898	0.7735 (0.7484–0.7985)	0.7762	0.7844 (0.7513–0.8174)	0.7886	0.7495 (0.7285–0.7705)	0.7495
sFlt-1								
22 weeks	0.8191 (0.7557–0.8824)	0.7935	0.7697 (0.7253–0.8141)	0.7773	0.7952 (0.7351–0.8553)	0.7915	0.7393 (0.7042–0.7743)	0.7495
32 weeks	0.9594 (0.9292–0.9895)	0.9760	0.9237 (0.8950–0.9525)	0.9390	0.9658 (0.9440–0.9876)	0.9770	0.8052 (0.7723–0.8382)	0.8245
22 and 32 weeks	0.9833 (0.9662–0.9999)	0.9922	0.9474 (0.9126–0.9823)	0.9699	0.9866 (0.9754–0.9978)	0.9936	0.8505 (0.8140–0.8870)	0.8600

Values in parentheses are 95%CIs.

and 30–34 weeks' gestation are given in Table S2. The distributions of $\log_{10}$ MoM values of sFlt-1 in pregnancies that developed PE and the standard deviations for $\log_{10}$ MoM values of sFlt-1 at 19–24 and 30–34 weeks were reported previously[21]. The estimated correlation for $\log_{10}$ MoM sFlt-1 at 19–24 and 30–34 weeks, from the pooled data of PE and unaffected groups, was 0.6838 (95% CI, 0.6720–0.6953).

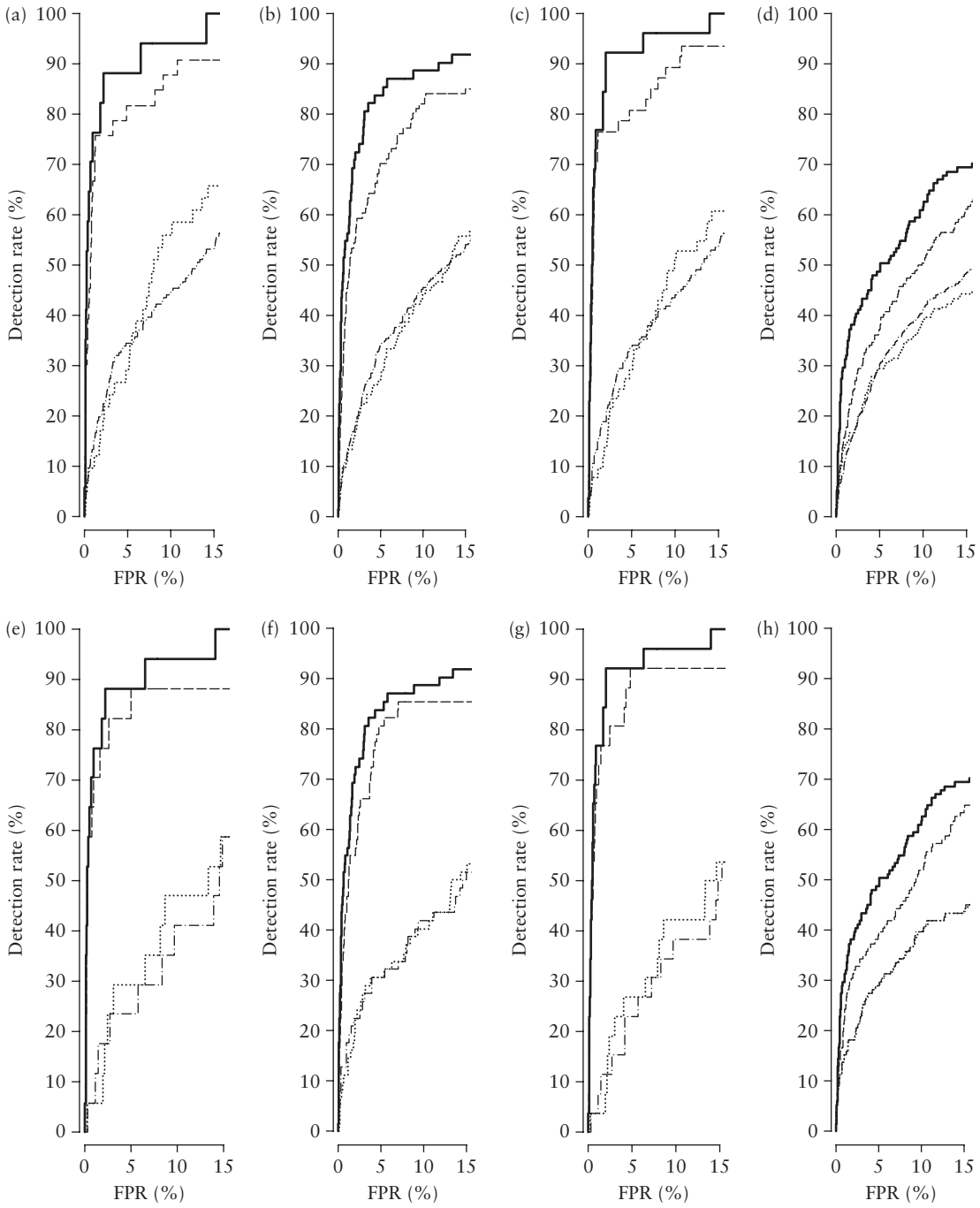

Figure 1 Receiver–operating characteristics curves for prediction of pre-eclampsia developing after third-trimester assessment by maternal factors (·–·–·) and by a combination of maternal factors with serum soluble fms-like tyrosine kinase-1 (sFlt-1) level at 19–24 weeks (·····), 30–34 weeks (– – –) and at both 19–24 and 30–34 weeks (——), in pregnancies delivering: (a,e) within 4 weeks of assessment; (b,f) within 6 weeks of assessment; (c,g) < 37 weeks; and (d,h) ≥ 37 weeks. (a–d) give results on all available data at 19–24 and/or 30–34 weeks and (e–h) give results from the pregnancies with complete data at both 19–24 and 30–34 weeks.

Empirical and model-based performance of screening for PE developing after the third-trimester visit by maternal factors and combinations of maternal factors and sFlt-1 at 19–24 weeks, sFlt-1 at 30–34 weeks and sFlt-1 at both 19–24 and 30–34 weeks are shown in Tables 2, 3 and S3 and Figures 1 and 2. Serum sFlt-1 at 19–24 weeks did not improve the prediction of PE provided by maternal factors alone. In contrast, screening by sFlt-1 at 30–34 weeks improved substantially the performance achieved by maternal factors alone and this was further improved by the addition of sFlt-1 at 19–24 weeks. Screening by maternal factors and sFlt-1 at 30–34 weeks predicted 94% of preterm PE and 54% of term PE, at a FPR of 10%; this was improved to 99% and 64%, respectively, by the additional measurement of sFlt-1 at 19–24 weeks. The empirical performance of screening was compatible with the model-based results but, as expected, the latter tended to be optimistically biased.

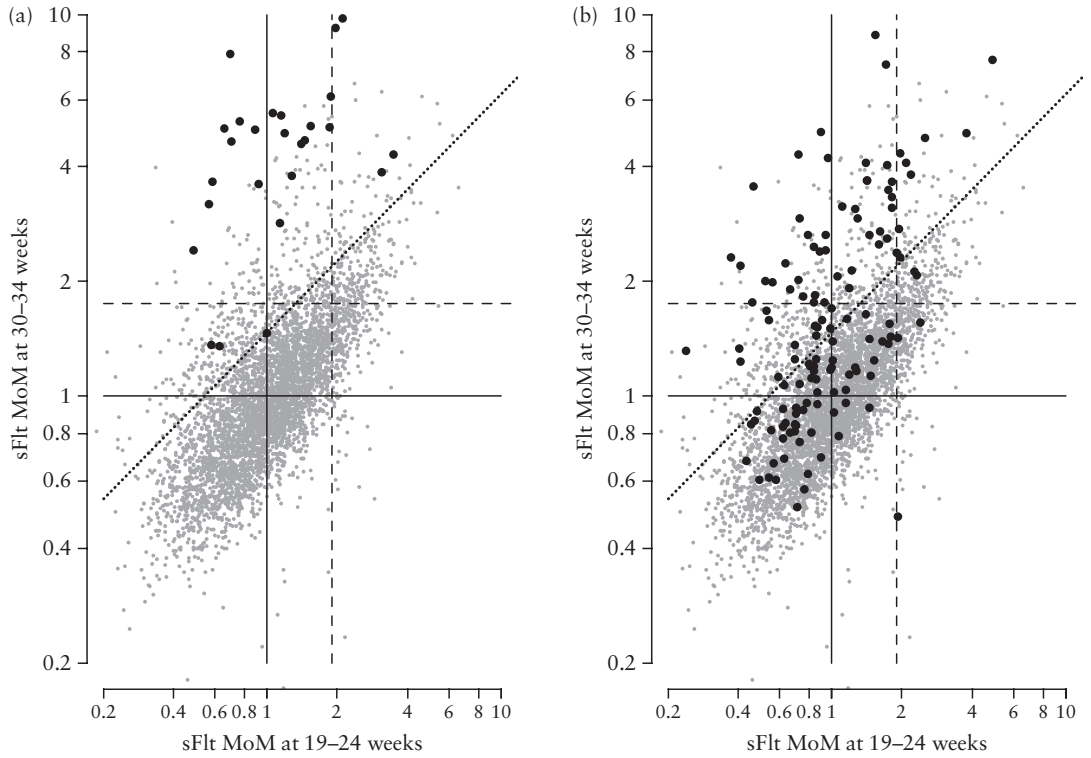

Figure 2 Relationship between multiples of the median (MoM) values of serum soluble fms-like tyrosine kinase-1 (sFlt-1) at 19–24 weeks and 30–34 weeks in unaffected pregnancies (•) and pregnancies that developed pre-eclampsia (•) and delivered after third-trimester assessment, either < 37 (a) or ≥ 37 (b) weeks' gestation. The dashed vertical and horizontal lines represent 90th percentiles of unaffected pregnancies for sFlt-1 at 19–24 weeks and 30–34 weeks, respectively. The diagonal dotted line is the likelihood ratio contour corresponding to the 90th percentile of unaffected pregnancies for sFlt-1 at both 19–24 and 30–34 weeks.

DISCUSSION

The findings of this study demonstrate that, in routine screening for PE by maternal serum sFlt-1 at 30–34 weeks' gestation, it is possible to identify most pregnancies that will develop preterm PE and many of those that develop term PE. In contrast, serum sFlt-1 in the second trimester is not a useful marker for PE developing after 32 weeks. However, the performance of screening for PE by serum sFlt-1 in the early third trimester is improved by inclusion of measurements from the second trimester. This is because, in both the unaffected pregnancies and in those that develop PE, there is a high correlation between second- and early third-trimester measurements of serum sFlt-1.

The findings of combining second- and early third-trimester serum sFlt-1 in screening for PE are compatible with those of combining first- and second-trimester serum PAPP-A in screening for trisomy 21[11,12]. These results provide further evidence in favor of the concept that certain combinations of highly correlated markers, some of which individually have poor discriminatory power, can improve the overall performance of screening. Our findings in pregnancies complicated by PE are consistent with those of previous studies investigating high-risk pregnancies which reported that measurement of serum sFlt-1 is accurate in identifying the subgroup that will develop severe PE requiring delivery within the subsequent few weeks[2–5].

The strengths of this screening study for PE are first, examination of a population of pregnant women attending for routine care at two gestational-age ranges which are widely used for assessment of fetal anatomy and growth, second, recording of data on maternal characteristics and medical history to define the prior risk, third, use of automated machines to provide accurate measurement of sFlt-1, fifth, expression of the values of sFlt-1 as MoMs after adjustment for factors that affect the measurements, sixth, use of Bayes' theorem to combine the prior risk with sFlt-1 to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy. A limitation of the study is that of optimistic bias in performance due to deriving and testing a model using the same dataset. We used five-fold cross validation to reduce such bias.

Extensive research in the last decade has led to the development of a two-stage strategy for identification of pregnancies at risk of PE[22]. The first stage involves screening at 11–13 weeks with the aim of predicting preterm PE because the prevalence of this condition can potentially be reduced substantially by the prophylactic use of low-dose aspirin started before 16 weeks' gestation[23,24]. The second stage involves screening in the second and third trimesters to predict both preterm PE and term PE, because close monitoring of such pregnancies for earlier diagnosis of the clinical signs of the disease could potentially improve perinatal outcome through such interventions as the administration of antihypertensive

medication and early delivery[25]. The potential value of novel treatments, including the administration of statins and vascular endothelial growth factor or extracorporeal removal of sFlt-1, is currently under investigation[26–28].

Measurement of sFlt-1 will inevitably be included in an integrated assessment combining biophysical and biochemical markers at around 32 weeks' gestation for the prediction of PE developing during the third trimester of pregnancy[6,10]. Although measurement of sFlt-1 at a similar combined clinic at 22 weeks does not improve the prediction of PE[8], consideration should be given to including such measurement to improve the prediction achieved at 32 weeks. The extent to which such a strategy will prove to be cost-effective remains to be determined.

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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 Maternal and pregnancy characteristics in total study population of women with singleton pregnancy screened for pre-eclampsia (PE) with data on maternal factors

Table S2 Maternal and pregnancy characteristics of 4589 singleton pregnancies screened for pre-eclampsia (PE) with complete data on serum soluble fms-like tyrosine kinase-1 measured at both 19–24 and 30–34 weeks' gestation

Table S3 Empirical and model-based performance of screening for pre-eclampsia (PE) by maternal factors and a combination of maternal factors and serum soluble fms-like tyrosine kinase-1 (sFlt-1) in 4589 singleton pregnancies with complete data on sFlt-1 measured at both 19–24 and 30–34 weeks' gestation

