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Maternal serum PAPP-A and free β-hCG at 12, 22 and 32 weeks' gestation in screening for pre-eclampsia

A. WRIGHT*, L. GUERRA†, M. PELLEGRINO†, D. WRIGHT* and K. H. NICOLAIDES†

**Institute of Health Research, University of Exeter, Exeter, UK; †Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK*

KEYWORDS: Bayes' theorem; free β-human chorionic gonadotropin; impaired placentation; pre-eclampsia; pregnancy-associated plasma protein-A; pyramid of pregnancy care; screening

ABSTRACT

Objective *To examine the distribution of maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) at 12, 22 and 32 weeks' gestation in singleton pregnancies which develop pre-eclampsia (PE) and examine the performance of these biomarkers in screening for PE.*

Methods *Serum PAPP-A and free β-hCG were measured in 94 989 cases at 11–13 weeks, 7597 at 19–24 weeks and 8088 at 30–34 weeks' gestation. Bayes' theorem was used to combine the* a-priori *risk from maternal characteristics and medical history with PAPP-A and free β-hCG. The empirical and model-based performance of screening for preterm PE requiring delivery < 37 weeks' gestation and term PE with delivery ≥ 37 weeks was estimated.*

Results *Combined screening with maternal factors and serum PAPP-A at 11–13 and 30–34 weeks and with maternal factors and serum free β-hCG at 19–24 and 30–34 weeks improved the prediction provided by maternal factors alone for preterm PE. The detection rate, at a 10% false-positive rate, for preterm PE by screening with maternal factors was about 45% which improved to 51% and 53% by combined screening with PAPP-A at 11–13 weeks and 30–34 weeks, respectively, and 55% and 54% by combined screening with free β-hCG at 19–24 weeks and 30–34 weeks, respectively. Measurement of serum PAPP-A and free β-hCG was not useful in the prediction of term PE.*

Conclusions *Measurement of serum PAPP-A and free β-hCG could improve the prediction of preterm PE provided by maternal characteristics and medical history alone. Copyright © 2016 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Maternal serum levels of pregnancy-associated plasma protein-A (PAPP-A) in the first trimester of pregnancy are decreased in pregnancies with fetal trisomies 21, 18 or 13 and in those with impaired placentation resulting in pre-eclampsia (PE) and delivery of small-for-gestational-age (SGA) neonates[1–5]. There is also some evidence that serum PAPP-A is reduced in the second trimester in pregnancies that develop PE, but the levels are increased in cases with established disease[6–9]. Maternal serum levels of free β-human chorionic gonadotropin (β-hCG) in the first trimester of pregnancy are increased in pregnancies with fetal trisomy 21 and decreased in trisomies 18 and 13[1,2]. Serum free β-hCG is increased in pregnancies with established PE and in the third trimester before clinical onset of the disease, but is decreased or unaltered at 11–13 weeks' gestation[10–14].

We have proposed that the best approach to screening for PE is to use Bayes' theorem to combine the *a-priori* risk from maternal characteristics and medical history with the measurement of biomarkers[15–17]. Our approach assumes that, if the pregnancy was to continue indefinitely, all women would develop PE and whether they do so or not before a specified gestational age depends on competition between delivery before or after development of PE. The effect of maternal factors and biomarkers is to modify the mean of the distribution of gestational age at delivery with PE so that in pregnancies at low risk of PE the gestational-age distribution is shifted to the right with the implication that, in most pregnancies, delivery will actually occur before development of PE. In high-risk pregnancies, the distribution is shifted to the left and the smaller the mean gestational age the higher is the risk of PE.

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK (e-mail: kypros@fetalmedicine.com)

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Table 1 Maternal and pregnancy characteristics in women with singleton pregnancy screened at different gestational periods for prediction of pre-eclampsia (PE)

	11–13 weeks		*19–24 weeks*		*30–34 weeks*	
Characteristic	*Normal (n = 92 800)*	*PE (n = 2189)*	*Normal (n = 7396)*	*PE (n = 201)*	*Normal (n = 7895)*	*PE (n = 193)*
Maternal age (years)	31.7 (27.4–35.4)	31.6 (27.2–36.1)	30.9 (26.4–34.6)	31.7 (26.5–35.7)	30.9 (26.6–34.7)	31.5 (27.0–34.8)
Maternal weight (kg)	65.5 (58.7–75.1)	71.8 (62.2–85.0)*	71.0 (63.0–82.0)	77.5 (69.0–91.0)*	76.7 (68.5–87.2)	83.0 (72.5–97.0)*
Maternal height (cm)	165 (160–169)	163 (159–168)*	165 (160–169)	164 (160–168)	165 (160–169)	164 (159–169)*
BMI (kg/m^2)	24.2 (21.8–27.7)	26.8 (23.4–31.7)*	26.1 (23.5–29.9)	28.7 (25.7–33.2)*	28.2 (25.4–32.0)	31.1 (27.9–35.7)*
GA (weeks)	12.7 (12.3–13.1)	12.7 (12.3–13.1)*	21.8 (21.2–22.1)	21.9 (21.1–22.1)	32.2 (32.0–32.5)	32.1 (32.0–32.4)*
Racial origin		*		*		*
Caucasian	68 124 (73.4)	1210 (55.3)	5664 (76.6)	124 (61.7)	5958 (75.5)	118 (61.1)
Afro-Caribbean	14 844 (16.0)	761 (34.8)	1146 (15.5)	65 (32.3)	1331 (16.9)	63 (32.6)
South Asian	4897 (5.3)	128 (5.9)	309 (4.2)	7 (3.5)	290 (3.7)	9 (4.7)
East Asian	2527 (2.7)	39 (1.8)	134 (1.8)	3 (1.5)	143 (1.8)	2 (1.0)
Mixed	2408 (2.6)	51 (2.3)	143 (1.9)	2 (1.0)	173 (2.2)	1 (0.5)
Medical history						
CH	993 (1.1)	239 (10.9)*	82 (1.1)	23 (11.4)*	95 (1.2)	26 (13.5)*
DM	718 (0.8)	50 (2.3)*	72 (1.0)	8 (4.0)*	75 (1.0)	3 (1.6)
SLE/APS	181 (0.2)	14 (0.6)*	10 (0.1)	0 (0)	15 (0.2)	0 (0)
Family history of PE	3643 (3.9)	168 (7.7)*	217 (2.9)	9 (4.5)	228 (2.9)	7 (3.6)
Mode of conception		*				
IVF	89 380 (96.3)	2066 (94.4)	7155 (96.7)	191 (95.0)	7639 (96.8)	186 (96.4)
Ovulation drugs	2217 (2.4)	94 (4.3)	174 (2.4)	5 (2.5)	184 (2.3)	3 (1.6)
Spontaneous	1203 (1.3)	29 (1.3)	67 (0.9)	5 (2.5)	72 (0.9)	4 (2.1)
Obstetric history		*		*		*
Parous						
No previous PE	44 929 (48.4)	1339 (61.2)	3480 (47.1)	130 (64.7)	3842 (48.7)	115 (59.6)
Previous PE	45 333 (48.9)	543 (24.8)	3674 (49.7)	44 (21.9)	3793 (48.0)	54 (28.0)
Nulliparous	2538 (2.7)	307 (14.0)	242 (3.3)	27 (13.4)	260 (3.3)	24 (12.4)
Interpregnancy interval (years)	2.9 (1.9–4.8)	3.9 (2.3–6.9)*	3.1 (2.0–4.9)	4.3 (2.5–6.5)*	3.1 (2.1–5.1)	3.65 (2.4–6.5)*

Data are given as median (interquartile range) or *n* (%). Comparisons with normal group: chi-square or Fisher's exact tests for categorical variables and Mann–Whitney *U*-test for continuous variables: *$P < 0.05$. APS, antiphospholipid syndrome; BMI, body mass index; CH, chronic hypertension; DM, diabetes mellitus; GA, gestational age at screening; IVF, *in-vitro* fertilization; SLE, systemic lupus erythematosus.

The objectives of this study were to present the distribution of serum PAPP-A and free β-hCG values at 11–13, 19–24 and 30–34 weeks' gestation in pregnancies that develop PE and examine the performance of screening for PE by serum PAPP-A and free β-hCG at these stages in pregnancy.

METHODS

Study population

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending three routine hospital visits at King's College Hospital (between March 2006 and July 2014), University College London Hospital (between April 2009 and July 2013), and Medway Maritime Hospital (between April 2010 and July 2014), UK. In the first visit, at 11 + 0 to 13 + 6 weeks' gestation, we recorded maternal characteristics and medical history and performed combined screening for aneuploidies[2]. The second visit, at 19 + 0 to 24 + 6 weeks' gestation, and the third at 30 + 0 to 34 + 6 weeks, included ultrasound examination of the fetal anatomy and estimation of fetal size. Gestational age was determined by the measurement of fetal crown–rump length at 11–13 weeks or fetal head circumference at 19–24 weeks[18,19].

Written informed consent was obtained from the women agreeing to participate in a study on adverse pregnancy outcome, which was approved by the ethics committee of each participating hospital. The inclusion criteria for this study were singleton pregnancy delivering phenotypically normal live birth or stillbirth at or after 24 weeks' gestation. Pregnancies with aneuploidy or major fetal abnormalities, and those ending in termination, miscarriage or fetal death before 24 weeks' gestation were excluded.

Patient characteristics

Patient characteristics included maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous/assisted conception requiring the use of ovulation drugs/*in-vitro* fertilization), medical history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus (SLE) or antiphospholipid syndrome (APS), family history of PE in the mother of the patient and obstetric history including

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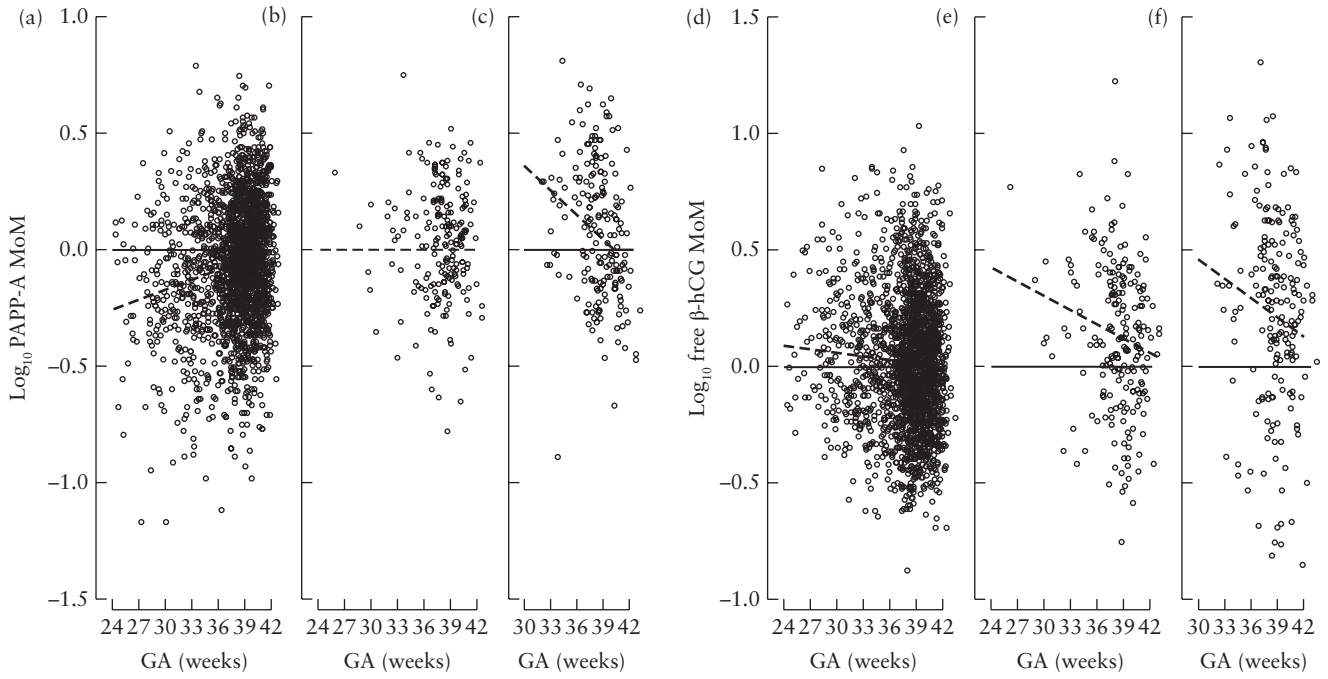

Figure 1 Relationship between maternal serum pregnancy-associated plasma protein-A (PAPP-A) (a–c) and free beta-human chorionic gonadotropin (β-hCG) (d–f) multiples of the median (MoM) and gestational age at delivery (GA) in pregnancies with pre-eclampsia, with screening at: (a,d) 11–13, (b,e) 19–24 and (c,f) 30–34 weeks' gestation. Regression lines (– –) are shown.

parity (parous/nulliparous if no previous pregnancy at or after 24 weeks), previous pregnancy with PE, gestational age at delivery and interval in years between birth of the last child and estimated date of conception of the current pregnancy. Maternal height was measured in the first visit and maternal weight in each visit.

Measurement of maternal serum PAPP-A and free β-hCG

In the patients included in this study, maternal serum PAPP-A and free β-hCG were measured at each visit by automated biochemical analyzers within 10 min of blood sampling. The first-trimester samples were analyzed using the DELFIA Xpress system (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) and in the second and third trimesters the analysis was by the Cobas e411 system (Roche Diagnostics Ltd., Penzberg, Germany).

Outcome measures

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women. The obstetric records of all women with pre-existing or pregnancy-associated hypertension were examined to determine if the condition was PE, as defined by the International Society for the Study of Hypertension in Pregnancy[20]. The outcome measures for this study were PE delivering < 32, at $32 + 0$ to $36 + 6$, < 37 and ≥ 37 weeks' gestation.

Statistical analysis

Competing-risks model

The distribution of gestational age at delivery with PE was defined by two components: first, the prior distribution based on maternal characteristics[15] and second, the distribution of serum PAPP-A and free β-hCG multiples of the median (MoM) values with gestational age at delivery in pregnancies affected by PE. The values of PAPP-A and free β-hCG were $\log_{10}$ transformed to achieve homogeneity of variance and approximate Gaussian distributional form. Each measured value in the unaffected and PE pregnancies was expressed as a MoM, adjusting for those characteristics found to provide a substantive contribution to the $\log_{10}$-transformed value[21,22]. Risks of PE were obtained by applying Bayes' theorem to derive the posterior distribution of gestational age at delivery with PE from the maternal factors specific prior distribution[15] and the likelihood function of PAPP-A and free β-hCG. The likelihood function comprises the regression of $\log_{10}$ MoM PAPP-A and free β-hCG on gestational age at delivery with PE.

Model-based estimates of screening performance using Bayes' theorem

To provide model-based estimates of screening performance, the following procedure was adopted. First, we obtained the dataset of 123 406 singleton pregnancies with available data on maternal factors[15]. Second, for each of the records, PAPP-A and free β-hCG MoM values

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Table 2 Empirical and model-based detection rates of screening for pre-eclampsia (PE) by maternal factors and by a combination of maternal factors and serum pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG) at 11–13, 19–24 and 30–34 weeks' gestation

	Detection rate of PE delivering:							
	< 32 weeks		*32 + 0 to 36 + 6 weeks*		*< 37 weeks*		*≥ 37 weeks*	
Screening	*Empirical (95% CI) (%) (n/N)*	*Model (%)*	*Empirical (95% CI) (%) (n/N)*	*Model (%)*	*Empirical (95% CI) (%) (n/N)*	*Model (%)*	*Empirical (95% CI) (%) (n/N)*	*Model (%)*
11–13 weeks								
FPR = 5%								
Maternal	41 (33–49) 64/158	41	31 (27–36) 138/441	31	34 (30–38) 202/599	34	27 (24–29) 422/1590	27
PAPP-A	45 (37–53) 71/158	47	37 (32–42) 163/441	35	39 (35–43) 234/599	38	27 (25–30) 436/1590	27
β-hCG	39 (32–47) 62/158	42	31 (27–36) 137/441	32	33 (29–37) 199/599	34	27 (24–29) 422/1590	27
FPR = 10%								
Maternal	51 (43–59) 81/158	52	44 (40–49) 196/441	44	46 (42–50) 277/599	46	37 (35–40) 594/1590	37
PAPP-A	58 (50–66) 92/158	61	51 (46–56) 224/441	48	53 (49–57) 316/599	51	38 (36–41) 606/1590	38
β-hCG	53 (44–61) 83/158	53	44 (40–49) 196/441	44	47 (43–51) 279/599	47	37 (34–39) 586/1590	37
19–24 weeks								
FPR = 5%								
Maternal	43 (10–82) 3/7	41	33 (20–50) 14/42	31	35 (22–50) 17/49	34	25 (18–33) 38/152	27
β-hCG	57 (18–90) 4/7	53	43 (28–59) 18/42	38	45 (31–60) 22/49	42	24 (17–31) 36/152	27
FPR = 10%								
Maternal	71 (29–96) 5/7	52	52 (36–68) 22/42	44	55 (40–69) 27/49	46	35 (27–43) 53/152	37
β-hCG	57 (18–90) 4/7	67	57 (41–72) 24/42	51	57 (42–71) 28/49	55	37 (29–45) 56/152	38
30–34 weeks								
FPR = 5%								
Maternal					29 (15–46) 11/38	31	25 (18–32) 38/155	27
PAPP-A					39 (24–57) 15/38	39	25 (19–33) 39/155	28
β-hCG					47 (31–64) 18/38	42	24 (17–31) 37/155	31
Combined					61 (43–79) 23/38	50	28 (21–35) 43/155	33
FPR = 10%								
Maternal					42 (26–59) 16/38	44	34 (26–42) 52/155	37
PAPP-A					55 (38–71) 21/38	53	36 (29–44) 56/155	38
β-hCG					63 (46–78) 24/38	54	39 (31–47) 60/155	43
Combined					63 (46–78) 24/38	63	37 (30–46) 58/155	45

FPR, false-positive rate.

were simulated from the fitted multivariate Gaussian distribution for log-transformed MoM values. Third, risks were obtained using the competing-risks model from the simulated MoM values and the pregnancy characteristics. These three steps were applied to the pregnancies within the normal group with no restriction on the time of delivery. Fourth, for a given false-positive rate (FPR), risks from the normal group were used to define a risk cut-off. The proportion of PE risks was then used to obtain an estimate of the associated detection rate (DR). The area under the receiver–operating characteristics curve was also calculated. The simulations were repeated 10 times to reduce variability due to the simulation process and provide suitably precise model-based estimates of performance.

Empirical performance of screening

Five-fold cross validation was used to assess the performance of screening for subgroups of PE according to gestational age at delivery, by models combining maternal factors with serum PAPP-A and free β-hCG. The data were divided into five equal subgroups, the model was then fitted five times to different combinations of four of the five subgroups and used to predict risk of PE in the remaining fifth of the data. In each case, the maternal-factor model and the regression models were fitted to the training dataset comprising four-fifths of the data and used to produce risks for the hold-out sample comprising the remaining fifth of the data.

The statistical software package R was used for data analyses[23] and the survival package[24] was used for fitting the maternal-factors model.

RESULTS

The characteristics of the study population of singleton pregnancies with measurements of serum PAPP-A and free β-hCG are summarized in Table 1.

The median PAPP-A MoM in unaffected pregnancies was 1.000 (95% CI, 0.996–1.004). In the PE group, PAPP-A MoM was decreased at 11–13 weeks (0.850 (95% CI, 0.830–0.870)), not significantly altered at 19–24 weeks (1.019 (95% CI, 0.997–1.040)) and increased at 30–34 weeks (1.236 (95% CI, 1.205–1.268)). The median β-hCG MoM in unaffected pregnancies was 1.000 (95% CI, 0.995–1.005). In

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the PE group, β-hCG MoM was not significantly altered at 11–13 weeks (1.014 (95% CI, 0.990–1.038)) and was increased at 19–24 weeks (1.288 (95% CI, 1.253–1.324)) and at 30–34 weeks (1.680 (95% CI, 1.621–1.742)).

The relationship of PAPP-A and free β-hCG MoM values with gestational age at delivery in pregnancies that developed PE is shown in Figure 1 and the regression equations are given in Table S1. The standard deviation for $\log_{10}$ PAPP-A and free β-hCG MoM in unaffected pregnancies and in those that developed PE are given in Table S2.

Empirical and model-based performance of screening for PE by maternal factors and serum PAPP-A and free β-hCG are shown in Tables 2 and S3. All model-based results were within the 95% CI of the empirical data. Combined screening with maternal factors and serum PAPP-A at 11–13 and 30–34 weeks and maternal factors and serum free β-hCG at 19–24 and 30–34 weeks improved the prediction of preterm PE provided by maternal factors alone.

DISCUSSION

Principal findings of the study

The findings of this study demonstrate that, in pregnancies that develop PE compared to unaffected pregnancies, maternal serum PAPP-A is decreased during the first trimester, is not significantly different in the second trimester and is increased in the early third trimester, and serum free β-hCG is not significantly different in the first trimester but is increased in the second and third trimesters, the deviation from normal increasing with increasing gestational age at screening. The separation in MoM values from normal is greater with an earlier than later gestational age at which delivery for PE becomes necessary; consequently, the performance of screening is superior for preterm PE than for term PE.

Strengths and limitations

The strengths of this screening study for PE in the three trimesters of pregnancy are first, examination of a large population of pregnant women attending for routine care, second, recording of data on maternal characteristics and medical history to identify known risk factors associated with PE, third, measurement of serum PAPP-A and free β-hCG by automated machines that provide reproducible results, fourth, expression of the values of serum PAPP-A and free β-hCG as MoMs after adjustment for factors that affect the measurements and fifth, use of Bayes' theorem to combine the prior risk from maternal factors with PAPP-A and free β-hCG to estimate patient-specific risks and the performance of screening for PE delivering at different stages of pregnancy.

A limitation of the study is that of optimistic bias in performance due to deriving and testing a model using the same dataset. We used five-fold cross validation to reduce such bias.

Clinical implications of the study

In a proposed new pyramid of pregnancy care[25], assessment at 12 weeks aims to identify those at high risk of developing preterm PE and through pharmacological intervention reduce the prevalence of the disease[26,27]. Assessment at 22 and/or 32 weeks aims to estimate the patient-specific risk of developing PE and, on the basis of such risk, define the timing and content of subsequent visits to help improve perinatal outcome.

At 11–13 weeks' gestation, serum free β-hCG is not a useful marker of PE. Serum PAPP-A improves the prediction of preterm PE provided by maternal factors alone but the improvement is small. We have reported previously that serum PAPP-A does not improve the performance provided by combined screening with maternal factors, uterine artery pulsatility index (UtA-PI), mean arterial pressure (MAP) and serum placental growth factor (PlGF), which detects 75% of preterm PE and 47% of term PE at a FPR of 10%[5].

At 19–24 weeks' gestation, serum PAPP-A is not a useful marker of PE. Serum free β-hCG improves the prediction of preterm PE provided by maternal factors alone, but the improvement is small. We have reported previously that effective screening for PE at this gestational age is provided by a combination of maternal factors, UtA-PI, MAP and PlGF, which detects 85% of preterm PE and 46% of term PE at a FPR of 10%[28].

At 30–34 weeks' gestation, combined screening by maternal factors, serum PAPP-A and free β-hCG could improve the prediction of preterm PE provided by maternal factors alone from 44% to 63%, at a FPR of 10%. However, such improvement is small by comparison with screening by a combination of maternal factors, UtA-PI, MAP, PlGF and serum soluble fms-like tyrosine kinase-1, which detects 99% of preterm PE and 66% of term PE at a FPR of 10%[29].

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 Regression equations of serum pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG) multiples of the median in pregnancies that developed pre-eclampsia

Table S2 Pooled standard deviations and correlations for $\log_{10}$ serum pregnancy-associated plasma protein-A (PAPP-A) and free beta human chorionic gonadotropin (β-hCG) multiples of the median in unaffected pregnancies and those that developed pre-eclampsia

Table S3 Model-based and empirical areas under the receiver–operating characteristics curve (AUC) in screening for pre-eclampsia (PE) delivering < 32, < 37 and ≥ 37 weeks' gestation by maternal factors and a combination of maternal factors and serum pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG) at 11–13, 19–24 and 30–34 weeks

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