Repeat measurements of uterine artery pulsatility index, mean arterial pressure and serum placental growth factor at 12, 22 and 32 weeks in the prediction of preeclampsia

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Biomarkers in prediction of preeclampsia

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ABSTRACT

Objective: To investigate the potential value of repeat measurements of uterine artery pulsatility index (UTPI), mean arterial pressure (MAP) and serum placental growth factor (PLGF) at 12, 22 and 32 weeks' gestation in the prediction of preeclampsia (PE) after 32 weeks.

Methods: The data were derived from prospective screening for adverse obstetric outcomes in women attending for their routine hospital visit at 11-13, 19-24 and / or 30-34 weeks' gestation in two maternity hospitals in England. UTPI, MAP and PLGF were measured. Bayes theorem was used to combine the *a priori* risk from maternal factors with UTPI, MAP and PLGF multiple of the median (MoM) values. The performance of screening for PE developing after the 30-34 weeks visit by UTPI, MAP and PLGF measured at 11-13, 19-24, 30-34 and their combinations was examined.

Results: Screening at 30-34 weeks by UTPI, MAP and PLGF detected, at 10% false positive rate, 79%, 86% and 92% of preterm-PE and 42%, 50% and 56% of term-PE. The addition of biomarker values obtained at 11-13 and / or 19-24 weeks was not associated with any improvement in the detection rate of preterm-PE; in the case of

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term-PE, there was a marginal (<2%) improvement in detection for UTPI and MAP and a modest improvement of about 5% for PLGF.

Conclusions: Measurements of UTPI, MAP and PLGF in the first- and / or second-trimester has a small or no effect in improving the prediction of PE provided by screening in the early third-trimester.

Introduction

Effective screening for preeclampsia (PE) is provided by the application of 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 [1-5]. Useful biomarkers at 11-13 and 19-24 weeks are uterine artery pulsatility index (UTPI), mean arterial pressure (MAP) and serum placental growth factor (PLGF), but not soluble fms-like tyrosine kinase-1 (SFLT) [2,3]. At 30-34 and 35-37 weeks, UTPI, MAP, PLGF and SFLT are useful biomarkers [4,5].

Routine screening for PE by maternal serum SFLT at 30-34 weeks' gestation can identify most pregnancies that will develop preterm-PE and many of those that develop term-PE [6]. We have recently shown that although serum SFLT at 19-24 weeks is not a useful marker for PE developing after 32 weeks, the performance of screening for PE by serum SFLT at 30-34 weeks was improved by inclusion of measurements from the second-trimester [6]. 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. These results provide evidence in favour 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 [7,8].

The objective of this study is to investigate whether the performance of screening for PE by the measurement of UTPI, MAP and PLGF at 30-34 weeks is improved by taking into account the measurements obtained in the first- and second-trimesters.

Methods

Study design and participants

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine second- and third-trimester hospital visit in pregnancy at King's College Hospital and Medway Maritime Hospital, UK. These visits, which were held at 11^{+0} - 13^{+6}, 19^{+0} - 24^{+6} and 30^{+0} - 34^{+6} weeks' gestation, included recording maternal characteristics and medical history,[1] ultrasound assessment of fetal growth, anatomy and wellbeing and measurement of UTPI, MAP and PLGF. Gestational age was determined from measurement of fetal crown-rump length (CRL) at 11-13 weeks or the fetal head circumference at 19-24 weeks [9,10]. The women were screened between December 2010 and August 2014 and gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

First and third trimester Dopper studies were carried out transabdominally but in the second trimester the transvaginal route was used because the cervical length was also measured. At 11-13 weeks, a sagittal section of the uterus was obtained, the cervical canal and internal cervical os were identified, the transducer was gently tilted from side to side and color flow mapping was used to identify each uterine artery along the side of the cervix and uterus at the level of the internal os [11,12]. At 19-24 weeks, women were asked to empty their bladder and were placed in the dorsal lithotomy position, the ultrasound probe was inserted into the vagina and advanced in turn into the left and right lateral fornix and the uterine arteries were identified using color Doppler at the level of the internal cervical os [13]. At 30- 37 weeks, color Doppler was used to identify each uterine artery at the apparent crossover with the external iliac arteries [12]. After

identification of each uterine artery, pulsed wave Doppler was used with the sampling gate set at 2 mm to cover the whole vessel. Care was taken to ensure that the angle of insonation was <30º and the peak systolic velocity was >60 cm/s to ensure that the uterine artery, rather than the arcuate artery, was examined. When three similar consecutive waveforms were obtained the PI was measured and the mean PI of the left and right arteries calculated.

Measurement of MAP was by validated automated devices (3BTO-A2, Microlife, Taipei, Taiwan). The women were in the sitting position, their arms were supported at the level of the heart, and a small (22 cm), normal (22 to 32 cm) or large (33 to 42 cm) adult cuff was used depending on the mid-arm circumference. After rest for five minutes, two recordings of blood pressure were made in both arms simultaneously. We calculated the final MAP as the average of all four measurements [14]. Maternal serum PLGF was measured by automated biochemical analyzers within 10 minutes of blood sampling (DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, USA or Cobas e411 system, Roche Diagnostics, Penzberg, Germany).

The inclusion criteria for this study were singleton pregnancies delivering a phenotypically normal live birth or stillbirth at any stage after the visit at 30-34 weeks; we excluded pregnancies with major fetal abnormalities. For the visit at 30-34 weeks, we selected all cases with measurement of UTPI, MAP and / or PLGF. For the visits at 11-13 and 19-24 weeks, we selected all patients with measurements of UTPI, MAP and / or PLGF at this visit who also attended for a visit at 30-34 weeks.

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]. 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 analyses

Performance of screening for each biomarker was assessed firstly, by modeling, whereby values on UTPI, MAP and PLGF were simulated for our 123,406 singleton pregnancies with available data on maternal factors [1] and secondly, by examining the empirical results in pregnancies with complete data for a given biomarker at 11-13, 19-24 and 30-34 weeks.

Competing risks model

This model assumes that if 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 women with specific maternal factors and biomarker multiple 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 defined by two components: firstly, the prior distribution based on maternal factors [1] and secondly, the conditional distribution of MoM biomarker values given the gestational age with PE and maternal factors. Values of UTPI, MAP,

PLGF 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 [16-18]. Multivariable Gaussian distributions were fitted to the first-, second- and third-trimester $\log_{10}$ MoM values of UTPI, MAP, PLGF 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.

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 [1]. Second, for each case of PE (n=2,748) and pregnancies unaffected by PE or PIH (n=117,710), the MoM values for first-, second- and third-trimester UTPI, MAP, PLGF were simulated from the fitted multivariate Gaussian distribution for log transformed MoM values. Third, risks were obtained using the competing risk 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 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 DR. The area under the receiver operating characteristic curve (AUROC) 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.

Empirical performance of screening

Ten-fold cross validation was used to assess the empirical performance of screening for PE by maternal factors and the combination of maternal factors and each of the three biomarkers. The data were divided into ten equal subgroups, the model was then fitted ten times to different combinations of nine of the ten subgroups and used to predict risk of PE in the remaining tenth of the data. In each case, the regression models and the covariance matrix were fitted to the training data set comprising nine tenths on the data and used to produce risks for the hold out sample comprising the remaining tenth of the data. Our fitted model [1] for maternal factors was assumed for the *prior* distribution of time to delivery with PE, assuming no other cause of delivery.

Mahalanobis distances

The Mahalanobis distance provides a simple measure of the separation between the fitted distributions of log transformed MoM values in unaffected pregnancies and those that developed PE. For a single marker the Mahalanobis distance is the difference in means between the PE and unaffected groups divided by the standard deviation. For two or more markers it is a generalization of this that takes account of the correlations. We present Mahalanobis distances between the distribution of log transformed MoM values in unaffected pregnancies and those requiring delivery with PE at 34.2 weeks, the median gestation for pregnancies delivered preterm for PE.

The statistical software package R was used for data analyses [19]. The survival package [20] was used for fitting the maternal factors model and the package pROC [21] was used for the receiver operating characteristic (ROC) curve analysis.

Results

The characteristics of the total population of 123,406 pregnancies with maternal factors are given in Table S1 and pregnancies with complete data for a given biomarker at 11-13, 19-24 and 30-34 weeks are given in Table S2. The distributions of $\log_{10}$ MoM values of UTPI, MAP and PLGF in pregnancies that developed PE and the standard deviations for $\log_{10}$ MoM values of each biomarker at 11-13, 19-24 and 30-34 weeks were reported previously.[22-24] Estimated correlations for $\log_{10}$ MoM values of each biomarker at 11-13, 19-24 and 30-34 weeks, from the pooled data of the PE and unaffected groups, are given in Table S3.

Empirical and model-based performance of screening for PE developing after the third-trimester visit by maternal factors and combinations of maternal factors with each biomarker at 11-13, 19-24 and 30-34 weeks are shown in Table 1. The empirical performance of screening was consistent with the model-based results. The performance of screening for preterm-PE at 30-34 weeks by UTPI, MAP or PLGF was not improved by the addition of the respective biomarker values obtained at 11-13 and / or 19-24 weeks; in the case of term-PE, there was a small improvement in performance of screening.

The relationship between MoM values of UTPI, MAP and PLGF at 19-24 weeks and 30-34 weeks in unaffected pregnancies and pregnancies that developed PE and delivered after the third trimester assessment at <37 weeks' gestation are shown in Figure 1; the values are compared to those of SFLT that were reported in a previous study [6]. In the case of UTPI, MAP and PLGF, combined screening at both 19-24 and 30-34 weeks has no or only marginal benefit over screening at 30-34 weeks alone. In contrast, screening by SFLT at 30-34 weeks is substantially improved by the addition of data from 19-24 weeks [6].

The Mahalanobis distances between unaffected and PE pregnancies for the biomarkers at 11-13 weeks, 19-24 weeks, 30-34 weeks and their combination are shown in Table 2. In the case of UTPI, there is slightly more separation between unaffected and PE pregnancies at 19-24 than at 30-34 weeks and there is little benefit from their combination. In the case of MAP and PLGF there is substantially more separation at 30-34 weeks than at 11-13 or 19-24 weeks and there is little benefit from combining the measurements at 30-34 weeks with those taken at earlier gestational ages. As reported in previous studies, SFLT has very little separation at 11-13 or 19-24 weeks, but at 30-34 weeks the separation is larger than that for any other marker; inclusion of the measurements at 19-24 weeks increases the separation substantially.[6,25]

Discussion

Principal findings of this study

The findings of this study demonstrate that in routine screening for PE by UTPI, MAP and serum PLGF at 30-34 weeks' gestation the detection rate (DR) of preterm-PE and term-PE, at a given false positive rate (FPR), is not substantially improved by inclusion of measurements from the first or second-trimester. This is different from the finding that the performance of screening by SFLT at 30-34 weeks is improved by the inclusion of measurements from the second-trimester [6].

The prevailing view in multi-marker screening tests for pregnancy complications is that firstly, the individual markers should have good discriminatory power and secondly, 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 individually have poor discriminatory power, can improve the overall performance of screening [7,8]. 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 [7,8]. This is the consequence of a high correlation 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.

In the case of SFLT there is a relatively high correlation between second- and third-trimester levels and, although the latter provides good discrimination between PE and unaffected pregnancies, the former does not. The combination of two measurements separates the unaffected pregnancies from those with PE substantially more than either of the individual measurements as illustrated in Figure 1. This is also reflected in the increase in Mahalanobis distance achieved by including the second-trimester measurements along with those in the third-trimester. In the case of MAP and UTPI there is a relatively low correlation between second- and third-trimester levels and the shift in the pregnancies that develop PE is similar in the two trimesters. For PLGF there is a relatively high correlation, but the shifts in means from PE are such that the second-trimester measurements add little to those in the third-trimester; this is shown in Figure 1 by the way in which the likelihood ratio contour is very similar to the 10th percentile of the third-trimester measurement.

Strengths and limitations

The strengths of this study are first, examination of a large population of pregnant women attending for routine care at three 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, expression of the values of UTPI, MAP and PLGF as MoMs after adjustment for factors that affect the measurements, fourth, use of Bayes theorem to combine the *prior* risk with biomarkers to estimate the performance of screening for PE. 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 ten-fold cross validation to reduce such bias.

Clinical implications of the study

Extensive research in the last decade has led to the development of a two stage strategy for identification of pregnancies at risk of PE [26]. The first stage, involves risk stratification at 11-13 weeks with the aim of predicting preterm-PE, because the prevalence of this condition can be potentially reduced substantially by the prophylactic use of low-dose aspirin started before 16 weeks' gestation [27,28]. The second-stage, involves risk stratification in the second- and third-trimesters to predict both preterm-PE and term-PE, with the aim of improving outcome through close monitoring of high-risk pregnancies to define the best time, place and mode of delivery.

Risk stratification at 11-13 weeks by a combination of maternal factors, UTPI, MAP and PLGF can predict 75% of cases of preterm-PE and about 45% of term-PE at FPR of 10% [2,29]; the respective values for risk stratification at 19-24 weeks are 85% and about 45% [3]. Risk stratification at 30-34 weeks by maternal factors, UTPI, MAP, PLGF and SFLT detects 99% of preterm-PE and 66% of term-PE [4]. The findings of this study suggest that in screening at 30-34 weeks for women who had prior screening in the first- and / or second-trimester there is no benefit in considering the results of UTPI, MAP or PLGF from such prior screening and only the results from testing at 30-34 weeks should be considered.

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Figure legend

Figure 1. Relationship between multiple of the median (MoM) values of UTPI, MAP, PLGF and SFLT^6 at 19-24 weeks and 30-34 weeks in unaffected pregnancies (grey dots) and pregnancies that developed preeclampsia (red dots) and delivered after the third trimester assessment at <37 weeks' gestation. For UTPI, MAP and SFLT, the interrupted blue vertical and horizontal lines represent the 90th percentiles of unaffected pregnancies for the biomarker at 19-24 weeks and at 30-34 weeks, separately. The diagonal blue line is the 90th percentile likelihood ratio contour of unaffected pregnancies for measurements taken at 19-24 and at 30-34 weeks combined. PLGF is reduced in PE and the 10th rather than the 90th percentiles are shown.

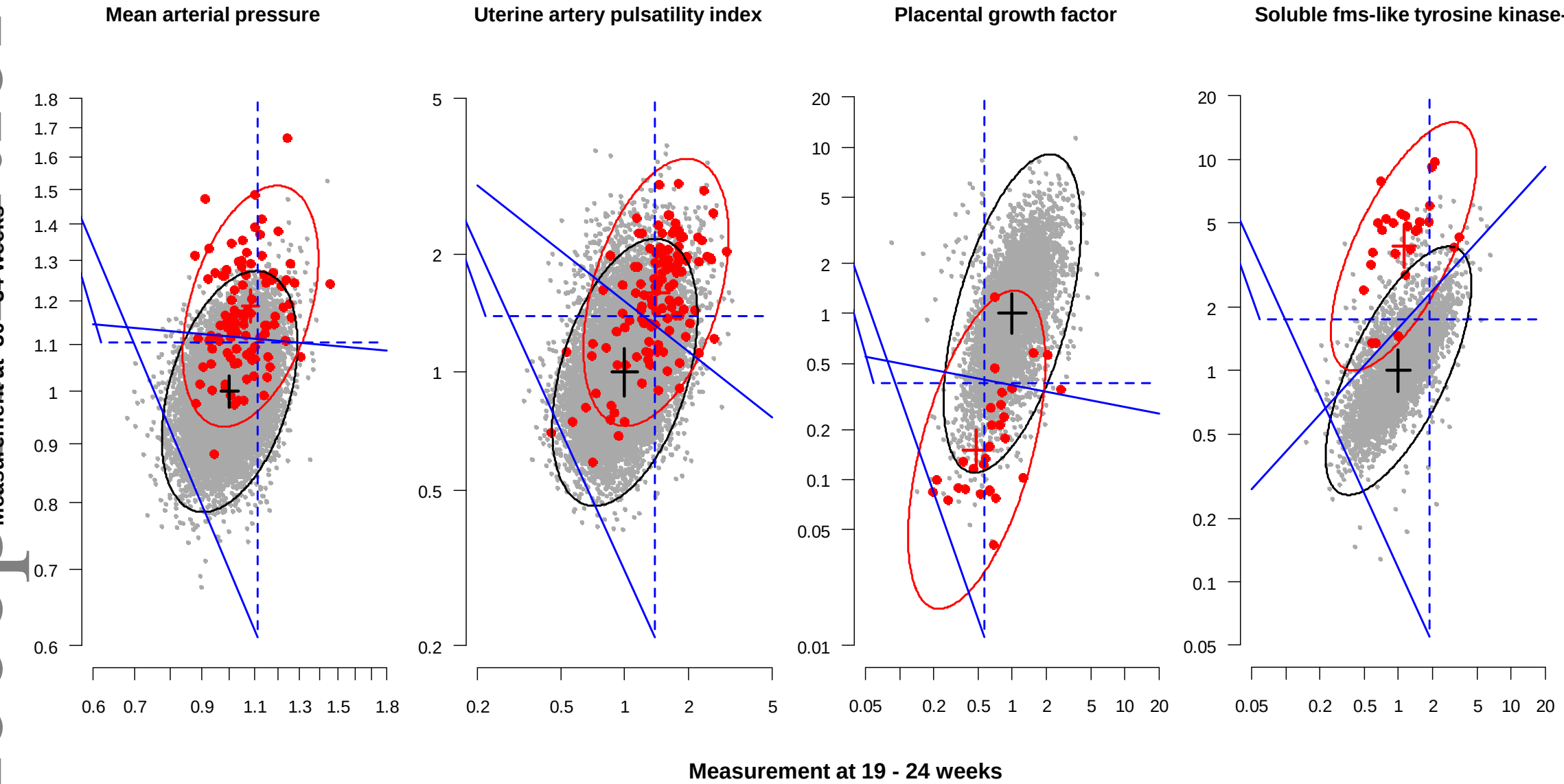

Table 1. Performance of screening for preeclampsia developing after the third trimester assessment, by maternal factors and combination of maternal factors and biomarkers. The numbers in bold in each cell are the model-based detection rates.

Method of screening	AUROC		False positive rate 5%				False positive rate 10%			
	Preeclampsia at:		Preeclampsia at <37 w		Preeclampsia at ≥37 w		Preeclampsia at <37 w		Preeclampsia at ≥37 w	
	<37 w	≥37 w	n/N	DR (95% CI)	n/N	DR (95% CI)	n/N	DR (95% CI)	n/N	DR (95% CI)
Maternal factors	0.7957	0.7495	31/85	36.5 (26.3, 47.6); **33.7**	100/363	27.5 (23.0, 32.5); **29.4**	39/85	45.9 (35.0, 57.0); **46.6**	140/363	38.6 (33.5, 43.8); **40.5**
MAP1	0.8490	0.7897	35/85	41.2 (30.6, 52.4); **43.8**	112/363	30.9 (26.1, 35.9); **33.0**	45/85	52.9 (41.8, 63.9); **57.3**	162/363	44.6 (39.4, 49.9); **45.8**
MAP2	0.8598	0.7805	34/85	40.0 (29.5, 51.2); **46.4**	107/363	29.5 (24.8, 34.5); **32.0**	44/85	51.8 (40.7, 62.7); **60.2**	153/363	42.1 (37.0, 47.4); **44.5**
MAP3	0.9534	0.8084	58/85	68.2 (57.2, 77.9); **78.1**	133/363	36.6 (31.7, 41.8); **37.6**	71/85	83.5 (73.9, 90.7); **86.1**	188/363	51.8 (46.5, 57.0); **50.3**
MAP2, MAP3	0.9536	0.8126	58/85	68.2 (57.2, 77.9); **78.1**	129/363	35.5 (30.6, 40.7); **38.3**	70/85	82.4 (72.6, 89.8); **86.2**	190/363	52.3 (47.1, 57.6); **50.9**
MAP1, MAP2, MAP3	0.9537	0.8195	58/85	68.2 (57.2, 77.9); **78.1**	131/363	36.1 (31.1, 41.3); **39.1**	70/85	82.4 (72.6, 89.8); **86.2**	185/363	51.0 (45.7, 56.2); **51.9**
Maternal factors	0.7957	0.7495	36/94	38.3 (28.5, 48.9); **33.7**	106/378	28.0 (23.6, 32.9); **29.4**	47/94	50.0 (39.5, 60.5); **46.6**	144/378	38.1 (33.2, 43.2); **40.5**
UTPI1	0.8440	0.7543	38/94	40.4 (30.4, 51.0); **44.0**	109/378	28.8 (24.3, 33.7); **29.5**	49/94	52.1 (41.6, 62.5); **57.0**	151/378	39.9 (35.0, 45.1); **41.1**
UTPI2	0.8947	0.7698	53/94	56.4 (45.8, 66.6); **57.5**	117/378	31.0 (26.3, 35.9); **31.1**	64/94	68.1 (57.7, 77.3); **69.6**	163/378	43.1 (38.1, 48.3); **43.1**
UTPI3	0.9280	0.7568	59/94	62.8 (52.2, 72.5); **69.6**	105/378	27.8 (23.3, 32.6); **29.8**	73/94	77.7 (67.9, 85.6); **79.0**	157/378	41.5 (36.5, 46.7); **41.7**
UTPI2, UTPI3	0.9351	0.7703	65/94	69.1 (58.8, 78.3); **72.1**	118/378	31.2 (26.6, 36.2); **31.3**	72/94	76.6 (66.7, 84.7); **80.8**	163/378	43.1 (38.1, 48.3); **43.2**
UTPI1, UTPI2, UTPI3	0.9351	0.7703	65/94	69.1 (58.8, 78.3); **72.1**	118/378	31.2 (26.6, 36.2); **31.3**	72/94	76.6 (66.7, 84.7); **80.8**	164/378	43.4 (38.3, 48.6); **43.3**
Maternal factors	0.796	0.750	9/26	35.0 (17.0, 56.0); **33.7**	30/110	27.0 (19.0, 37.0); **29.4**	13/26	50.0 (30.0, 70.0); **46.6**	41/110	37.0 (28.0, 47.0); **40.5**
PLGF1	0.870	0.771	11/26	42.0 (23.0, 63.0); **50.1**	31/110	28.0 (20.0, 38.0); **30.9**	16/26	62.0 (41.0, 80.0); **63.2**	43/110	39.0 (30.0, 49.0); **42.8**
PLGF2	0.905	0.750	11/26	42.0 (23.0, 63.0); **63.4**	32/110	29.0 (21.0, 39.0); **29.3**	17/26	65.0 (44.0, 83.0); **73.0**	43/110	39.0 (30.0, 49.0); **40.6**
PLGF3	0.972	0.835	20/26	77.0 (56.0, 91.0); **86.2**	48/110	44.0 (34.0, 53.0); **42.9**	25/26	96.0 (80.0, 100); **91.9**	59/110	54.0 (44.0, 63.0); **55.8**
PLGF2, PLGF3	0.972	0.854	20/26	77.0 (56.0, 91.0); **86.2**	46/110	42.0 (32.0, 52.0); **47.4**	25/26	96.0 (80.0, 100); **92.0**	65/110	59.0 (49.0, 68.0); **60.1**
PLGF1, PLGF2, PLGF3	0.972	0.857	20/26	77.0 (56.0, 91.0); **86.4**	45/110	41.0 (32.0, 51.0); **48.2**	25/26	96.0 (80.0, 100); **92.1**	61/110	55.0 (46.0, 65.0); **61.0**

AUROC = area under receiver operating characteristic curve obtained by modeling; DR = detection rate; CI = confidence interval; MAP = mean arterial pressure; UTPI = uterine artery pulsatility index; PLGF = placental growth factor; 1 = screening at 11-13 weeks; 2 = screening at 19-24 weeks; 3 = screening at 30-34 weeks.

Table 2. Mahalanobis distances between unaffected pregnancies and those that developed preterm-preeclampsia and delivered after the third trimester assessment.

Biomarker	Mahalanobis distance					
	1	2	1 and 2	3	2 and 3	1, 2 and 3
MAP	0.847	0.937	1.056	2.148	2.150	2.151
UTPI	0.778	1.308	1.320	1.816	1.912	1.912
PLGF	1.045	1.573	1.634	2.600	2.606	2.608
SFLT*	0.161	0.223	0.461	3.040	3.979	3.984

MAP = mean arterial pressure; UTPI = uterine artery pulsatility index; PLGF = placental growth factor; 1 = screening at 11-13 weeks; 2 = screening at 19-24 weeks; 3 = screening at 30-34 weeks.

* Date for SFLT were derived from previous publications [6]