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First-trimester markers for the prediction of pre-eclampsia in women with *a-priori* high risk

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KEYWORDS: Doppler; first trimester; high risk; PP13; pre-eclampsia; pulse-wave analysis

ABSTRACT

Objective *To investigate the predictive value of the combination of first-trimester serum placental protein 13 (PP13), uterine artery Doppler pulsatility index (PI) and pulse wave analysis (augmentation index at a heart rate of 75 beats per min (AIx-75)), and to evaluate concurrent and contingent strategies using this combination for assessing the risk of pre-eclampsia in high-risk women.*

Methods *In this nested case–control study, serum PP13, uterine artery mean PI and AIx-75 were measured at between 11 + 0 and 13 + 6 weeks' gestation in women at increased risk of pre-eclampsia. For each case of pre-eclampsia (*n = *42), five matched controls were randomly selected from the study group. Gestation specific multiples of the median (MoMs) were adjusted for body mass index, ethnicity, smoking, age and parity. MoMs were compared between cases and controls using the Wilcoxon rank sum test. Sensitivities and specificities were derived from receiver–operating characteristics curves.*

Results *Compared with controls, women who developed pre-eclampsia had lower PP13, higher uterine artery mean PI and higher AIx-75 (*P < *0.001). For a 10% false-positive rate, the best detection rate for pre-eclampsia (85.7% (95% CI, 71.5–94.6%)) and pre-eclampsia requiring delivery before 34 weeks (92.9% (95% CI, 66.1–99.8%)) was achieved by concurrent testing with all three markers. The best contingency screening sequences for pre-eclampsia were (AIx-75 → PP13 → mean PI) and (PP13 → AIx-75 → mean PI), with an 86% detection rate for false-positive rates of 9 and 10%, respectively. These two sequences would require 410 and 414 tests, respectively, compared with 756 tests in concurrent testing.*

Conclusion *Combination of first-trimester PP13, uterine artery mean PI and pulse-wave analysis is promising for the prediction of pre-eclampsia in women at increased* a-priori *risk and may be useful in clinical practice. Contingency screening achieved similar detection rates to concurrent testing, but required almost 50% fewer tests, making it a more cost-effective option. Copyright © 2010 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Pre-eclampsia is a multisystem disorder that originates in early pregnancy and leads to considerable maternal and fetal morbidity and mortality[1–3]. Women who develop pre-eclampsia are also at increased long-term risk of cardiovascular disease and stroke[4,5]. In spite of extensive research, as yet there is no accurate method of identifying women who will develop pre-eclampsia, and no effective prophylactic intervention.

Pre-eclampsia is associated with failure of adequate trophoblast invasion of the maternal spiral arteries, which begins in the first trimester[6]. This results in persistently increased resistance in these vessels throughout pregnancy. Maternal reaction to placental ischemia–reperfusion causes the maternal pre-eclamptic syndrome and may lead to intrauterine growth restriction[7–10].

In the first trimester, uterine artery Doppler pulsatility index (PI) alone has a sensitivity of around 25–30% for a 10% false-positive rate for the detection of pre-eclampsia.

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This sensitivity is slightly better for the prediction of early pre-eclampsia (40–50%)[11], but this can be improved considerably by the addition of measurements of maternal serum biochemistry[12–14]. Other serum markers, such as soluble endoglin and soluble fms-like tyrosine kinase-1, show promise when measured from the second trimester but currently have little predictive value in the first[15–18]. Some early studies suggested that first-trimester free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A show potential, but the low sensitivity demonstrated until now means that they remain unsuitable for widespread use in clinical practice[19].

Placental protein 13 (PP13) has recently shown promise as a marker for the prediction of pre-eclampsia. First-trimester serum PP13 levels combined with either first-trimester[12] or second-trimester[20] uterine artery Doppler PI can predict early (requiring delivery before 34 weeks' gestation) pre-eclampsia better than term pre-eclampsia. First-trimester serum PP13 levels alone can predict pre-eclampsia with moderate accuracy in medium- to low-risk populations[20–23]. Recently, we have shown that first-trimester PP13 alone can also predict pre-eclampsia with moderate accuracy in a high-risk population[24].

Vascular compliance can be assessed by analyzing the peripheral arterial pulse waveform measured by applanation tonometry. This technique, known as pulse-wave analysis (PWA), has been widely studied in the non-pregnant population[25–30]. However, there are few published studies on its use in pregnancy[31–34]. PWA is a non-invasive technique that can assess changes in arterial stiffness associated with conditions that can cause endothelial dysfunction, such as diabetes mellitus, atherosclerosis and renal disorders. Studies using PWA have confirmed increased arterial stiffness in women with clinically established pre-eclampsia[33,34], and using PWA we have recently shown that arterial stiffness is increased as early as the first trimester in women who later develop pre-eclampsia[35].

The aim of this study was to investigate the predictive value of the combination of first-trimester serum PP13, uterine artery Doppler PI and PWA (augmentation index at a heart rate of 75 beats per min (AIx-75)) in a cohort of women at *a-priori* high risk of developing pre-eclampsia.

METHODS

Study design and population

This nested case–control study was carried out at the Homerton University Hospital, London, UK, between January 2006 and September 2007. During this period, approximately 9000 deliveries took place. This hospital has an ethnically diverse population, including a high proportion of women of Afro-Caribbean background. We recruited women at increased risk of developing pre-eclampsia who were at between 11 + 0 and 13 + 6 weeks' gestation.

The inclusion criteria were at least one of the following risk factors for pre-eclampsia: a history of pre-eclampsia in a previous pregnancy, chronic hypertension, chronic renal disease, antiphospholipid syndrome, systemic lupus erythematosus, pregestational diabetes mellitus and obesity (body mass index (BMI) ≥ 30 kg/m^2). The diagnosis of chronic hypertension required the use of antihypertensive therapy prior to pregnancy or blood pressure measurements $\geq 140/90$ mmHg on at least two occasions at least 4 h apart, prior to 14 weeks' gestation. Chronic renal disease was diagnosed in women with significant proteinuria (≥ 300 mg in a 24-h urine collection) or serum creatinine greater than 110 μmol/L prior to 14 weeks' gestation. Exclusion criteria included multiple pregnancy, cases of major fetal anomaly, miscarriage or fetal death, women with HIV or hepatitis, and women with placenta previa or placental abruption. The primary outcome measure was the occurrence of pre-eclampsia. Written informed consent was obtained from each woman after she had received full written information about the research project. The study was approved by the Camden and Islington Community Local Research Ethics Committee.

Diagnosis of pre-eclampsia

The diagnosis of pre-eclampsia was made according to the guidelines of the International Society for the Study of Hypertension in Pregnancy. This definition requires two recordings of diastolic blood pressure ≥ 90 mmHg at least 4 h apart in a previously normotensive woman, and proteinuria ≥ 300 mg in 24 h, or two readings of at least ++ on dipstick analysis of a midstream or catheter specimen of urine (if no 24-h urine collection is available) after 20 weeks' gestation[36]. In women with chronic hypertension, the diagnosis of pre-eclampsia required new-onset proteinuria after 20 weeks' gestation. In women with chronic renal disease, diagnosis of pre-eclampsia required new-onset hypertension and worsening proteinuria (doubling of the 24-h urine protein) after 20 weeks' gestation. In women with both hypertension and proteinuria at recruitment, the diagnosis of pre-eclampsia required both worsening hypertension (a rise in systolic blood pressure of ≥ 30 mmHg and/or diastolic blood pressure ≥ 15 mmHg above baseline values) and worsening proteinuria (doubling of 24-h urine protein). The onset of pre-eclampsia was defined as the time of the first elevated blood pressure or urinary protein measurement fulfilling the criteria for diagnosis. The early-onset pre-eclampsia subgroup included women who required delivery before 34 weeks' gestation. Small-for-gestational-age (SGA) was defined as a birth weight below the 5th percentile for gestational age.

The total study included 414 women, all of whom were at high risk as defined previously. Women who were lost to follow-up ($n = 19$) were excluded, leaving 395 women eligible for further study. The prevalence of pre-eclampsia in the overall study population was 42/395 (10.6%).

We randomly selected five controls from the study group for each case of pre-eclampsia. Controls were matched to cases for gestational age (± 5 days) at the

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time of blood sampling. In order to minimize potential confounding due to sample decay, controls and cases were also matched for storage time of the blood samples (± 2 weeks). To qualify as controls, women had to have sufficient clinical data and a completed pregnancy uncomplicated by pre-eclampsia, gestational diabetes or an SGA fetus.

Demographic and clinical data including age, maternal weight and height, BMI, parity, ethnicity, smoking status, blood pressure and gestational age at the time of recruitment were collected. Gestational age was established on the basis of menstrual dates and confirmed by first-trimester ultrasonography. If there was a difference of 7 days or more in the gestational age calculated according to last menstrual period and by sonography, the value obtained by sonography was used.

Between 11 + 0 and 13 + 6 weeks' gestation the women had venous blood samples collected, uterine artery Doppler measured and PWA recorded at the same visit. The blood samples were centrifuged at 3000 rpm for 10 min and the serum was separated and frozen at −80°C for subsequent analysis.

Uterine artery Doppler

Doppler ultrasound of the uterine arteries was performed by a single investigator (A.K.) as previously described[11], and the average PI of the blood flow through both arteries was determined.

Pulse-wave analysis

All PWA measurements were performed by one operator (A.K.) as previously described[35]. Briefly, the radial artery waveform was recorded using applanation tonometry and the Sphygmocor® system (Atcor Medical, West Ryde, Australia) was used to analyze the radial artery wave contour[37,38]. The AIx, a measure of arterial stiffness, was calculated from the aortic waveform. Because there is a linear relationship between AIx and heart rate, AIx was standardized to a heart rate of 75 beats per minute (AIx-75)[39].

PP13 immunoassay

The maternal serum concentration of PP13 was measured using a solid-phase sandwich enzyme-linked immunosorbent assay with a pair of PP13-specific monoclonal antibodies, as previously described[40–42]. The laboratory personnel who performed the assays were blinded to the pregnancy outcome and the clinician recruiting the women took no part in analyzing the samples. For this study, we used an improved version of the assay kit developed with a specific sample dilution buffer formula to remove heterophilic antibodies. The kit-to-kit variation within the same batch was 8.8% and the batch-to-batch variation was 8.3%. The lower limit of detection was 8 pg/mL and all samples with readings below this threshold were assigned this value.

Uterine artery PI and AIx-75 were measured in the whole study group, with serum PP13 measurement restricted to the cases of pre-eclampsia and matched controls.

All the women were followed up until 4 weeks after delivery, and fetal and maternal outcome data were obtained from the women's medical and labor-ward records.

Statistical analysis

Baseline and delivery characteristics were compared between cases and controls using Fisher's exact test for categorical variables and independent *t*-test for continuous variables. Statistical modeling was performed to evaluate concurrent testing of PP13, mean uterine artery Doppler PI and AIx-75 in assessing the risk of developing pre-eclampsia.

The values of PP13, uterine artery Doppler mean PI and AIx-75 were not normally distributed. We therefore compared the results between women who developed pre-eclampsia and those who did not using the Wilcoxon rank sum test.

Multiple regression analysis was then performed to analyze covariates that could affect marker values including gestational age, BMI, ethnicity, smoking, maternal age and parity. A stepwise regression analysis was performed to assess which covariate(s) correlated significantly with each marker level. Covariates found to be significant from the step-wise regression analysis were further analyzed for possible significant interactions among them by specifying a regression equation that included each individual covariate and any interaction between covariate pairs. Accordingly, the following correlations were identified:

- For PP13: gestational age, $P < 0.001$; BMI, $P = 0.029$; ethnicity, $P = 0.005$; smoking, $P = 0.497$; maternal age, $P = 0.097$; parity, $P = 0.204$; BMI + ethnicity, $P = 0.001$; gestational age + BMI, $P = 0.025$.
- For uterine artery mean PI: gestational age, $P < 0.669$; BMI, $P = 0.052$; ethnicity, $P = 0.041$; smoking, $P = 0.481$; maternal age, $P = 0.553$; parity, $P = 0.910$; confounder interactions: none was significant.
- For AIx-75: gestational age, $P = 0.246$; BMI, $P = 0.905$; ethnicity, $P = 0.460$; smoking, $P = 0.047$; maternal age, $P = 0.211$; parity, $P = 0.235$; confounder interactions: none was significant.

PP13, mean PI and AIx-75 were then each converted into gestational week-specific multiples of the median (MoM) levels among the controls following the method described by Cuckle and Wald[43] and adapted for PP13 by Cuckle *et al.*[22,44,45] and by Spencer *et al.*[20,21]. Gestational age-adjusted MoMs were then sequentially adjusted to BMI, ethnicity, smoking, maternal age and parity.

The dataset used to 'fit' the regression models included individual subjects whose risk of pre-eclampsia we aimed to predict. To avoid potential bias due to 'over-fitting'

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of the models, the risk of pre-eclampsia for each woman was calculated using the 'out of sample' model, in which values were calculated by running the analysis repeatedly, each time excluding one woman from the group[46].

Next, the relationships between the adjusted MoMs of the three markers (PP13, mean PI and AIx-75) were analyzed to ensure that they were independent. The analysis was performed by logistic regression, assuming that the three markers were independent determinants of the risk of developing pre-eclampsia. To avoid over-fitting, the sensitivity and specificity were calculated for each subject using the logistic regression equation estimated without that subject in the learning data set (i.e. out-of-sample estimation).

Screening accuracy was assessed by measuring sensitivities and specificities derived from receiver–operating characteristics (ROC) curves prepared from the adjusted MoM values after correcting the regression models by the 'out of sample' approach. Overall accuracy was estimated with the area under the curve (AUC). We first examined the performance of concurrent screening, in which all three markers were measured in all patients. Secondly, we performed contingency screening, in which all women were tested for a single marker, with only those above a predetermined threshold undergoing testing of a second marker[12,45]. $P < 0.05$ was considered statistically significant. All *P*-values were two-tailed. Data were analyzed using SAS® 9.1.3 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

Forty-two women who developed pre-eclampsia, including 14 who developed early-onset pre-eclampsia, were matched to 210 controls. Baseline characteristics and clinical and delivery data for the cases and controls are presented in Tables 1 and 2. The baseline characteristics did not differ significantly between the two groups. Women who developed pre-eclampsia were significantly more likely to deliver smaller babies at lower gestational ages, and to deliver by Cesarean section ($P < 0.001$). Thirteen (31.0%) women with pre-eclampsia also had an SGA fetus.

MoMs of the markers studied

The MoM values for PP13, uterine artery Doppler mean PI and AIx-75, according to study groups and pre-eclampsia subtypes, are shown in Figure 1. PP13 MoMs were significantly lower ($P < 0.001$) and AIx-75 MoMs significantly higher ($P \leq 0.002$) in all pre-eclampsia subgroups (all those with pre-eclampsia, those with early-onset pre-eclampsia and those who had both pre-eclampsia and SGA) compared to controls. Mean PI MoMs were significantly higher ($P \leq 0.002$) in the pre-eclampsia subgroups (including those who also had SGA), except in women with term pre-eclampsia (onset after 37 weeks' gestation).

Table 1 Demographic characteristics of the study groups

Characteristic	*Control group* (n = *210*)	*Pre-eclampsia group* (n = *42*)	P
Maternal age (years)	30.1 ± 5.84	30.0 ± 5.00	0.878
BMI (kg/m^2)	26.6 ± 4.25	27.6 ± 3.34	0.142
Nulliparous	113 (53.8)	20 (47.6)	0.550
Ethnicity			
White	109 (51.9)	26 (61.9)	0.309
Black	75 (35.7)	15 (35.7)	1.00
Asian	23 (11.0)	1 (2.4)	0.144
Mixed	3 (1.4)	0 (0)	1.00
Obesity (BMI ≥ 30 kg/m^2)	57 (27.1)	14 (33.3)	0.454
Chronic hypertension	50 (23.8)	12 (28.6)	0.557
Pregestational diabetes	19 (9.0)	6 (14.3)	0.393
Renal disease	5 (2.4)	1 (2.4)	1.000
Previous pre-eclampsia	85 (40.5)	12 (28.6)	0.167
Systemic lupus erythematosus	5 (2.4)	1 (2.4)	1.000
Antiphospholipid syndrome	11 (5.2)	2 (4.8)	1.000
Smoker	23 (11.0)	2 (4.8)	0.272
Age > 36 years	34 (16.2)	5 (11.9)	0.641
GA at blood sampling (weeks)	12.6 (11–13.9)	12.6 (11–13.9)	1.0
Blood pressure at enrollment (mmHg)			
Systolic	110 (83–250)	115 (95–138)	0.367
Diastolic	69.5 (50–120)	69 (56–85)	0.707
Blood pressure at diagnosis of pre-eclampsia (mmHg)			
Systolic		158 (130–200)	
Diastolic		100.5 (90–131)	
Proteinuria at delivery (by dipstick)	0 (0–1)	2 (2–3)	

Values are expressed as *n* (%), mean ± SD or median (range). BMI, body mass index; GA, gestational age.

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Table 2 Pregnancy outcomes

Variable	*Control group* (n = *210)*	*Pre-eclampsia group* (n = *42)*	P
GA at delivery (weeks)	40.3 (32.0–42.3)	35.0 (24.9–38.4)	< 0.001
Birth weight (g)	3500 (1800–4500)	2400 (500–3200)	< 0.001
SGA	0	13 (31.0)	
Male neonate	105 (50.0)	19 (45.2)	0.678
Preterm delivery	3 (1.4)	34 (81.0)	< 0.001
Cesarean delivery	54 (25.7)	28 (66.7)	< 0.001

Values are expressed as median (range) or *n* (%). GA, gestational age; SGA, small-for-gestational age, defined as birth weight < 5th percentile for GA.

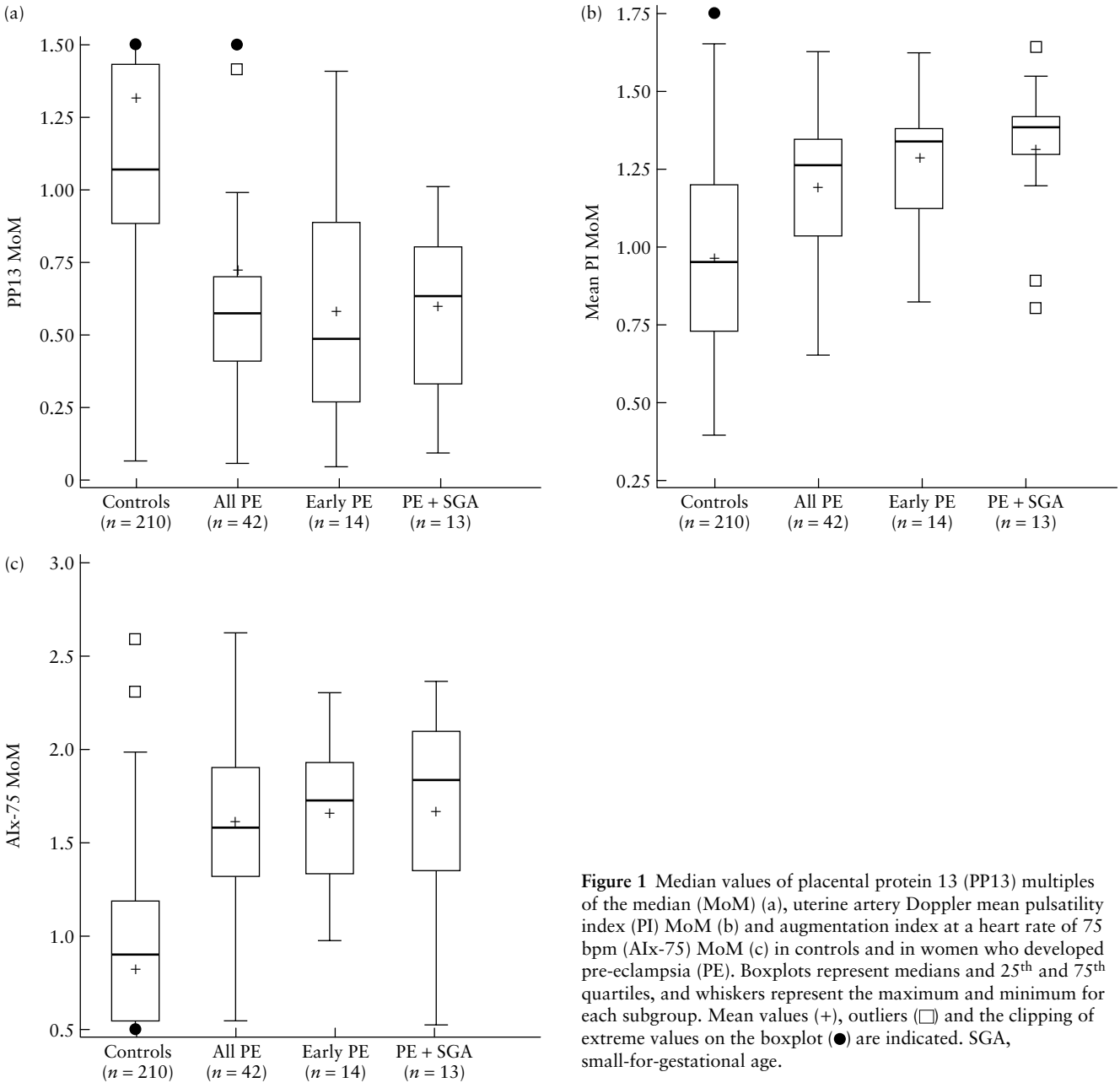

Figure 1 Median values of placental protein 13 (PP13) multiples of the median (MoM) (a), uterine artery Doppler mean pulsatility index (PI) MoM (b) and augmentation index at a heart rate of 75 bpm (AIx-75) MoM (c) in controls and in women who developed pre-eclampsia (PE). Boxplots represent medians and 25th and 75th quartiles, and whiskers represent the maximum and minimum for each subgroup. Mean values (+), outliers (□) and the clipping of extreme values on the boxplot (●) are indicated. SGA, small-for-gestational age.

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Performance of screening

Area under the curve

Figure 2 shows the ROC curves for PP13, uterine artery Doppler mean PI and AIx-75 MoMs individually for the prediction of pre-eclampsia and of early-onset pre-eclampsia. Table 3 provides the corresponding values of the AUCs, with 95% CIs, for the various pre-eclampsia subgroups.

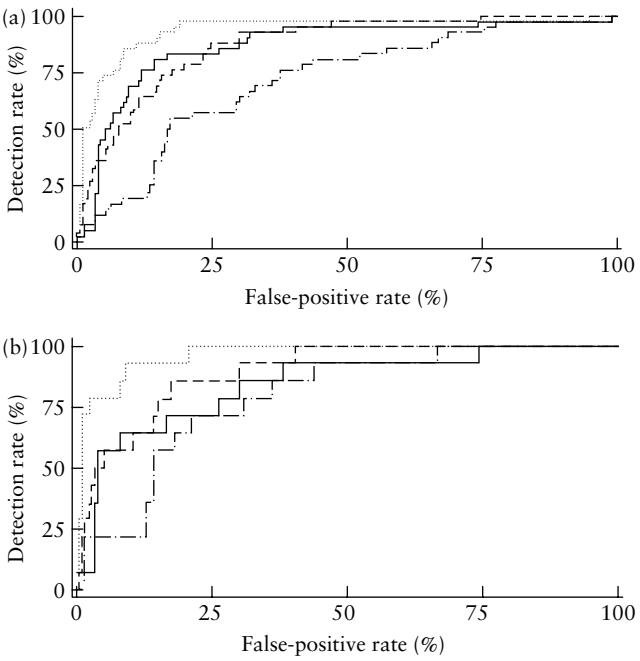

Figure 2 Receiver-operating characteristics (ROC) curves showing the sensitivity and specificity of first-trimester markers for the prediction of pre-eclampsia ($n = 42$) (a) and of pre-eclampsia requiring delivery before 34 weeks ($n = 14$) (b). Markers: placental protein 13 (PP13) (——), mean pulsatility index (PI) (—·—·), augmentation index at a heart rate of 75 bpm (AIx-75) (– – –) and PP13 + mean PI + AIx-75 (·····). ROC curves were generated based on multiples of the median corrected for gestational age, body mass index, ethnicity, smoking, age and parity.

Sensitivity and specificity

Table 4 shows the detection rates of the MoMs of the different markers for the prediction of pre-eclampsia, for a fixed false-positive rate of 10%. In the whole pre-eclampsia group, the detection rate using PP13 (69.0%; 95% CI, 52.9–82.4%) was not significantly higher than for AIx-75 (57.1%; 95% CI, 41.0–72.3%, $P = 0.21$); both markers performed significantly better than uterine artery Doppler mean PI (19.0%; 95% CI, 8.6–34.1%, $P < 0.001$).

In concurrent testing of pairs of markers, any pair combination provided better prediction than any individual marker (Table 4). Of the three potential pairs, the best was combined PP13 + AIx-75, which had a significantly higher detection rate compared to each individual marker and compared to the other pairs.

Concurrent testing using all three markers had significantly higher detection rates compared to testing using paired markers for all pre-eclampsia (AIx-75 + mean PI, $P < 0.001$; PP13 + mean PI, $P < 0.005$; AIx-75 + PP13, $P < 0.005$) and early pre-eclampsia (AIx-75 + mean PI, $P < 0.005$; PP13 + mean PI, $P < 0.005$; AIx-75 + PP13; $P < 0.001$) (Table 4).

Contingency screening

In contingency screening, the best two orders of testing for all PE were (AIx-75 → PP13 → mean PI) and (PP13 → AIx-75 → mean PI). Both yielded very similar results – an 86% detection rate for false-positive rates of 9 and 10%, respectively. In the first sequence, 410 tests would be performed and in the second sequence 414 tests. This compares with 756 tests (252 each) performed in concurrent testing. Other contingent scenarios yielded inferior results.

DISCUSSION

In this screening study of women with increased *a-priori* risk of pre-eclampsia, we found that concurrent

Table 3 Area under the receiver–operating characteristics curves (AUC) of first-trimester markers for the subsequent development of pre-eclampsia

	AUC (95% CI)		
Parameter	*All PE* (n = *42*)	*Early PE* (n = *14*)	*All PE + SGA* (n = *13*)
PP13	0.87 (0.81–0.93)	0.84 (0.73–0.96)	0.88 (0.80–0.95)
Mean PI	0.72 (0.64–0.80)	0.79 (0.69–0.89)	0.76 (0.64–0.89)
AIx-75	0.87 (0.82–0.92)	0.90 (0.83–0.96)	0.86 (0.74–0.98)
PP13 + mean PI	0.88 (0.82–0.94)*§	0.90 (0.82–0.98)‡§	0.90 (0.82–0.97)*†
PP13 + AIx-75	0.93 (0.88–0.98)†‡	0.94 (0.88–1.00)§§	0.97 (0.94–1.00)§§
Mean PI + AIx-75	0.89 (0.85–0.94)§*	0.94 (0.88–0.99)§§	0.92 (0.83–1.00)§†
PP13 + mean PI + AIx-75	0.94 (0.89–0.99)§§§	0.97 (0.93–1.00)§§§	0.98 (0.95–1.00)§§§

No significant difference, †$P < 0.05$, ‡$P < 0.005$ and §$P < 0.001$ compared to the AUC of the individual marker alone respective to the order of the parameters included in each group as listed in the left-hand column. Accordingly, the symbols §§§ next to each AUC value for the three markers in the bottom row indicates $P < 0.001$ compared to PP13 alone, to mean PI alone and to AIx-75 alone, whereas the symbols § next to the value for all PE in the mean PI + AIx-75 row means $P < 0.001$ compared to mean PI alone but no significant difference from AIx-75 alone. AIx-75, augmentation index at heart rate of 75 bpm; PE, pre-eclampsia; PI, pulsatility index; PP13, placental protein 13; SGA, small-for-gestational age.

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Table 4 Sensitivity of first-trimester markers for the prediction of development of pre-eclampsia for a fixed false-positive rate of 10%

	Sensitivity (% (95% CI))		
Parameter	*All PE (n = 42)*	*Early PE (n = 14)*	*All PE + SGA (n = 13)*
PP13	69.0 (52.9–82.4)	64.3 (35.1–87.2)	61.5 (31.6–86.1)
Mean PI	19.0 (8.6–34.1)	21.4 (4.7–50.8)	15.4 (1.9–45.4)
AIx-75	57.1 (41.0–72.3)	57.1 (28.9–82.3)	61.5 (31.6–86.1)
PP13 + mean PI	71.4 (55.4–84.3)	78.6 (49.2–95.3)	76.9 (46.2–95.0)
PP13 + AIx-75	81.0 (65.9–91.4)	85.7 (57.2–98.2)	92.3 (64.0–99.8)
Mean PI + AIx-75	57.1 (41.0–72.3)	78.6 (49.2–95.3)	76.9 (46.2–95.0)
PP13 + mean PI + AIx-75	85.7 (71.5–94.6)	92.9 (66.1–99.8)	92.3 (64.0–99.8)

AIx-75, augmentation index at heart rate of 75 bpm; PE, pre-eclampsia; PI, pulsatility index; PP13, placental protein 13; SGA, small-for-gestational age.

first-trimester testing of PP13, uterine artery mean PI and AIx-75 predicted pre-eclampsia with very high sensitivity and specificity. The prediction of early onset pre-eclampsia (requiring delivery before 34 weeks' gestation) using this approach achieved even greater accuracy (92.9%). Most perinatal mortality and morbidity associated with pre-eclampsia result from early-onset disease. This study suggests that testing of these three markers concurrently can achieve clinically useful prediction of this most relevant form of the disease.

PWA measures arterial stiffness by assessing the central (aortic) waveform. The technique is non-invasive, inexpensive, easy to perform in the outpatient setting and easy to learn. In this study the single most accurate predictor of pre-eclampsia was AIx-75. We have previously shown[35] that arterial stiffness is raised as early as the first trimester in women destined to develop pre-eclampsia, suggesting that this technique may have potential, either alone or in combination with other markers, as a first-trimester screening tool for pre-eclampsia. Increased Alx-75 among this group could be an indication of a pre-disposition to vascular disease, elevated blood pressure and pre-eclampsia.

PP13 is a homodimer of 16-kDa subunits linked by disulfide bonds[41]. Produced mainly by syncytiotrophoblast, it is found primarily at the maternal–fetal interface[42,47,48]. Its role in placental development is still not entirely understood, but several studies suggest that it is involved in placentation[40–42,47,48]. The current literature suggests that prediction of pre-eclampsia using PP13 can be improved by concurrent testing with uterine artery Doppler mean PI[12,20,21,45].

Examination of the uterine arteries is feasible in the first trimester in women at 11–14 weeks' gestation[11]. In general terms, prediction of pre-eclampsia using first-trimester mean PI performs less well than in the second trimester, although prediction improves with increasing severity of the disease.

Concurrent testing of the combination of all three first-trimester markers (PP13, mean PI and AIx-75) achieved high detection rates for women who subsequently developed pre-eclampsia. These detection rates were better than the equivalent rates for any pair of markers. Each of these three parameters assesses different aspects of the pathological process of pre-eclampsia: placental function, spiral artery remodeling and maternal vascular and endothelial dysfunction. The fact that prediction using all three markers was superior to any pair supports the view that independent multi-marker analysis is necessary for accurate prediction of this multisystem disorder.

Contingency screening achieved detection rates very similar to those of concurrent testing, the two best orders of testing being (AIx-75 → PP13 → mean PI) and (PP13 → AIx-75 → mean PI). However, contingency screening required almost 50% fewer tests than concurrent testing. Clearly, this has significant financial implications, and contingency screening appears to be a more cost-effective option in this group of women at high risk of developing pre-eclampsia. The optimal sequence may be AIx-75 → PP13 → mean PI. Pulse-wave analysis (measuring Alx-75) is a simple and inexpensive investigation, so it may be the most appropriate initial screen. PP13 is a simple, reproducible blood test, making it a good option for the second test in the sequence. The addition of mean PI to these two markers improved detection rates only marginally, so would benefit relatively few women in the clinical setting. Moreover, first-trimester Doppler scanning requires considerable skill and training; contingency screening would minimize the proportion of women requiring this test.

In general, detection rates for pre-eclampsia (early or term) associated with SGA were similar to those for early-onset disease. This reflects the fact that, in our study, women with *a-priori* high risk had a high incidence of early, preterm and severe pre-eclampsia, much of it associated with SGA. This is also consistent with a previous report that found that, compared to women with normal pregnancy outcomes, PP13 levels are lowest in combined pre-eclampsia and SGA[22].

The prevalence of pre-eclampsia in this study was high (10.6%), and a high proportion of those with the disease (a third) had early-onset pre-eclampsia. This may be explained by the high-risk nature of our study population. Approximately 35% of the pre-eclampsia group had risk factors known to be associated with a high frequency of early pre-eclampsia (systemic lupus erythematosus, antiphospholipid syndrome, chronic hypertension and

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renal disease; Table 1). The high proportion of Afro-Caribbean ethnicity may also have contributed to the relatively high proportion of early-onset pre-eclampsia. It is possible that the extra surveillance in this study led to early diagnosis, so that early pre-eclampsia was more likely to be correctly labeled as such. Another study[49] of women with pre-existing hypertension found an incidence of superimposed pre-eclampsia of 22%, almost half of which group (44%) had early-onset disease, which is consistent with our findings.

The inclusion criteria for this study specified that women should be at *a-priori* high risk of developing pre-eclampsia. As with any screening test, it is likely that the various combinations of screening markers described in this study would perform less well in populations with a lower *a-priori* risk of developing pre-eclampsia. In this study, we fixed the false-positive rate at 10%, which enabled us to achieve very high detection rates. This was because the implications of a false-positive result are relatively benign, namely increased surveillance of blood pressure, proteinuria and fetal growth, and the potential use of low-dose aspirin.

The strengths of our study were the focus on high-risk women and the use of a well-defined gestational age period. We adjusted gestational-week specific PP13 MoMs for BMI at enrollment, ethnicity, smoking, maternal age and parity. Earlier studies[23,44,45] suggested that adjustment for all these confounders improves the predictive accuracy of PP13 MoMs.

There is currently no effective preventive measure for pre-eclampsia, even in women identified as being at high risk; low-dose aspirin taken throughout pregnancy results in only a modest (approximately 10%) reduction in its incidence[50,51]. Nevertheless, early identification of women at increased risk is of value; targeted surveillance and intervention may lead to improved outcomes[52,53]. Predicting pre-eclampsia in the first, as opposed to the second, trimester means that any preventive strategy can be instituted in early pregnancy. Given that the disease process is already established by the mid second trimester, it seems likely that the earlier such a measure is instituted, the greater its chances of success. Early prediction will also facilitate the investigation of prophylactic interventions in the future.

We have not assessed the cost implications of adding PWA to PP13 and uterine artery Doppler measurements in the first trimester, at a time when most women will be having an ultrasound scan in any case. A cost–benefit analysis of the various combinations of these three markers, and comparing concurrent with contingency screening, would be helpful in identifying the most clinically feasible screening tool.

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