

# Hypertensive disorders in pregnancy: screening by biophysical and biochemical markers at 11–13 weeks

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**KEYWORDS:** first trimester; mean arterial pressure; placental growth factor; screening for pre-eclampsia; uterine artery Doppler

## ABSTRACT

**Objective** To examine the performance of screening for pre-eclampsia (PE) and gestational hypertension (GH) by a combination of maternal factors and various biophysical and biochemical markers at 11–13 weeks' gestation.

**Methods** This was a case–control study of 26 cases of early PE, 90 of late PE, 85 of GH and 201 unaffected controls. Maternal history was recorded, the uterine artery with the lowest pulsatility index (L-PI) and mean arterial pressure (MAP) were measured and stored plasma and serum were analyzed for placental growth factor (PLGF), inhibin-A, activin-A, tumor necrosis factor receptor-1, matrix metalloproteinase-9, pentraxin-3 and P-selectin.

**Results** Multivariate logistic regression analysis demonstrated that significant prediction for early PE was provided by maternal factors, MAP, uterine artery L-PI and serum PLGF. Significant prediction of late PE was provided by maternal factors, MAP, uterine artery L-PI, PLGF, activin-A and P-selectin. For GH significant prediction was provided by maternal factors, MAP, uterine artery L-PI and activin-A. In screening by a combination of maternal factors, biophysical and biochemical markers the estimated detection rates, at a 5% false-positive rate, were 88.5% (95% CI, 69.8–97.4%) for early PE, 46.7% (95% CI, 36.1–57.5%) for late PE and 35.3% (95% CI, 25.2–46.4%) for GH.

**Conclusion** Combined biophysical and biochemical testing at 11–13 weeks could effectively identify women at high risk for subsequent development of hypertensive disorders in pregnancy. Copyright © 2010 ISUOG. Published by John Wiley & Sons, Ltd.

## INTRODUCTION

Pre-eclampsia (PE), which affects about 2% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality<sup>1–3</sup>. Hypertension developing in the second half of pregnancy is subdivided according to the presence or absence of co-existing significant proteinuria into PE and gestational hypertension (GH). Recent evidence suggests that PE can be further subdivided into early PE and late PE, the former being associated with a higher incidence of fetal growth restriction and both short- and long-term maternal mortality and morbidity<sup>4–7</sup>.

We performed a screening study for hypertensive disorders in 8366 singleton pregnancies at 11–13 weeks' gestation, including 205 that developed PE or GH. Multiple regression analysis was used to derive the patient-specific risk for early PE, late PE and GH by combining the disease-specific maternal factor-derived risk with measurements of the mean arterial pressure (MAP) and the uterine artery pulsatility index (PI) recorded from the artery with the lowest PI (L-PI)<sup>8</sup>. The estimated detection rates of early PE, late PE and GH were 89, 57 and 50%, respectively, at a 10% false-positive rate and 78, 42 and 36%, respectively, at a 5% false-positive rate.

The underlying mechanism for the development of PE is thought to be impaired trophoblastic invasion of the maternal spiral arteries and their conversion from narrow muscular vessels to wide non-muscular channels independent of maternal vasomotor control<sup>9–12</sup>. Impaired placental perfusion is thought to lead to placental ischemia and damage with release of inflammatory factors that cause platelet activation and endothelial dysfunction and consequent development of the clinical symptoms of the disease<sup>13–15</sup>. In previous case–control studies we investigated the maternal serum or plasma concentration at 11–13 weeks of biochemical factors thought to be involved in placentation or in the cascade of events leading

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from impaired placentation to the development of the clinical symptoms of the disease. These included pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PIGF), inhibin-A, activin-A, tumor necrosis factor receptor-1 (TNF-R1), matrix metalloproteinase-9 (MMP-9), pentraxin-3 (PTX-3) and P-selectin<sup>16–23</sup>.

The aim of this study was to examine the performance of screening for hypertensive disorders in pregnancy by a combination of the *a-priori* risk derived from the combination of maternal factors, MAP and uterine artery L-PI with various combinations of the biochemical markers.

## METHODS

In our center we perform screening for adverse pregnancy outcomes in women attending for routine assessment of risk for chromosomal abnormalities by measurement of fetal nuchal translucency thickness and maternal serum PAPP-A and free  $\beta$ -human chorionic gonadotropin at 11 + 0 to 13 + 6 weeks of gestation<sup>24,25</sup>. In this case-control study we recorded maternal characteristics and medical history, measured the uterine artery PI by transabdominal color Doppler ultrasonography<sup>26</sup>, measured the MAP by automated devices<sup>27</sup> and stored plasma and serum at  $-80^{\circ}\text{C}$  for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

For the measurement of uterine artery PI by transabdominal ultrasound, a sagittal section of the uterus was obtained and the cervical canal and internal cervical os were identified. Subsequently 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. Pulsed-wave Doppler was used with the sampling gate set at 2 mm to cover the whole vessel and care was taken to ensure that the angle of insonation was less than  $30^{\circ}$ . When three similar consecutive waveforms had been obtained the uterine artery PI was measured from the left and right arteries. All ultrasound and Doppler studies were carried out by sonographers who had received the appropriate Certificate of Competence in the 11–13-weeks scan and Doppler of The Fetal Medicine Foundation ([www.fetalmedicine.com](http://www.fetalmedicine.com)). The results of the Doppler studies were not given to the women or their doctors and did not influence the subsequent management of the pregnancies.

Blood pressure (BP) was taken by automated devices (3BTO-A2, Microlife, Taipei, Taiwan), which were calibrated before and at regular intervals (about 1000 inflations) during the study. The recordings were made by doctors who had received appropriate training in the use of these machines. The women were seated, their arms were supported at the level of the heart and either a small (<22 cm), normal (22–32 cm) or large (33–42 cm) adult cuff was used depending on the mid-arm circumference. After rest for 5 min BP was measured in both arms simultaneously and a series of recordings were made at 1-min intervals until variations between consecutive readings fell

within 10 mmHg in systolic BP and 6 mmHg in diastolic BP in both arms. When this point of stability had been reached we calculated the MAP of each arm as the average of the last two stable measurements, and as recommended we took the arm with the highest final MAP.

This study is part of a research program on the early prediction of pregnancy complications. In previous publications we have reported the findings from case-control studies on PAPP-A, PIGF, inhibin-A, activin-A, TNF-R1, MMP-9, PTX-3 and P-selectin<sup>16–23</sup>. In this study we present the combined data.

## Maternal serum biochemistry

None of the samples was previously thawed and refrozen. Maternal serum PAPP-A was measured using the DELFIA XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). Serum PIGF was measured by a quantitative enzyme linked immunoassay (ELISA) technique using Quantikine® human PIGF immunoassay (R&D Systems Europe Ltd., Abingdon, UK). Plasma inhibin-A was measured by an ELISA technique using DSL-10-28 100 inhibin-A immunoassay (Diagnostic systems Laboratories, Inc., Webster, TX, USA). Serum total activin-A concentration was measured by solid phase sandwich ELISA using Oxford Bio-Innovation total activin-A immunoassay kits (Oxford Bio-Innovation Ltd, Oxfordshire, UK). Serum TNF-R1 was measured using Quantikine® ELISA kits (R&D Systems Europe Ltd., Abingdon, UK). Serum MMP-9 was measured by a quantitative sandwich enzyme immunoassay using Quantikine® human MMP-9 immunoassay (R&D Systems Europe Ltd.). Plasma PTX-3 was measured by an ELISA kit (R&D Systems Europe Ltd.). Plasma P-selectin was measured by an ELISA technique using human soluble P-selectin/CD62P immunoassay (R&D Systems Europe Ltd.).

## Case-control population

The base cohort study population, in which the present case-control study was nested, was examined between March 2006 and November 2007 and contained 165 (2.0%) cases who developed PE, including 37 (0.4%) who required delivery before 34 weeks (early PE) and 128 (1.5%) with late PE, 140 (1.7%) with GH and 8061 (96.4%) cases who were unaffected by PE or GH. Stored maternal blood was available from 26 of the 37 cases who developed early PE, 90 of the 128 with late PE and 85 of the 140 with GH. Each case of PE and GH was matched with one control from whom blood was collected on the same day and who delivered a phenotypically normal neonate appropriate in size for gestational age at term and did not develop any hypertensive disorder of pregnancy.

## Outcome measures

The definitions of PE and GH used were those of the International Society for the Study of Hypertension in Pregnancy<sup>28</sup>, according to which in GH the diastolic BP should be 90 mmHg or more on at least two occasions 4 h apart developing after 20 weeks of gestation in previously normotensive women in the absence of significant proteinuria, and in PE there should be GH with proteinuria of 300 mg or more in 24 h or two readings of at least ++ on dipstick analysis of midstream or catheter urine specimens if no 24-h collection is available. In PE superimposed on chronic hypertension significant proteinuria (as defined above) should develop after 20 weeks of gestation in women with known chronic hypertension (a history of hypertension before conception or the presence of hypertension at the booking visit before 20 weeks of gestation in the absence of trophoblastic disease) for the diagnosis to be established.

Data on pregnancy outcome were collected from the hospital maternity records or the women's general medical practitioners. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine whether the condition was chronic hypertension, PE or GH.

## Statistical analysis

The measured MAP, uterine artery L-PI and PAPP-A were converted to multiples of the expected normal median (MoM) corrected for fetal crown-rump length (CRL), maternal age, body mass index (BMI) or weight, smoking, parity, racial origin and method of conception, as previously described<sup>26,27,29</sup>. The measured concentration of each of the biochemical markers was log transformed to make their distribution Gaussian. Multiple regression analysis was then used to determine which of the factors among the maternal characteristics and fetal CRL were significant predictors of each biochemical marker in the control group, and from the regression model the value in each case and control was expressed as a MoM. Comparison between each hypertensive disorder group and controls was by chi square or Fisher's exact test for categorical variables and Mann-Whitney-*U* test for continuous variables, both with post-hoc Bonferroni correction (critical statistical significance  $P < 0.0167$ ). The *a-priori* risks for early PE, late PE and GH based on maternal factors and a combination of maternal factors, MAP and uterine artery L-PI were determined as previously described and were then logarithmically transformed<sup>8,30</sup>. Backward stepwise logistic regression analysis was used to determine if the log MoM of each of the biochemical markers, log transformed *a-priori* risk for the disorder based on maternal factors only or log transformed combined *a-priori* risk based on maternal factors, MAP and uterine artery L-PI, had significant contribution in predicting early PE, late PE and GH. The performance of screening was estimated by receiver-operating characteristics (ROC) curves.

The statistical software package SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

## RESULTS

### Patient characteristics

The maternal characteristics of the early PE, late PE and GH groups are compared with those of the controls in Table 1. In the early-PE group, compared to controls, there was a higher prevalence of Black women, chronic hypertensives on antihypertensive medication and women with PE in their previous pregnancies. In the late-PE group there was a higher prevalence of Black women, women with a previous history of miscarriage or termination prior to 24 weeks of gestation and women with a maternal history of PE, and their BMI was higher. In the GH group there was a higher prevalence of women with previous PE and their BMI was higher.

### Control group

Multiple regression analysis in the control group demonstrated that for the log value of each biochemical marker significant independent contributions were provided by maternal characteristics and gestation. In each patient we used the following formulae to derive the expected log values of each biochemical marker and then expressed the observed value as a MoM of the expected (Table 2):

Log expected PIGF

$$\begin{aligned}
 &= 1.166 \text{ (standard error (SE), 0.131)} \\
 &+ 0.009 \text{ (SE, 0.002)} \times \text{CRL in mm} \\
 &- 0.003 \text{ (SE, 0.001)} \times \text{weight in kg} \\
 &+ (0.200 \text{ (SE, 0.049)} \text{ if smoking, 0 if not}) \\
 &+ (0.159 \text{ (SE, 0.034)} \text{ if Black,} \\
 &0 \text{ if other racial origins);} \\
 &R^2 = 0.255, P < 0.0001.
 \end{aligned}$$

Log expected inhibin-A = 2.613 (SE, 0.080)

$$\begin{aligned}
 &- 0.003 \text{ (SE, 0.001)} \times \text{weight in kg} \\
 &+ (0.118 \text{ (SE, 0.37)} \text{ if Black,} \\
 &0 \text{ if other racial origins);} \\
 &R^2 = 0.082, P < 0.0001.
 \end{aligned}$$

Log expected activin-A = 0.187 (SE, 0.093)

$$\begin{aligned}
 &+ 0.008 \text{ (SE, 0.002)} \times \text{age in years} \\
 &- 0.003 \text{ (SE, 0.001)} \times \text{weight in kg} \\
 &+ (0.109 \text{ (SE, 0.030)} \text{ if Black,} \\
 &0 \text{ if other racial origins);} \\
 &R^2 = 0.139, P < 0.0001.
 \end{aligned}$$

**Table 1** Maternal characteristics in the four outcome groups

| Maternal variable                        | Unaffected<br>(n = 201) | Early pre-eclampsia<br>(n = 26) | Late pre-eclampsia<br>(n = 90) | Gestational hypertension<br>(n = 85) |
|--|-------------------------|---------------------------------|--------------------------------|--------------------------------------|
| Maternal age (years)                     | 32.1 (28.7–35.5)        | 32.7 (27.4–38.7)                | 31.5 (26.3–36.3)               | 33.4 (30.1–35.8)                     |
| Body mass index (kg/m <sup>2</sup> )     | 25.1 (22.9–28.7)        | 27.2 (23.7–32.0)                | 27.1 (23.8–33.4)*              | 26.7 (24.2–31.4)*                    |
| Racial origin                            |                         |                                 |                                |                                      |
| White                                    | 139 (69.2)              | 11 (42.3)*                      | 40 (44.4)‡                     | 63 (74.1)                            |
| Black                                    | 40 (19.9)               | 11 (42.3)†                      | 38 (42.2)‡                     | 17 (20.0)                            |
| Indian or Pakistani                      | 15 (7.5)                | 2 (7.7)                         | 7 (7.8)                        | 0                                    |
| Chinese or Japanese                      | 2 (1.0)                 | 0                               | 1 (1.1)                        | 1 (1.2)                              |
| Mixed                                    | 5 (2.5)                 | 2 (7.7)                         | 4 (4.4)                        | 4 (4.7)                              |
| Parity                                   |                         |                                 |                                |                                      |
| Nulliparous                              | 60 (29.9)               | 9 (34.6)                        | 39 (43.3)                      | 31 (36.5)                            |
| Miscarriage/termination before 24 weeks  | 19 (9.5)                | 4 (15.4)                        | 20 (22.2)*                     | 16 (18.8)                            |
| Parous—no previous PE                    | 115 (57.2)              | 6 (23.1)†                       | 22 (24.4)‡                     | 29 (34.1)†                           |
| Parous—previous PE                       | 7 (3.5)                 | 7 (26.9)†                       | 9 (10.0)                       | 9 (10.6)*                            |
| Cigarette smoker                         | 16 (8.0)                | 0                               | 6 (6.7)                        | 7 (8.2)                              |
| Family history of PE                     |                         |                                 |                                |                                      |
| Mother                                   | 6 (3.0)                 | 3 (11.5)                        | 11 (12.2)*                     | 8 (9.4)                              |
| Sister                                   | 3 (1.5)                 | 3 (11.5)                        | 1 (1.1)                        | 0                                    |
| Conception                               |                         |                                 |                                |                                      |
| Spontaneous                              | 193 (96.0)              | 23 (88.5)                       | 86 (95.6)                      | 82 (96.5)                            |
| Ovulation induction                      | 7 (3.5)                 | 2 (7.7)                         | 3 (3.3)                        | 0                                    |
| In-vitro fertilization                   | 1 (0.5)                 | 1 (3.8)                         | 1 (1.1)                        | 3 (3.5)                              |
| Medical history                          |                         |                                 |                                |                                      |
| None                                     | 194 (96.5)              | 21 (80.8)*                      | 85 (94.4)                      | 82 (96.5)                            |
| Chronic hypertension                     | 1 (0.5)                 | 4 (15.4)†                       | 4 (4.4)                        | 0                                    |
| Diabetes mellitus                        | 2 (1.0)                 | 0                               | 0                              | 2 (2.4)                              |
| Anti-phospholipid syndrome/thrombophilia | 3 (1.5)                 | 1 (3.8)                         | 1 (1.1)                        | 1 (1.2)                              |
| Other                                    | 1 (0.5)                 | 0                               | 0                              | 0                                    |
| Medication during pregnancy              |                         |                                 |                                |                                      |
| None                                     | 181 (90.0)              | 22 (84.6)                       | 83 (92.2)                      | 73 (85.9)                            |
| Antihypertensives                        | 2 (1.0)                 | 2 (7.7)*                        | 2 (2.2)                        | 0                                    |
| Insulin                                  | 1 (0.5)                 | 0                               | 0                              | 2 (2.4)                              |
| Aspirin                                  | 3 (1.5)                 | 1 (3.8)                         | 0                              | 3 (3.5)                              |
| Other                                    | 14 (7.0)                | 1 (3.8)                         | 5 (5.6)                        | 7 (8.2)                              |

Data are given as *n* (%) or median (interquartile range). Comparison between each hypertensive disorder group and controls was by chi square or Fisher's exact test for categorical variables and Mann–Whitney-*U* test for continuous variables, both with post-hoc Bonferroni correction (critical statistical significance  $P < 0.0167$ ). \* $P < 0.0167$ . † $P < 0.001$ . ‡ $P < 0.0001$ .

Log expected TNF-R1 = 2.979 (SE, 0.031)

+ 0.001 (SE, 0.0004) × weight in kg

+ (−0.036 (SE, 0.014) if Black,

0 if other racial origins);

$R^2 = 0.065$ ,  $P = 0.001$ .

Log expected MMP-9 = 2.674 (SE, 0.018)

+ (−0.092 (SE, 0.040) if Black,

0 if other racial origins);

$R^2 = 0.026$ ,  $P = 0.022$ .

Log expected PTX-3 = −0.098 (SE, 0.086)

− 0.003 (SE, 0.001) × weight in kg

+ (−0.125 (SE, 0.060) if Indian or

Pakistani, 0 if other racial origins);

$R^2 = 0.047$ ,  $P = 0.009$ .

Log expected P-selectin = 1.321 (SE, 0.079)

+ 0.002 (SE, 0.001) × CRL in mm

+ (−0.056 (SE, 0.024) if Black,

− 0.264 (SE, 0.095) if Chinese or Japanese,

0 if other racial origins)

+ (−0.185 (SE, 0.053) if conceived with  
ovulation drug, 0 if not);

$R^2 = 0.112$ ,  $P < 0.0001$ .

#### Patient-specific risks

The patient-specific risk for each hypertensive disorder is calculated from the formula: risk = odds/(1+odds), where odds =  $e^Y$ . The Y for each hypertensive disorder was derived from backward stepwise multivariate logistic regression analysis of log MoM of each of the biochemical markers, log transformed *a-priori* risk for the disorder based on maternal factors only or log transformed

Table 2 Data for each marker in the four outcome groups

|                            | Control                | Early pre-eclampsia    | Late pre-eclampsia     | Gestational hypertension |
|----------------------------|------------------------|------------------------|------------------------|--------------------------|
| Mean arterial pressure     |                        |                        |                        |                          |
| MoM                        | 0.99 (0.95–1.05)       | 1.16 (1.08–1.25)*      | 1.09 (1.02–1.13)*      | 1.08 (1.02–1.14)*        |
| mmHg                       | 84.2 (80.5–89.5)       | 98.0 (91.8–106.5)      | 93.8 (87.0–98.7)       | 93.3 (86.5–98.3)         |
| Lowest uterine artery PI   |                        |                        |                        |                          |
| MoM                        | 1.05 (0.85–1.31)       | 1.65 (1.31–1.82)*      | 1.26 (0.92–1.55)†      | 1.12 (0.87–1.38)         |
| Unit                       | 1.43 (1.18–1.84)       | 2.29 (1.87–2.45)       | 1.73 (1.26–2.18)       | 1.53 (1.19–1.87)         |
| PAPP-A                     |                        |                        |                        |                          |
| MoM                        | 1.00 (0.69–1.45)       | 0.62 (0.42–1.11)†      | 0.96 (0.61–1.33)       | 0.86 (0.62–1.39)         |
| mU/L                       | 2.79 (1.78–4.57)       | 2.63 (0.95–3.36)       | 2.79 (1.57–4.32)       | 2.01 (1.52–3.45)         |
| Placental growth factor    |                        |                        |                        |                          |
| MoM                        | 0.96 (0.75–1.31)       | 0.59 (0.49–0.78)*      | 0.85 (0.55–1.03)*      | 0.93 (0.69–1.18)         |
| pg/mL                      | 34.2 (26.2–50.0)       | 23.0 (15.04–33.5)      | 30.1 (21.5–37.2)       | 29.6 (23.8–42.4)         |
| Inhibin-A                  |                        |                        |                        |                          |
| MoM                        | 0.98 (0.73–1.41)       | 1.54 (0.94–2.03)‡      | 1.23 (0.88–1.66)†      | 1.07 (0.82–1.40)         |
| pg/mL                      | 245.9 (175.1–340.7)    | 378.8 (243.6–530.0)    | 317.2 (217.8–433.3)    | 254.3 (199.5–333.7)      |
| Activin-A                  |                        |                        |                        |                          |
| MoM                        | 1.02 (0.77–1.29)       | 1.12 (0.93–1.61)       | 1.30 (0.94–1.73)*      | 1.11 (0.90–1.48)         |
| pg/mL                      | 1.80 (1.43–2.43)       | 2.42 (1.80–2.94)       | 2.29 (1.82–3.02)       | 2.00 (1.49–2.66)         |
| TNF-R1                     |                        |                        |                        |                          |
| MoM                        | 1.00 (0.87–1.13)       | 1.10 (0.91–1.32)       | 1.08 (0.94–1.19)‡      | 1.03 (0.95–1.15)         |
| pg/mL                      | 1178.2 (1012.4–1299.0) | 1293.0 (1096.3–1457.6) | 1260.1 (1083.1–1403.9) | 1204.3 (1104.7–1408.0)   |
| Matrix metalloproteinase-9 |                        |                        |                        |                          |
| MoM                        | 1.03 (0.71–1.41)       | 1.23 (0.92–1.71)       | 1.20 (0.96–1.62)‡      | 1.09 (0.82–1.37)         |
| pg/mL                      | 454.9 (325.6–658.9)    | 530.2 (387.6–768.1)    | 536.1 (417.1–699.9)    | 495.4 (380.8–641.0)      |
| Pentraxin-3                |                        |                        |                        |                          |
| MoM                        | 0.97 (0.74–1.21)       | 1.39 (0.82–2.01)       | 1.12 (0.78–1.62)       | 1.11 (0.82–1.55)         |
| ng/mL                      | 0.48 (0.38–0.61)       | 0.57 (0.42–0.87)       | 0.48 (0.37–0.78)       | 0.50 (0.39–0.69)         |
| P-selectin                 |                        |                        |                        |                          |
| MoM                        | 1.02 (0.83–1.24)       | 1.25 (0.87–1.51)       | 1.24 (1.00–1.46)*      | 1.11 (0.95–1.30)‡        |
| ng/mL                      | 29.7 (22.9–35.0)       | 35.0 (25.1–40.1)       | 35.3 (27.0–40.4)       | 32.7 (27.1–38.7)         |

Data given as median (interquartile range). Comparison between each hypertensive disorder group and controls was by Mann–Whitney-U test, with post-hoc Bonferroni correction (critical statistical significance  $P < 0.0167$ ). \* $P < 0.0001$ . † $P < 0.001$ . ‡ $P < 0.0167$ . MoM, multiples of the expected median; PAPP-A, pregnancy associated plasma protein-A; PI, pulsatility index; TNF-R1, tumor necrosis factor receptor-1.

combined *a-priori* risk based on maternal factors, MAP and uterine artery L-PI.

In the case of early PE when the *a-priori* risk was based only on the maternal factors, significant prediction was provided by PlGF, inhibin-A and TNF-R1 but not PAPP-A, activin-A, MMP-9, PTX-3 or P-selectin:

$$\begin{aligned}
 Y &= 3.022 \text{ (SE, 1.342)} + 2.652 \text{ (SE, 0.610)} \\
 &\times \log \text{ maternal factor-derived } a\text{-priori risk for} \\
 &\text{early-PE} - 6.056 \text{ (SE, 1.543)} \times \log \text{ PlGF MoM} \\
 &+ 3.103 \text{ (SE, 1.258)} \times \log \text{ inhibin-A MoM} \\
 &+ 9.753 \text{ (SE, 3.577)} \times \log \text{ TNF-R1 MoM;} \\
 R^2 &= 0.523, P < 0.0001.
 \end{aligned}$$

In the case of early PE when the *a-priori* risk was based on the combination of maternal factors, MAP and uterine artery L-PI significant prediction was provided only by PlGF. Inhibin-A and TNF-R1 did not remain significant predictors together with PAPP-A, activin-A,

MMP-9, PTX-3 or P-selectin:

$$\begin{aligned}
 Y &= 2.547 \text{ (SE, 0.858)} + 2.518 \text{ (SE, 0.482)} \\
 &\times \log \text{ combined } a\text{-priori risk for early-PE} \\
 &- 6.012 \text{ (SE, 2.018)} \times \log \text{ PlGF MoM;} \\
 R^2 &= 0.698, P < 0.0001.
 \end{aligned}$$

In the case of late PE when the *a-priori* risk was based only on the maternal factors, significant prediction was provided by PlGF, activin-A, MMP-9 and P-selectin but not PAPP-A, inhibin-A, TNF-R1 or PTX-3:

$$\begin{aligned}
 Y &= 3.810 \text{ (SE, 0.842)} + 2.898 \text{ (SE, 0.472)} \\
 &\times \log \text{ maternal factor-derived } a\text{-priori risk for} \\
 &\text{late-PE} - 3.171 \text{ (SE, 0.807)} \times \log \text{ PlGF MoM} \\
 &+ 3.792 \text{ (SE, 0.979)} \times \log \text{ activin-A MoM} \\
 &+ 2.013 \text{ (SE, 0.817)} \times \log \text{ MMP-9 MoM} + 5.242 \\
 &\text{(SE, 1.364)} \times \log \text{ P-selectin MoM;} \\
 R^2 &= 0.495, P < 0.0001.
 \end{aligned}$$

In the case of late PE when the *a-priori* risk was based on the combination of maternal factors, MAP and uterine artery L-PI significant prediction was provided by PIGF, activin-A and P-selectin. MMP-9 did not remain a significant predictor together with PAPP-A, inhibin-A, TNF-R1 or PTX-3:

$$Y = 3.490 \text{ (SE, 0.658)} + 2.717 \text{ (SE, 0.381)} \\ \times \log \text{ combined } a\text{-priori risk for} \\ \text{late-PE} - 2.966 \text{ (SE, 0.844)} \times \log \text{ PIGF MoM} \\ + 3.937 \text{ (SE, 1.045)} \times \log \text{ activin-A MoM} \\ + 4.190 \text{ (SE, 1.408)} \times \log \text{ P-selectin MoM}; \\ R^2 = 0.558, P < 0.0001.$$

In the case of GH when the *a-priori* risk was based only on the maternal factors, significant prediction was provided by activin-A and P-selectin but not PIGF, PAPP-A, inhibin-A, MMP-9, TNF-R1 or PTX-3:

$$Y = 2.815 \text{ (SE, 0.773)} + 2.119 \text{ (SE, 0.434)} \\ \times \log \text{ maternal factor-derived } a\text{-priori risk for GH} \\ + 2.389 \text{ (SE, 1.111)} \times \log \text{ P-selectin MoM} \\ + 1.730 \text{ (SE, 0.842)} \times \log \text{ activin-A MoM}; \\ R^2 = 0.186, P < 0.0001.$$

In the case of GH when the *a-priori* risk was based on the combination of maternal factors, MAP and uterine artery L-PI significant prediction was provided only by activin-A. P-selectin did not remain a significant predictor together with PIGF, PAPP-A, inhibin-A, MMP-9, TNF-R1 or PTX-3:

$$Y = 3.309 \text{ (SE, 0.621)} + 2.405 \text{ (SE, 0.356)} \\ \times \log \text{ combined } a\text{-priori risk for GH} \\ - 2.103 \text{ (SE, 0.891)} \times \log \text{ activin-A MoM}; \\ R^2 = 0.295, P < 0.0001.$$

### Performance of screening

The areas under the ROC curve and detection rates of early PE, late PE and GH in screening by maternal factors

only, a combination of maternal factors with biochemical markers, a combination of maternal factors, MAP and uterine artery L-PI and a combination of maternal factors, MAP, uterine artery L-PI and biochemical markers are given in Tables 3 and 4 and Figure 1.

### Example

For example, in a Black woman in her first pregnancy, who is 28 years old, has a weight of 55 kg and a height of 165 cm (BMI of 20 kg/m<sup>2</sup>), does not smoke and at 12 weeks of gestation (CRL 65 mm), her PIGF is 40 pg/mL, inhibin-A is 250 pg/mL and TNF-R1 is 1300 pg/mL, the risk for early-PE is 30.6%.

Maternal factor-derived *a-priori* risk for early PE:

$$Y = -5.674 + 1.267 \text{ (Black race)} \\ + 0 \text{ (history of chronic hypertension)} \\ + 0 \text{ (spontaneous conception)} \\ + 0 \text{ (nulliparous)} \\ = -4.406$$

$$\text{Odds} = e^Y = 0.012$$

$$A\text{-priori risk} = \text{odds}/(1 + \text{odds}) = 0.012$$

$$\text{Log expected PIGF} = \\ 1.166 - 0.009 \times 65 \text{ (CRL in mm)} \\ - 0.003 \times 55 \text{ (weight in kg)} \\ + 0.200 \times 0 \text{ (not smoking)} \\ + 0.159 \text{ (Black race)} \\ = 1.724$$

$$\text{Log PIGF MoM} = -0.122$$

$$\text{Log expected inhibin-A} = \\ 2.613 - 0.003 \times 55 \text{ (weight in kg)} \\ + 0.118 \text{ (Black race)} \\ = 2.542$$

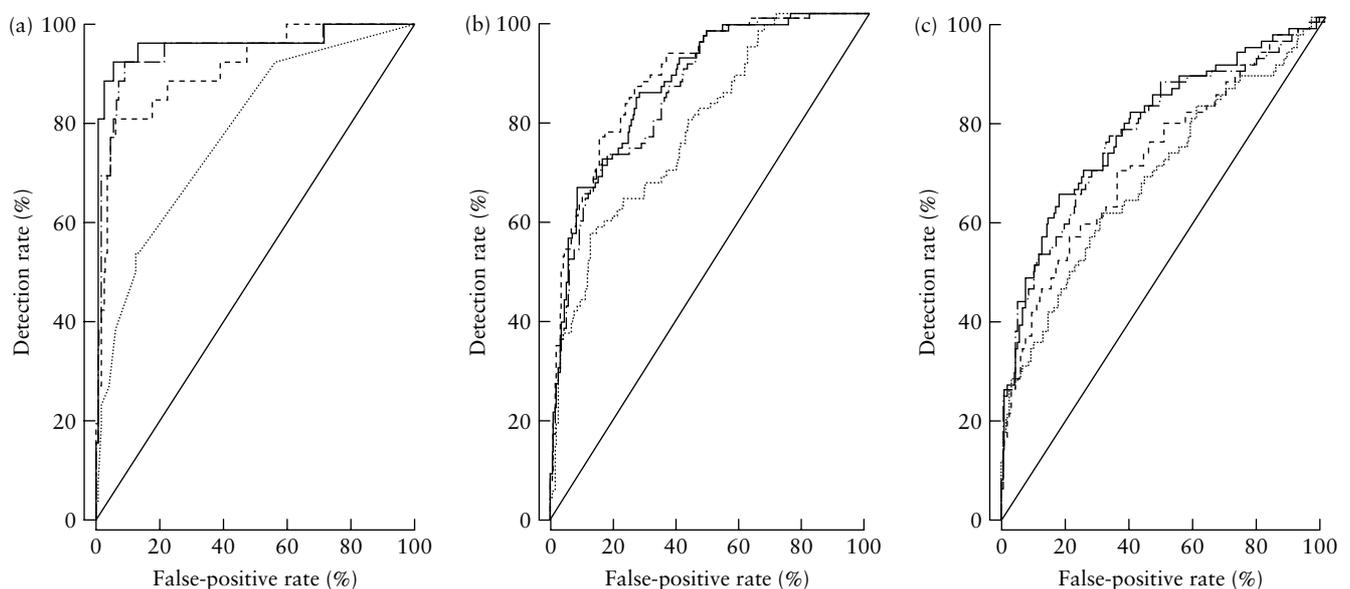
$$\text{Log inhibin-A MoM} = -0.144$$

**Table 3** Performance of screening for pre-eclampsia and gestational hypertension by maternal factors only, a combination of maternal factors with biochemistry, a combination of maternal factors, lowest uterine artery pulsatility index (L-PI) and mean arterial pressure (MAP) and a combination of maternal factors, uterine artery L-PI, MAP and biochemistry as shown by area under receiver-operating characteristics (ROC) curve

| Screening test                         | Area under ROC curve (95% CI) |                     |                          |
|--|-------------------------------|---------------------|--------------------------|
|  | Early pre-eclampsia           | Late pre-eclampsia  | Gestational hypertension |
| Maternal factors                       | 0.715 (0.652–0.773)           | 0.778 (0.726–0.824) | 0.677 (0.619–0.731)      |
| Maternal factors plus:                 |                               |                     |                          |
| Biochemistry                           | 0.908 (0.863–0.942)           | 0.876 (0.833–0.912) | 0.715 (0.659–0.767)      |
| Uterine artery L-PI, MAP               | 0.933 (0.892–0.962)           | 0.835 (0.787–0.876) | 0.759 (0.705–0.807)      |
| Uterine artery L-PI, MAP, biochemistry | 0.959 (0.925–0.981)           | 0.862 (0.817–0.900) | 0.782 (0.730–0.829)      |

**Table 4** Performance of screening for pre-eclampsia and gestational hypertension by maternal factors only, a combination of maternal factors with biochemistry, a combination of maternal factors, lowest uterine artery pulsatility index (L-PI) and mean arterial pressure (MAP) and a combination of maternal factors, uterine artery L-PI, MAP and biochemistry as shown by detection rate for a fixed false-positive rate (FPR)

|  | Detection rate (% (95% CI)) for fixed FPR |                  |                    |                  |                          |                  |
|--|---|------------------|--------------------|------------------|--------------------------|------------------|
|  | Early pre-eclampsia                       |                  | Late pre-eclampsia |                  | Gestational hypertension |                  |
|  | FPR 5%                                    | FPR 10%          | FPR 5%             | FPR 10%          | FPR 5%                   | FPR 10%          |
| Maternal factors                       | 39.5 (5.0–69.0)                           | 47.0 (12.5–79.0) | 36.7 (26.8–47.5)   | 47.5 (24.0–67.5) | 28.2 (19.0–39.0)         | 35.0 (17.0–56.0) |
| Maternal factors plus:                 |   |                  |                    |                  |                          |                  |
| Biochemistry                           | 76.9 (56.3–91.0)                          | 80.8 (60.6–93.4) | 53.3 (42.5–63.9)   | 64.4 (53.7–74.3) | 27.1 (18.0–37.8)         | 38.8 (28.4–50.0) |
| Uterine artery L-PI, MAP               | 77.5 (40.0–96.0)                          | 87.5 (54.0–99.5) | 40.0 (10.0–67.5)   | 57.0 (27.5–80.0) | 37.0 (12.5–62.0)         | 48.2 (37.3–59.3) |
| Uterine artery L-PI, MAP, biochemistry | 88.5 (69.8–97.4)                          | 92.3 (74.8–98.8) | 46.7 (36.1–57.5)   | 65.6 (54.8–75.3) | 35.3 (25.2–46.4)         | 49.4 (38.4–60.5) |



**Figure 1** Receiver-operating characteristics curves of maternal factors (.....), maternal factors with biochemistry (-.-.-.), maternal factors with mean arterial pressure (MAP) and uterine artery lowest pulsatility index (L-PI) (---) and maternal factors with MAP, uterine artery L-PI and biochemistry (—) in the prediction of early pre-eclampsia (a), late pre-eclampsia (b) and gestational hypertension (c).

$$\text{Log expected TNF-R1} =$$

$$2.613 - 0.003 \times 55 \text{ (weight in kg)}$$

$$+ 0.118 \text{ (Black race)}$$

$$= 3.016$$

$$\text{Log TNF-R1 MoM} = 0.098$$

$$= 0.418$$

$$\text{Odds} = e^Y = 1.520$$

$$A\text{-posteriori risk} = \text{odds}/(1 + \text{odds}) = 0.603$$

If her uterine artery L-PI were 1.6 and her MAP 85 mmHg, the risk of developing early PE becomes 4.7%:

*A-posteriori* risk for early PE based on maternal factors and biochemical markers:

$$Y = 3.022 + 2.652 \times -1.919(\text{log maternal factor-derived } a\text{-priori risk for early-PE})$$

$$- 6.056 \times -0.122 \text{ (log PlGF MoM)}$$

$$+ 3.103 \times -0.144 \text{ (log inhibin-A MoM)}$$

$$+ 9.753 \times 0.098 \text{ (log TNF-R1 MoM)}$$

$$\text{Log expected uterine artery L-PI} =$$

$$0.348 - 0.002 \times 65 \text{ (CRL in mm)}$$

$$+ 0.035 \text{ (Black race)}$$

$$- 0.002 \times 20 \text{ (BMI in kg/m}^2\text{)}$$

$$- 0.001 \times 28 \text{ (age in years)}$$

$$= 0.182$$

$$\text{Log uterine artery L-PI MoM} = 0.023$$

$$\begin{aligned}
 \text{Log expected MAP} &= \\
 &1.861 - (0.0002 \times 65 \text{ (CRL in mm)}) \\
 &+ (0.003 \times 20 \text{ (BMI in kg/m}^2\text{)}) \\
 &+ (0.0004 \times 28 \text{ (age in years)}) \\
 &+ 0 \text{ (smoking)} \\
 &- 0.005 \text{ (Black race)} \\
 &= 1.909 \\
 \text{Log MAP MoM} &= 0.021
 \end{aligned}$$

*A-posteriori* risk for early PE based on maternal factors, biophysical and biochemical markers:

$$\begin{aligned}
 Y &= 2.547 + 2.518 \times -2.501 \text{ (log combined} \\
 &\text{ } a\text{-priori risk for early PE)} \\
 &- 6.012 \times -0.122 \text{ (log PIGF MoM)} \\
 &= -3.014 \\
 \text{Odds} &= e^Y = 0.049 \\
 \text{ } A\text{-posteriori risk} &= \text{odds}/(1 + \text{odds}) = 0.047 = 4.7\%.
 \end{aligned}$$

## DISCUSSION

This study has demonstrated an approach for combining biophysical and biochemical parameters for identifying, in the first trimester of pregnancy, women who are at high risk for the subsequent development of PE or GH. The approach is similar to that used for combining maternal age with sonographic and maternal serum biochemical markers in early screening for chromosomal defects<sup>24,25</sup>.

The *a-priori* risk in combined screening for PE and GH was estimated by multivariate logistic regression analysis of maternal characteristics<sup>30</sup>. The risk for early PE was higher in Black than White women and in those with chronic hypertension or a history of PE in previous pregnancies. The risk for late PE and GH increased with maternal age and BMI, and was higher for women with a family or prior history of PE. The biophysical components of the new combined screening test are impedance to flow in the uterine arteries and MAP. Uterine artery L-PI was significantly increased in those who subsequently developed PE and this was particularly pronounced in those with early PE. In contrast, MAP was significantly increased in both those who developed PE and those who developed GH<sup>8</sup>.

The biochemical factors we investigated are thought to be involved in placentation or in the cascade of events leading from impaired placentation to the development of the clinical symptoms of the disease. Previous studies in women with established PE reported that the maternal blood concentrations of these biochemical factors is altered in comparison with normotensive controls<sup>31–36</sup>. Our results demonstrate that these changes are evident from the first trimester of pregnancy and several weeks

before the clinical onset of the disease. In pregnancies developing PE the maternal serum concentrations of PIGF and PAPP-A were reduced. These proteins are produced by trophoblast, and their reduced maternal serum concentration presumably reflects impaired placentation. Their performance in screening for PE is similar<sup>16,17</sup> and it is not surprising that PAPP-A does not remain a significant predictor in the presence of PIGF, as PIGF has a narrower confidence interval. In contrast, the concentrations of inhibin-A and activin-A, which are also produced by trophoblast, were increased, which may reflect a placental compensatory mechanism to promote trophoblastic invasion in cases where this process is impaired. Reduced placental perfusion is thought to lead to placental ischemia and damage with release of inflammatory factors, such as TNF-R1 and MMP-9, which in turn cause platelet activation and endothelial dysfunction, as demonstrated by increased levels of P-selectin and PTX-3, respectively. The development of the clinical symptoms of the disease is thought to be the consequence of platelet and endothelial dysfunction<sup>13–15</sup>.

In general, altered maternal blood concentrations of biochemical markers were observed in pregnancies developing PE but not in those developing non-proteinuric pregnancy hypertension. We have also observed that some of the markers showed a significant relationship with early PE and some with late PE. These differences may simply reflect the fact that there is a wide spectrum in impaired placentation and the consequent clinical presentation of the disease. Alternatively, these differences may support emerging evidence that PE is a common clinical expression of distinct pathophysiological processes. Additionally, the deviations from normal were greater in association with early compared to late PE. These findings are compatible with the observation that the degree of impaired placentation, reflected in increased uterine artery PI, was greatest in early PE compared with late PE or GH. The estimated detection rate of the combined screening test was about 90% for early PE, 45% for late PE and 35% for GH, at a false-positive rate of 5%. The high detection rate for early PE is important because it is this rather than late PE or GH that is associated with an increased risk for perinatal mortality and morbidity and both short- and long-term maternal complications<sup>4–7</sup>.

The ability to predict very early in pregnancy those women at high risk for PE might lead to decreased maternal and fetal morbidity through closer surveillance by physicians experienced or specialized in high-risk obstetrics, as well as delivery at tertiary care centers<sup>37</sup>. The results presented here are generated from a case-control study; therefore it is necessary to examine the performance of the proposed models in screening in prospective studies in unselected low-risk populations. The proposed combined screening test could also be used for effective identification of the high-risk group for future studies investigating the potential role of pharmacological interventions, starting from the first trimester, to improve placentation and reduce the prevalence of the disease.

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