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A novel approach to first-trimester screening for early pre-eclampsia combining serum PP-13 and Doppler ultrasound

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KEYWORDS: contingency screening; PP-13; pre-eclampsia; screening; uterine artery Doppler

ABSTRACT

Objective *To investigate the value of maternal serum placental protein 13 (PP-13) measurement and uterine artery Doppler during first-trimester screening in the prediction of early pre-eclampsia.*

Methods *This was a nested case-control prospective study of pregnancies at 11 + 0 to 13 + 6 weeks of gestation. The pulsatility index (PI) of blood flow in the uterine arteries and the maternal serum concentration of PP-13 were measured in 10 women who went on to develop pre-eclampsia that necessitated delivery before 34 weeks, and in 423 unaffected women. Results were expressed as multiples of the gestation-specific median in controls (MoM). A logistic regression model was used to predict detection and false-positive rates.*

Results *In the cases that developed pre-eclampsia requiring delivery before 34 weeks, compared with the unaffected pregnancies, the median uterine artery PI was higher (1.43 MoM) and the median serum PP-13 level was lower (0.07 MoM; P < 0.001, Wilcoxon rank sum test for both). Modeling predicted that for a 90% detection rate of pre-eclampsia requiring delivery before 34 weeks, the false-positive rate of screening by PP-13 was 12%, by uterine artery PI was 31% and by a combination of the two methods was 9%. A policy of contingency screening, whereby all women are screened by maternal serum PP-13 and only the 14% at highest risk are then screened by Doppler, achieved a detection rate of 90% with an overall false-positive rate of 6%.*

Conclusion *Effective screening for pre-eclampsia requiring delivery before 34 weeks can potentially be provided by assessment of a combination of maternal serum PP-13 and uterine artery Doppler in the first trimester of pregnancy.* *Copyright © 2005 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Pre-eclampsia is a leading cause of maternal morbidity and mortality, especially when it occurs before 34 weeks[1,2]. There is evidence that it is a consequence of failure of trophoblastic invasion of the maternal spiral arteries[3,4]. The use of Doppler ultrasound to assess impedance to blood flow in the maternal uterine arteries can identify pregnancies with impaired trophoblastic invasion. Screening studies at 20–24 weeks of gestation have reported that the detection rate of pregnancies that subsequently develop pre-eclampsia requiring early delivery is 50–70%, for a false-positive rate of 5%[5]. However, such late identification of the high-risk group may be responsible for failure of therapeutic intervention, such as the prophylactic use of low-dose aspirin, in reducing the risk of pre-eclampsia[6,7]. In contrast, there is some evidence that aspirin administered from the first trimester can significantly reduce the risk for the subsequent development of pre-eclampsia requiring early delivery[8,9]. It is therefore important to develop a method for the effective identification of the high-risk group early in pregnancy. Although it is possible to predict pre-eclampsia requiring early delivery by Doppler assessment in the first trimester, such screening is associated with a very high false-positive rate[10].

Placental protein 13 (PP-13) is a 32-kD dimer protein, which has recently been prepared in recombinant form[11,12]. This placental product is thought to be involved

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in implantation and maternal artery remodeling[11–13]. Preliminary data suggest that the gene for PP-13 is down regulated in women with pre-eclampsia requiring early delivery and that in pregnancies resulting in early pre-eclampsia there is impaired placental functional responsiveness to PP-13 during the first trimester of pregnancy[11,14].

The aim of our study was to examine the potential value of first-trimester maternal serum PP-13 measurement in predicting pre-eclampsia requiring delivery before 34 weeks and to determine if combined with uterine artery Doppler it could form the basis of an effective screening test.

METHODS

Women undergoing Down-syndrome screening at the Harris Birthright Research Centre for Fetal Medicine at 11 + 0 to 13 + 6 gestational weeks were recruited into the study[15]. Doppler ultrasound of the uterine arteries was performed as previously described[10], and the average pulsatility index (PI) of the blood flow through both arteries was determined. A maternal serum sample was collected and stored at −70°C. When pregnancy outcome was known from hospital medical records, samples were retrieved from storage for PP-13 assays in the 10 pregnancies which ended in pre-eclampsia requiring delivery before 34 weeks together with 423 unaffected controls. Controls were chosen that had a similar gestational age and duration of storage to those of the study group. Pre-eclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy[16] and early pre-eclampsia was defined as pre-eclampsia necessitating delivery before 34 weeks' gestation.

The maternal serum concentration of PP-13 was measured, without knowledge of pregnancy outcome, using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) with a pair of PP-13-specific monoclonal antibodies, as described previously[11,12]. Optical density was measured at 450 nm and translated to a quantitative amount using a calibration curve consisting of PP-13 standards (0–500 pg/mL) from a recombinant PP-13[11]. At maternal PP-13 serum concentrations of 10–500 pg/mL the intra- and interassay variations were $< 5.4\%$ and $< 9.4\%$, respectively. The lower limit of detection was 5 pg/mL and all samples with lower readings were assigned this value.

Statistical analysis

Baseline characteristics were compared using Fisher's exact test for categorical variables and an independent *t*-test for continuous variables.

The measured PP-13 and PI were expressed in multiples of the gestation-specific median value found in the unaffected controls (MoM). PP-13 levels below the sensitivity of the assay were assigned the lower limit of detection (5 pg/mL). MoM comparisons between cases and controls were made using the Wilcoxon rank-sum test.

Statistical modeling was performed to evaluate the screening potential of combining uterine artery PI and serum PP-13[17]. This involved estimating the likelihood ratio (LR) for the chance of a woman developing pre-eclampsia requiring delivery before 34 weeks given her marker levels relative to the chance of a normal outcome. Examination of the MoM values showed that neither marker fitted a Gaussian distribution, either untransformed or after log transformation. Therefore, logistic regression was performed in order to model LRs, assuming that both markers were independent determinants of risk. The logistic regressions provided the odds ratio (OR) for pre-eclampsia requiring early delivery given a woman's marker value. We then computed $LR_{marker} = OR_{marker}/P$, where P is the odds of pre-eclampsia requiring early delivery, which was 1 : 142 in the population from which the cases and controls were drawn. The combined LR, assuming independence was given by:

$$LR_{combined} = LR_{PP\text{-}13} \times LR_{PI} = OR_{PP\text{-}13} \times OR_{PI}/P^2.$$

To avoid over-fitting, the $OR_{PP\text{-}13}$ and OR_{PI} were computed for each subject using the logistic regression equation estimated without that subject in the learning dataset (i.e. out-of-sample estimation).

Screening accuracy was assessed by applying receiver–operating characteristics (ROC) curve methodology to the LRs. Overall accuracy was estimated with the area under the ROC curve and associated 95% CIs. The true-positive rate was fixed at 90% and the false-positive rate computed for screening with PP-13 and PI both alone and jointly.

We examined the performance of both concurrent screening, in which PP-13 and PI were measured in all patients, and contingency screening, in which a woman's LR on the first marker determined whether she was sufficiently at risk to require assessment with the second marker[18,19]. We simulated two contingency scenarios: in the first PP-13 was the contingency screen and PI the final screen, while in the second this order was reversed.

Data were analyzed using SAS® (32-33) and SPSS® (SPSS version 9.01, 1999, SPSS Inc., Chicago, IL, USA).

RESULTS

The demographic characteristics of the group with pre-eclampsia requiring early delivery and the unaffected group are compared in Table 1. In the unaffected group, the median values of PI for 11, 12 and 13 weeks were 1.54 ($n = 130$), 1.63 ($n = 243$) and 1.39 ($n = 50$), respectively, and the respective medians for PP-13 were 126.1, 124.0 and 121.3 pg/mL. The respective 25th, 50th and 75th percentiles in MoMs were 0.85, 1.00 and 1.25 for PI and 0.40, 0.79 and 1.27 for PP-13. There was no significant association between PP-13 and PI in MoMs ($r = 0.03$). Neither PP-13 nor PI was significantly associated with

Table 1 Comparison of demographic characteristics in pregnancies with pre-eclampsia requiring early delivery and unaffected controls

	Mean (range) or n *(%)*		
Variable	*Pre-eclampsia* (n = *10)*	*Controls* (n = *423)*	P
Maternal age (years)	32.7 (26.6–39.0)	30.1 (15.0–44.5)	0.153
Maternal height (cm)	165.0 (158.0–178.0)	164.2 (133–185.4)	0.730
Maternal weight (kg)	73.7 (50.8–99.0)	66.7 (40.0–129.0)	0.096
Nulliparity	6 (60.0)	198 (46.8)	0.527
Caucasian	3 (30.0)	250 (59.1)	0.101

maternal age, parity or weight and it was therefore not necessary to adjust for these variables.

In the cases that developed pre-eclampsia requiring early delivery, compared with the unaffected pregnancies, the median uterine artery PI was higher (1.43 MoM; $P < 0.001$) and the median serum PP-13 level was lower (0.07 MoM; $P < 0.001$) (Figure 1, Table 2). Serum PP-13 levels were below the sensitivity of the assay in seven (70%) of the cases that developed pre-eclampsia requiring early delivery and in 25 (5.9%) of the controls.

Figure 2 shows the model-predicted ROC curves for the risks based on the equation. For a 90% detection rate of pre-eclampsia requiring early delivery, the false-positive rate of screening was 12% (95% CI, 9–16%) by PP-13, it was 31% (95% CI, 26–36%) by uterine artery PI and it was 9% (95% CI, 6–12%) by a combination of the two methods. Conversely, for a 10% false-positive rate, the detection rates would have been 80% for PP-13 (95% CI, 44–98%), 40% for PI (95% CI, 12–74%) and 90% for the markers combined (95% CI, 55–100%).

In contingency screening, the overall detection rate of 90% would be achieved by testing all women for PP-13 and carrying out a Doppler examination on the 14% with the highest risk; with such a policy the

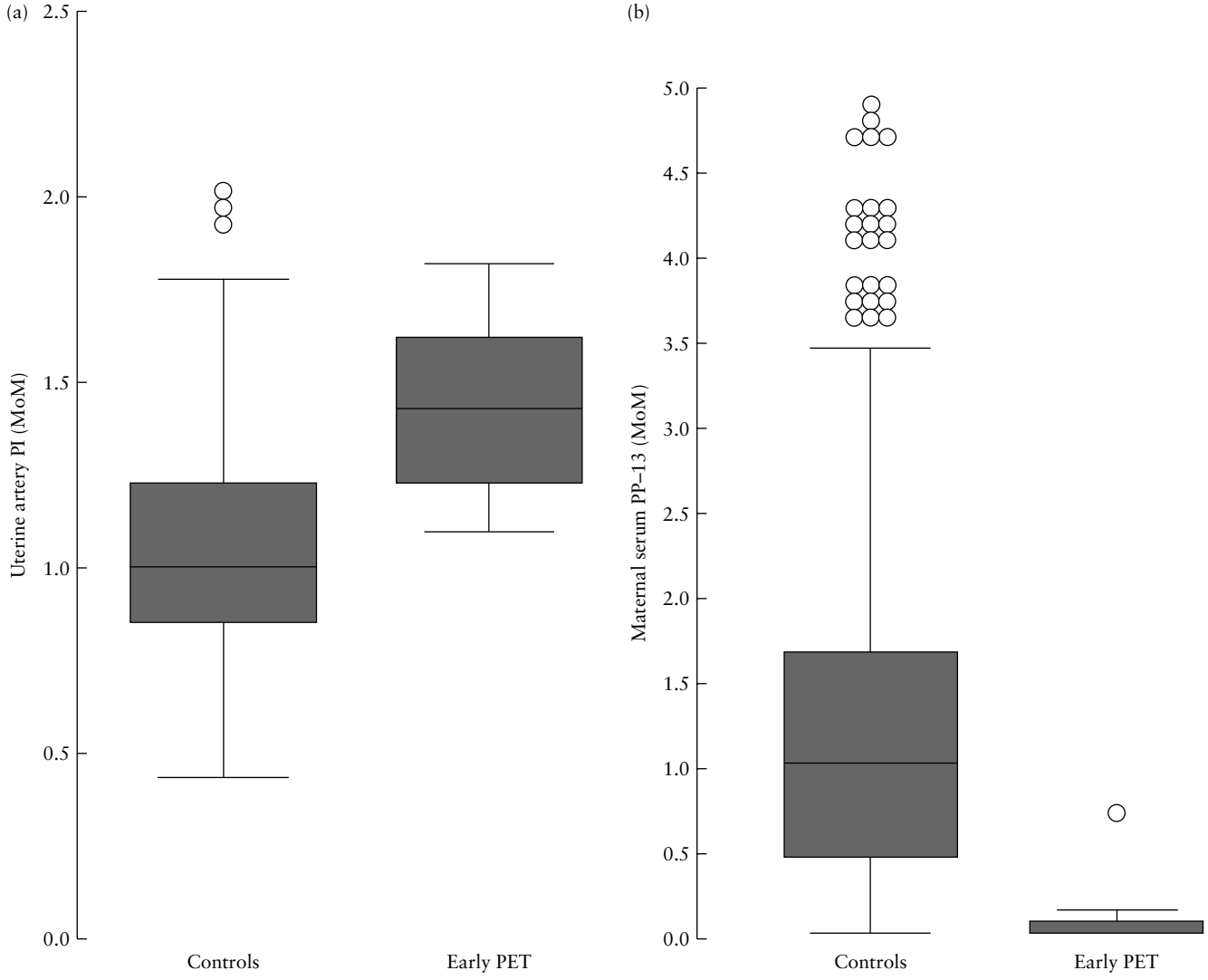

Figure 1 Box and whisker plots of (a) uterine artery pulsatility index (PI) and (b) maternal serum PP-13 in pregnancies complicated by pre-eclampsia requiring early delivery (early PET) and unaffected controls. Boxes show median and interquartile range. Whiskers represent the 2.5th and 97.5th centiles, and circles are outliers.

Table 2 Uterine artery pulsatility index (PI) and maternal serum PP-13 in cases subsequently developing pre-eclampsia requiring early delivery

Uterine artery PI			*Maternal serum PP-13*		
Case	*MoM*	*Normal (%)**	*Case*	*MoM*	*Normal (%)†*
1	1.96	1	1	0.03	5
2	1.74	1	5	0.03	5
3	1.65	2	2	0.07	7
4	1.58	3	4	0.07	7
5	1.43	9	8	0.07	7
6	1.42	9	9	0.07	7
7	1.29	18	10	0.07	7
8	1.24	25	3	0.11	9
9	1.20	28	6	0.17	12
10	1.12	36	7	0.73	36

MoM, multiples of the median for gestation of the unaffected pregnancies. *Percentage of normal pregnancies with a MoM value above that of the case in the first column. †Percentage of normal pregnancies with a MoM value below that of the case in the fourth column.

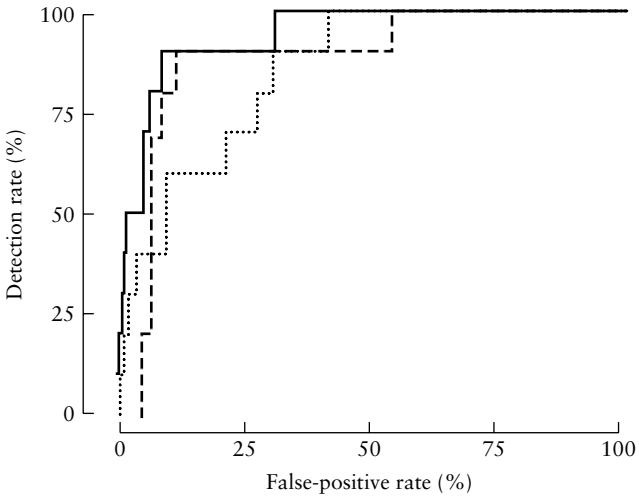

Figure 2 Receiver–operating characteristics curves for maternal serum PP-13 alone (dashed line), uterine artery pulsatility index alone (dotted line) and their combination (solid line) in the prediction of pre-eclampsia requiring early delivery.

overall false-positive rate would be 6% (95% CI, 4–8%). Alternatively, a policy with the ultrasound examination being performed on all women and measurement of PP-13 being reserved for the 32% with the highest risk would have an overall false-positive rate of 16% (95% CI, 12–20%).

DISCUSSION

The findings of this study demonstrate that in women who subsequently develop pre-eclampsia requiring delivery before 34 weeks, the maternal serum PP-13 concentration at 11 + 0 to 13 + 6 weeks of gestation is significantly lower than it is in normal pregnancies. These data are compatible with the observations that pre-eclampsia requiring early delivery is associated with down regulation of the gene for PP-13[14] and with impaired placental functional responsiveness to PP-13[11].

The data show that measurement of maternal serum PP-13 in early pregnancy, in combination with Doppler assessment of impedance to flow in the uterine arteries, may provide effective screening for this pregnancy complication. For a 10% false-positive rate, the detection rate of pre-eclampsia requiring early delivery was about 80% for serum PP-13, 40% for uterine artery PI and 90% for the markers combined.

The same high detection rate of 90% could be achieved with contingency screening, whereby maternal serum PP-13 is measured in all patients and uterine artery Doppler is reserved for a subgroup constituting one sixth of the total population. Such a pragmatic approach is more likely to achieve widespread clinical application, than would combined screening for the whole population, and would be associated with major economic savings. Certainly in the UK, the National Screening Committee has recommended that by April 2007 all pregnant women should be offered the option of screening for trisomy 21 by a combination of maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) and the measurement of fetal nuchal translucency thickness at 11 + 0 to 13 + 6 weeks. Within the context of such screening it would be relatively inexpensive to include measurement of PP-13. In contrast to biochemical testing, which could be undertaken in all good laboratories, the expertise for Doppler assessment of the uterine arteries is less readily available, and is currently confined to specialist centers.

We have also demonstrated an approach for the calculation of patient-specific risk for the development of pre-eclampsia requiring early delivery. This approach, which has been used widely in the assessment of risk for trisomy 21, is useful both for epidemiologists in estimating the effectiveness of screening and for patients and their doctors in planning the subsequent management of the pregnancy. At present there is no proven effective method for the prevention of pre-eclampsia and routine antenatal care has evolved with the aim of identifying women at risk and offering them more intensive antenatal care. In the UK, the National Institute for Clinical Excellence (NICE) has issued guidelines on routine antenatal care, recommending that a woman's level of risk for pre-eclampsia should be evaluated so that a plan for her schedule of antenatal appointments can be formulated[1]. Nevertheless, there is evidence that the prophylactic use of low-dose aspirin, starting from the first trimester of pregnancy, could reduce the prevalence of the disease[8,9].

This study has demonstrated the potential value of first-trimester screening for pre-eclampsia requiring early delivery by a combination of maternal serum testing and sonographic assessment of placental perfusion. The extent to which these results can be confirmed in large-scale multicenter studies and whether early therapeutic intervention in the high-risk group can reduce the prevalence of the disease remain to be determined.

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