Ultrasound Obstet Gynecol 2021; **57**: 257–265
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.23531

Prediction of pre-eclampsia in twin pregnancy by maternal factors and biomarkers at 11–13 weeks' gestation: data from EVENTS trial

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KEYWORDS: calibration; competing-risks model; first-trimester screening; mean arterial pressure; performance of screening; placental growth factor; pre-eclampsia; survival model; twin pregnancy; uterine artery pulsatility index

CONTRIBUTION

What are the novel findings of this work?
In screening for pre-eclampsia (PE) by maternal characteristics and medical history in twin pregnancy, using the competing-risks model, the effect of twins in shifting the distribution of gestational age at delivery with PE in singletons to the left is not constant but rather it increases with increasing prior mean gestational age, so that the shift to the left is greater if the prior mean is high and less if the prior mean is low. The slope of the regression lines for $\log_{10}$ multiples of the median (MoM) values of uterine artery pulsatility index (UtA-PI), mean arterial pressure (MAP) and serum placental growth factor (PlGF) according to gestational age at delivery with PE in twin pregnancies is steeper than in singleton pregnancies, indicating greater deviation from normal for early gestations and less deviation for later gestations; this finding is consistent with the fact that twin pregnancies deliver earlier and have a much higher incidence of preterm PE than do singletons.

What are the clinical implications of this work?
In the assessment of risk for PE in twin pregnancies, we can use the same model for the prior distribution of gestational age at delivery with PE based on maternal characteristics and medical history as reported previously, but in the calculation of the posterior distribution it is necessary to use the new $\log_{10}$ MoM values of UtA-PI, MAP and PlGF according to gestational age at delivery with PE.

ABSTRACT

Objectives *First, to validate a previously developed model for screening for pre-eclampsia (PE) by maternal characteristics and medical history in twin pregnancies; second, to compare the distributions of mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI), serum placental growth factor (PlGF) and serum pregnancy-associated plasma protein-A (PAPP-A) in twin pregnancies that delivered with PE to those in singleton pregnancies and to develop new models based on these*

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Accepted: 20 October 2020

results; and, third, to examine the predictive performance of these models in screening for PE with delivery at < 32 and < 37 weeks' gestation.

Methods *Two datasets of prospective non-intervention multicenter screening studies for PE in twin pregnancies at 11 + 0 to 13 + 6 weeks' gestation were used. The first dataset was from the EVENTS (Early vaginal progesterone for the preVention of spontaneous prEterm birth iN TwinS) trial and the second was from a previously reported study that examined the distributions of biomarkers in twin pregnancies. Maternal demographic characteristics and medical history from the EVENTS-trial dataset were used to assess the validity of risks from our previously developed model. The combined data from the first and second datasets were used to compare the distributional properties of log_{10} multiples of the median (MoM) values of UtA-PI, MAP, PlGF and PAPP-A in twin pregnancies that delivered with PE to those in singleton pregnancies and develop new models based on these results. The competing-risks model was used to estimate the individual patient-specific risks of delivery with PE at < 32 and < 37 weeks' gestation. Screening performance was measured by detection rates (DR) and areas under the receiver-operating-characteristics curve.*

Results *The EVENTS-trial dataset comprised 1798 pregnancies, including 168 (9.3%) that developed PE. In the validation of the prior model based on maternal characteristics and medical history, calibration plots demonstrated very good agreement between the predicted risks and the observed incidence of PE (calibration slope and intercept for PE < 32 weeks were 0.827 and 0.009, respectively, and for PE < 37 weeks they were 0.942 and −0.207, respectively). In the combined data, there were 3938 pregnancies, including 339 (8.6%) that developed PE and 253 (6.4%) that delivered with PE at < 37 weeks' gestation. In twin pregnancies that delivered with PE, MAP, UtA-PI and PlGF were, at earlier gestational ages, more discriminative than in singleton pregnancies and at later gestational ages they were less so. For PAPP-A, there was little difference between PE and unaffected pregnancies. The best performance of screening for PE was achieved by a combination of maternal factors, MAP, UtA-PI and PlGF. In screening by maternal factors alone, the DR, at a 10% false-positive rate, was 30.6% for delivery with PE at < 32 weeks' gestation and this increased to 86.4% when screening by the combined test; the respective values for PE < 37 weeks were 24.9% and 41.1%.*

Conclusions *In the assessment of risk for PE in twin pregnancy, we can use the same prior model based on maternal characteristics and medical history as reported previously, but in the calculation of posterior risks it is necessary to use the new distributions of log_{10} MoM values of UtA-PI, MAP and PlGF according to gestational age at delivery with PE.* © *2020 International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

In twin pregnancies, the rate of pre-eclampsia (PE) is about 9%, which is 3-times higher than in singleton pregnancies, but since twins are delivered at an earlier gestational age than singletons, comparison of the overall rates of PE between twin and singleton pregnancies underestimates the relative risk of preterm PE in twins, which is 9-times higher[1]. In singleton pregnancies, effective first-trimester screening for PE is provided by the combination of maternal characteristics and medical history with mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI), serum placental growth factor (PlGF) and serum pregnancy-associated plasma protein-A (PAPP-A) within the framework of a competing-risks model[2–7]. In the competing-risks approach, each woman has a personalized distribution of gestational age at delivery with PE; in pregnancies at low risk for PE, the mean gestational age at delivery with PE is increased, with the implication that in most pregnancies delivery from other causes occurs before development of PE; in high-risk pregnancies, the mean gestational age at delivery with PE is decreased so delivery with PE occurs more often[7].

We have proposed previously that the same competing-risks model based on maternal characteristics and medical history (prior model), developed in singleton pregnancies, can be adapted for use in twins; in dichorionic and monochorionic twin pregnancies with the same characteristics as singleton pregnancies, the distribution of gestational age of delivery with PE was shifted to the left by 8 and 10 weeks, respectively[8]. However, in a subsequent validation study, we found that the observed incidence of PE was lower than the predicted one and such overestimation of risk was particularly marked for early PE[9]. Consequently, we developed a new model in which the effect of twins in shifting the distribution of gestational age at delivery with PE in singletons to the left was not the same for all gestational ages but rather the shift depended on the singleton prior mean; the shift to the left was greater if the prior mean was high and less if the prior mean was low[10]. In another screening study at 11–13 weeks' gestation in twin pregnancies, we measured UtA-PI, MAP, PlGF and PAPP-A and found that, in the PE group, compared to those that remained normotensive, MAP and UtA-PI were increased and serum PlGF was decreased, whereas serum PAPP-A was not significantly different[11]. The distribution of biomarkers according to gestational age at delivery with PE was similar to the previously reported fitted regression relationships for singleton pregnancies with PE[3] and it was therefore assumed that the same model could be used for both singleton and twin pregnancies[11].

In this study, we used data of twin pregnancies from the EVENTS (Early vaginal progesterone for the preVention of spontaneous prEterm birth iN TwinS) trial[12]. The EVENTS trial was a multicenter study in which unselected twin pregnancies were randomized to vaginal progesterone (600 mg per day from 11–14 until 34 weeks' gestation), as compared with placebo; progesterone did not reduce the incidence of early

spontaneous preterm birth[12]. The objectives of the current study were, first, to examine the predictive performance of the new model in screening for PE by maternal factors in twin pregnancies[10] in a validation dataset; second, to combine the data of biomarkers with those from our previous study[11], compare the distributions of UtA-PI, MAP, PlGF and PAPP-A in twin pregnancies that delivered with PE to those in singleton pregnancies and develop new models based on these results[3]; and, third, to examine the predictive performance of this model in screening for PE with delivery at < 32 weeks' gestation and at < 37 weeks.

METHODS

Study population

Two datasets of twin pregnancies were used for this study. The first dataset was from the EVENTS study, which was conducted at 22 maternity hospitals in England, Spain, Bulgaria, Italy, Belgium and France between May 2017 and April 2019[12]. All women found at a routine visit at 11 + 0 to 13 + 6 weeks' gestation to have a dichorionic or monochorionic diamniotic twin pregnancy with two live fetuses and no major fetal abnormality were invited to participate in a screening study on the prediction of adverse pregnancy outcome, irrespective of their decision to participate in the progesterone trial or not. The women gave written informed consent to participate in the study, which was approved by the relevant research ethics committee and competent authority in each country in which the trial was conducted. The second dataset was from prospective screening for adverse obstetric outcomes in women attending for their first routine hospital visit at King's College Hospital, London and Medway Maritime Hospital, Gillingham, UK, between January 2006 and December 2015[11]. The women gave written informed consent to participate in the study, which was approved by the NHS Research Ethics Committee.

In both datasets, assessment at 11 + 0 to 13 + 6 weeks' gestation included: first, recording of maternal characteristics and medical history; second, measurement of MAP using validated automated devices and standardized protocol[13]; third, measurement of the left and right UtA-PI using transabdominal color Doppler ultrasound and calculation of the mean UtA-PI[14]; and, fourth, measurement of serum concentrations of PlGF and PAPP-A using an automated biochemical analyzer (first dataset: DELFIA Xpress system, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA; second dataset: BRAHMS KRYPTOR compact PLUS, Thermo Fisher Scientific, Hennigsdorf, Germany or Cobas e411 system, Roche Diagnostics, Penzberg, Germany). Gestational age was determined by the measurement of fetal crown–rump length[15] of the larger twin. Chorionicity was determined by examining the intertwin membrane at its junction with the placenta[16].

Patient characteristics included maternal age, racial origin (white, black, South Asian, East Asian or mixed), method of conception (spontaneous or assisted conception requiring *in-vitro* fertilization or the use of ovulation drugs), cigarette smoking during pregnancy, history of chronic hypertension, diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, family history of PE in the mother of the patient and obstetric history including parity (parous or nulliparous if no previous pregnancy at ≥ 24 weeks), previous pregnancy with PE, gestational age at delivery and birth weight of the neonate in the last pregnancy and interval in years between birth of the last child and estimated date of conception of the current pregnancy.

The inclusion criteria for the study were delivery of a phenotypically normal liveborn or stillborn neonate at ≥ 24 weeks' gestation. We excluded pregnancies with aneuploidy or major fetal abnormality, those ending in termination, miscarriage or fetal death before 24 weeks and those with an interval of > 3 days between death of one fetus and live birth of the second twin.

Outcome measures

Outcome measures were delivery with PE at < 32 and < 37 weeks' gestation. 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 chronic hypertension or pregnancy-associated hypertension were examined to determine the diagnosis of PE. This was based on the finding of new-onset hypertension (systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure of ≥ 90 mmHg on at least two occasions 4 h apart developing after 20 weeks' gestation in a previously normotensive woman) or chronic hypertension and at least one of the following: proteinuria (≥ 300 mg/24 h, protein-to-creatinine ratio ≥ 30 mg/mmol or ≥ 2+ on dipstick testing), renal insufficiency with serum creatinine > 97 μmol/L in the absence of underlying renal disease, hepatic dysfunction with blood concentration of transaminases more than twice the upper limit of normal (≥ 65 IU/L for our laboratory), thrombocytopenia (platelet count < 100 000/μL), neurological complications (for example, cerebral or visual symptoms), or pulmonary edema[17].

Statistical analysis

Data were expressed as median (interquartile range) for continuous variables and as *n* (%) for categorical variables. Student's *t*-test and chi-square test or Fisher's exact test were used for comparing outcome groups for continuous and categorical data, respectively.

Validation of prior model based on maternal characteristics and medical history

We used the dataset from the EVENTS trial[12] to validate the previously reported prior model for prediction of PE by maternal characteristics and medical history[10]. Calibration was assessed visually by plotting the observed incidence against that predicted for delivery with PE at

< 32 and < 37 weeks' gestation. The plots were produced by grouping the data into bins according to risk. The observed incidence in each group was then plotted against the incidence predicted by the model (i.e. the mean risks within each group). Calibration-in-the-large is a measure of whether the risks are generally too high or too low. To quantify this, logistic regression models were fitted with PE < 32 and < 37 weeks as outcomes and the logit risk as a predictor. First, we estimated the intercept from a logistic regression of incidence on the logit of risk with the slope fixed at 1. If there is a general tendency for underestimation, such that the observed incidence is larger than that predicted, the intercept will be positive. Conversely, for overestimation, the intercept will be negative. Secondly, we refitted the model for the slope to assess calibration across the range of risks. If the risk is well calibrated, then the slope should be 1.0.

Distribution of biomarkers

The measured values of biomarkers were converted to multiples of the medians (MoMs) to remove the effects of characteristics such as gestational age, weight, race, method of conception, medical conditions, elements from obstetric history associated with the individual being measured and characteristics associated with the instrument used for the measurement[4]. In the PE group, the mean $\log_{10}$ MoM was assumed to depend linearly on gestational age at delivery and this linear relationship was assumed to continue until a mean $\log_{10}$ MoM of zero, beyond which the mean was fixed at zero. Multivariate Gaussian distributions were fitted to the $\log_{10}$ MoM values of the biomarkers and a common covariance matrix was assumed for these distributions. Analysis of residuals was used to check the adequacy of the model and assess the effects of maternal factors on $\log_{10}$-transformed MoM values in pregnancies with PE. The regression lines of mean $\log_{10}$ MoMs according to gestational age at delivery with PE in twin pregnancies were compared to those in singleton pregnancies from our previous publication[3].

Performance of screening

The competing-risks model was used to estimate the individual patient-specific risks of delivery with PE at < 32 and < 37 weeks' gestation by a combination of maternal demographic characteristics and medical history with biomarkers[7]. The posterior distribution of gestational age at delivery with PE was obtained using Bayes' theorem by multiplying the prior probability density from maternal factors by the likelihood function from biomarker MoM values. The areas under the receiver-operating-characteristics (ROC) curves and detection rates (DRs) of delivery with PE, at a 10% false-positive rate (FPR), were assessed for various combinations of MAP, UtA-PI, serum PlGF and serum PAPP-A with maternal factors.

The statistical software package R was used for data analyses[18].

RESULTS

Study population

Maternal and pregnancy characteristics of the datasets from the EVENTS trial[12] and the previous study[11] are summarized in Table 1. The population was divided into those that developed PE and those that remained normotensive; pregnancies that developed gestational hypertension were excluded from the analysis. In the PE group, compared to the non-PE group, there was higher median maternal age, weight and body mass index, longer interpregnancy interval, a higher incidence of chronic hypertension, diabetes mellitus, systemic lupus erythematosus or antiphospholipid syndrome, conception by *in-vitro* fertilization and nulliparous or parous women with a previous pregnancy affected by PE, and a lower incidence of cigarette smokers.

The study population of 3938 twin pregnancies included a total of 339 (8.6%) cases that developed PE, and in 253 (6.4%) cases there was delivery with PE at < 37 weeks' gestation. In a previous study of 61 174 singleton pregnancies, we reported that 1770 (2.9%) developed PE and 493 (0.8%) delivered with PE at < 37 weeks[4]. Therefore, in twin pregnancies, compared to singleton pregnancies, the overall incidence of PE was about 3-times higher, but the incidence of delivery with PE at < 37 weeks was 8-times higher.

In the first dataset, from the EVENTS trial[12], data on maternal characteristics, MAP, UtA-PI and PAPP-A were available for 1798 pregnancies but serum PlGF was measured in only 1319 of the cases. In the second dataset[11], data on maternal characteristics were available for 2140 pregnancies, but measurements of biomarkers were carried out for only some of the patients (UtA-PI, $n = 1704$; MAP, $n = 1227$; PlGF, $n = 1316$; PAPP-A, $n = 1926$).

Validation of prior model based on maternal characteristics and medical history

Calibration plots of the predictive performance of the competing-risks model based on maternal characteristics and medical history for delivery with PE at < 32 and < 37 weeks' gestation are shown in Figure 1. The calibration slope and intercept for PE < 32 weeks were 0.827 (95% CI, 0.313 to 1.341) and 0.009 (95% CI, −0.503 to 0.522), respectively; the calibration slope and intercept for PE < 37 weeks were 0.942 (95% CI, 0.654 to 1.230) and −0.207 (95% CI, −0.389 to −0.025), respectively. These results demonstrate good agreement between the predicted risk and observed incidence of PE.

Distribution of biomarkers

The mean $\log_{10}$ MoM values for first-trimester biomarkers in twin pregnancies that developed PE and the common standard deviations and correlations are shown in Table 2. The fitted regression relationships between gestational

Table 1 Maternal and pregnancy characteristics of twin pregnancies in study population of EVENTS trial[12] and in combined dataset of EVENTS trial and previous study[11], according to development of pre-eclampsia (PE)

	Dataset from EVENTS trial (n = *1798*)			*Combined dataset* (n = *3938*)		
Characteristic	*Normal* (n = *1630*)	*PE* (n = *168*)	P	*Normal* (n = *3599*)	*PE* (n = *339*)	P
Age (years)	33.9 (30.2–37.3)	34.8 (30.3–38.5)	0.256	33.3 (29.3–36.7)	34.3 (30.1–37.8)	0.001
Weight (kg)	67.0 (59.4–78.0)	68.4 (59.0–81.9)	0.202	67.1 (60.0–78.0)	70.1 (61.1–82.25)	0.007
Height (cm)	165 (161–170)	163 (159–168)	0.002	165 (161–170)	164 (160–168)	0.006
Body mass index (kg/m^2)	24.5 (21.9–28.1)	25.8 (22.5–30.6)	0.005	24.6 (22.1–28.4)	26.0 (22.8–30.2)	0.00003
Gestational age (days)	91.7 (88.6–94.9)	92.3 (88.9–94.7)	0.410	91.0 (88.2–93.8)	91.0 (88.2–94.1)	0.996
Racial origin			0.637			0.172
White	1320 (81.0)	140 (83.3)		2848 (79.1)	264 (77.9)	
Black	193 (11.8)	20 (11.9)		489 (13.6)	57 (16.8)	
South Asian	76 (4.7)	5 (3.0)		150 (4.2)	11 (3.2)	
East Asian	13 (0.8)	2 (1.2)		42 (1.2)	5 (1.5)	
Mixed	28 (1.7)	1 (0.6)		70 (1.9)	2 (0.6)	
Medical history						
Chronic hypertension	20 (1.2)	4 (2.4)	0.375	37 (1.0)	17 (5.0)	< 0.00001
Diabetes mellitus Type 1	5 (0.3)	3 (1.8)	0.009	16 (0.4)	5 (1.5)	0.004
Diabetes mellitus Type 2	7 (0.4)	2 (1.2)	0.009	13 (0.4)	4 (1.2)	0.004
SLE/APS	4 (0.2)	3 (1.8)	0.016	7 (0.2)	4 (1.2)	0.006
Smoker	107 (6.6)	8 (4.8)	0.457	297 (8.3)	16 (4.7)	0.028
Family history of PE	29 (1.8)	3 (1.8)	0.913	111 (3.1)	12 (3.5)	0.851
Method of conception			0.006			0.010
Natural	1051 (64.5)	88 (52.4)		2435 (67.7)	202 (59.6)	
In-vitro fertilization	475 (29.1)	68 (40.5)		1014 (28.2)	120 (35.4)	
Ovulation drugs	104 (6.4)	12 (7.1)		150 (4.2)	17 (5.0)	
Parity			< 0.00001			< 0.00001
Nulliparous	870 (53.4)	125 (74.4)		1893 (52.6)	239 (70.5)	
Parous with no previous PE	732 (44.9)	34 (20.2)		1628 (45.2)	79 (23.3)	
Parous with previous PE	28 (1.7)	9 (5.4)		78 (2.2)	21 (6.2)	
Interpregnancy interval (years)	2.9 (1.6–4.9)	3.1 (1.9–7.55)	0.284	3.0 (1.8–5.0)	3.9 (2.2–7.0)	0.004
Chorionicity			0.086			0.026
Dichorionic	1291 (79.2)	143 (85.1)		2870 (79.7)	288 (85.0)	
Monochorionic	339 (20.8)	25 (14.9)		729 (20.3)	51 (15.0)	

Data are given as median (interquartile range) or *n* (%). Comparisons between outcome groups were by chi-square or Fisher's exact test for categorical variables and Mann–Whitney *U*-test for continuous variables. APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus.

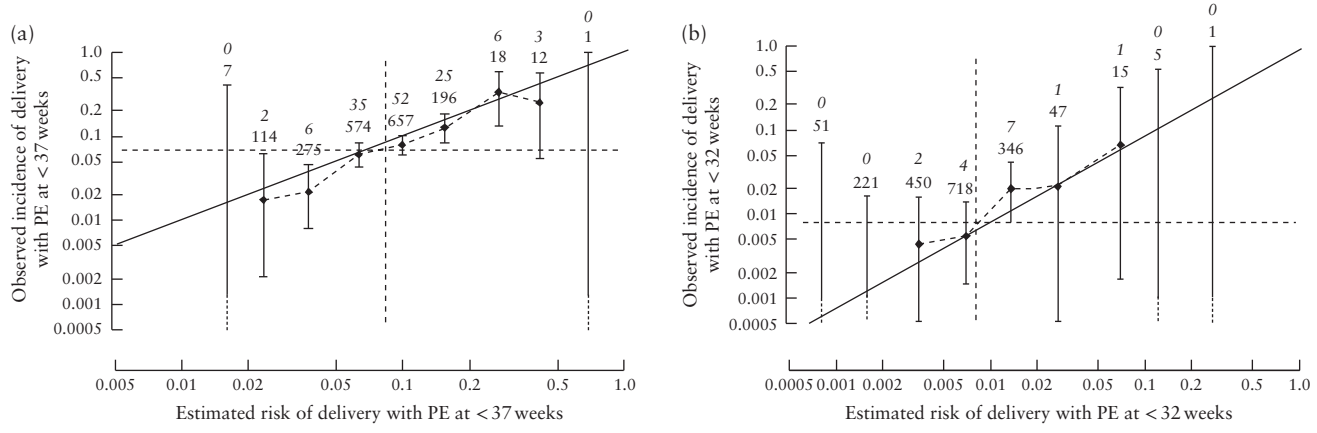

Figure 1 Calibration plots for delivery with pre-eclampsia (PE) at < 37 weeks' gestation (a) and < 32 weeks (b), in screening by combination of maternal factors, uterine artery pulsatility index, mean arterial pressure and serum placental growth factor in EVENTS trial dataset[12]. Observed incidence is given as median with 95% CI (vertical solid lines). Numbers in italics are number of women who developed PE, and numbers below are total number within each estimated-risk group. Diagonal line is line of perfect agreement. Overall mean risk is shown by vertical dashed line and overall incidence by horizontal dashed line. Slope and intercept were 0.942 (95% CI, 0.654 to 1.230) and −0.207 (95% CI, −0.389 to −0.025) for delivery with PE at < 37 weeks (a), and 0.827 (95% CI, 0.313 to 1.341) and 0.009 (95% CI, −0.503 to 0.522) for delivery with PE at < 32 weeks (b), respectively.

age at delivery with PE and biomarker MoMs in twin pregnancies are compared with those in singleton pregnancies from a previous study[3] in Figure 2. In both twin and singleton pregnancies, all markers showed greater separation at earlier than later gestations and this is reflected in their superior performance in detection of PE at < 32 weeks compared with PE at < 37 weeks (Table 3).

The slope of the regression lines for $\log_{10}$ MoM values of MAP, UtA-PI and PlGF in twin pregnancies was steeper than in singleton pregnancies (Figure 2) and this is reflected in the gestational age at which these regression lines intersect the line for 1 MoM (Table 2). Thus, for regression lines for $\log_{10}$ MAP MoM, the gestational age at intersection of the 1 MoM line was 41.7 weeks for twin pregnancies and 53.3 weeks for singleton pregnancies; the respective values for $\log_{10}$ UtA-PI MoM were 34.0 and 41.2 weeks and for $\log_{10}$ PlGF MoM they were 38.3 and 42.8 weeks. For PAPP-A, there was minimal separation of the regression lines for $\log_{10}$ MoM values in PE compared to unaffected twin pregnancies.

Table 2 Fitted regression models for first-trimester biomarker $\log_{10}$ multiples of the median (MoM) values on mean gestational age at delivery with pre-eclampsia in twin pregnancy, along with standard deviations and correlations of first-trimester biomarker $\log_{10}$ MoM values in twin pregnancies

Parameter	*Value in twins (95% CI)*	*Value in singletons**
MAP		
Intercept	0.1262 (0.0631 to 0.1905)	0.0890
Slope	−0.0030 (−0.0048 to −0.0012)	−0.0017
Intersection of 1 MoM	41.7 (39.1 to 50.5)	53.3
UtA-PI		
Intercept	1.2121 (0.4074 to 1.7028)	0.5861
Slope	−0.0357 (−0.0516 to −0.0104)	−0.0142
Intersection of 1 MoM	34.0 (33.1 to 39.3)	41.2
PlGF		
Intercept	−1.3613 (−2.1374 to −0.8231)	−0.9235
Slope	0.0355 (0.0204 to 0.0604)	0.0216
Intersection of 1 MoM	38.3 (35.5 to 40.8)	42.8
PAPP-A		
Intercept	−0.1710 (−0.5417 to −0.0651)	−0.5927
Slope	0.0034 (0.0009 to 0.0136)	0.0138
Intersection of 1 MoM	50.0 (38.9 to 75.0)	42.8
Standard deviation		
MAP	0.0358 (0.0348 to 0.0369)	—
UtA-PI	0.1311 (0.1275 to 0.1349)	—
PlGF	0.2158 (0.2099 to 0.2220)	—
PAPP-A	0.2089 (0.2032 to 0.2149)	—
Correlations		
MAP and UtA-PI	−0.0386 (−0.078 to 0.0009)	—
MAP and PlGF	−0.0432 (−0.0826 to −0.0037)	—
MAP and PAPP-A	−0.0270 (−0.0665 to 0.0125)	—
UtA-PI and PlGF	−0.1580 (−0.1963 to −0.1192)	—
UtA-PI and PAPP-A	−0.1172 (−0.1560 to −0.0780)	—
PlGF and PAPP-A	0.2345 (0.1968 to 0.2715)	—

*Values for singletons reported in previous study[3]. MAP, mean arterial pressure; PAPP-A, pregnancy-associated plasma protein-A; PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

Performance of screening for pre-eclampsia

Detection rates, at a 10% FPR, and AUCs in screening for PE by maternal factors and biomarkers are given in Table 3; Figure 3 shows the corresponding ROC curves. Serum PAPP-A did not provide any useful prediction of PE. The best performance of screening for PE was achieved by a combination of maternal factors, MAP, UtA-PI and PlGF. In screening by maternal factors alone, the DR, at a 10% FPR, was 30.6% for delivery with PE at < 32 weeks' gestation and this increased to 86.4% when screening by the combined test; the respective values for PE at < 37 weeks were 24.9% and 41.1%. This performance of screening was achieved at a risk cut-off of 1 in 6 for PE at < 37 weeks.

In a previous study of 61 174 singleton pregnancies undergoing first-trimester screening by maternal factors, MAP, UtA-PI and PlGF, the risk cut-off for PE at < 37 weeks that would result in a FPR of 10% was 1 in 70, and, at this cut-off, the DRs of delivery with PE at < 32 and < 37 weeks were 90% and 75%, respectively[4]. Screening in twin pregnancies with the same risk cut-off of 1 in 70 as in singletons would detect all cases of PE < 37 weeks, but at a FPR of 94% (Figure 3). Alternatively, in twin pregnancies, a DR of 75% for delivery with PE at < 37 weeks can be achieved at a risk cut-off of 1 in 15 and a FPR of 40%.

DISCUSSION

Main findings of study

There are five main findings of this study. First, the overall incidence of PE in twin pregnancies was about 3-times higher than in singleton pregnancies and the incidence of preterm PE was 8-times higher; this finding confirms our original suggestion that comparison of the overall rates of PE between twin and singleton pregnancies underestimates the relative risk of preterm PE in twins, because they are delivered at an earlier gestational age than singletons[1]. Second, in the validation of the prior model based on maternal characteristics

and medical history, calibration plots demonstrated very good agreement between the predicted risks and observed incidence of PE; this finding provides support for the model in which the effect of twins in shifting the distribution of gestational age at delivery with PE in singletons to the left is greater if the prior mean is high and less if the mean is low[10]. Third, the slope of the regression lines for $\log_{10}$ MoM values of MAP, UtA-PI and PlGF in twin pregnancies that developed PE was steeper than in singleton pregnancies, indicating greater deviation from normal for early gestations and lesser deviation for later gestations; this finding is consistent

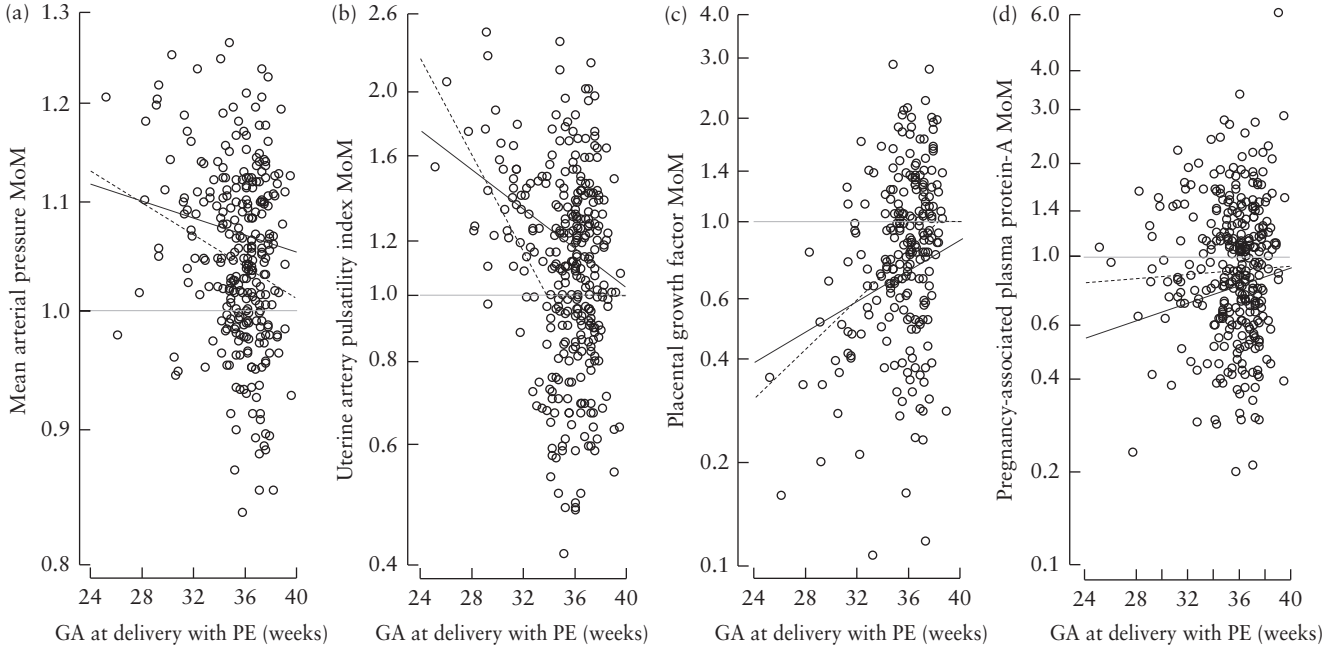

Figure 2 Scatter diagrams and regression lines (----) for relationship between mean arterial pressure (a), uterine artery pulsatility index (b), serum placental growth factor (c) and serum pregnancy-associated plasma protein-A (d) multiples of the median (MoM) and gestational age (GA) at delivery with pre-eclampsia (PE) in twin pregnancies. Solid lines are regression lines for singleton pregnancies from previous publication[3].

Table 3 Performance of screening for delivery with pre-eclampsia at < 37 and < 32 weeks' gestation, at 10% false-positive rate, by maternal factors (MF) combined with mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI), serum placental growth factor (PlGF) and pregnancy-associated plasma protein-A (PAPP-A) in twin pregnancy

Method of screening	*Study population* (n)	*AUC (95% CI)*	*Cases detected* (n/N)	*Detection rate (95% CI) (%)*
Pre-eclampsia < 37 weeks				
MF	3938	0.742 (0.710–0.773)	63/253	24.9 (19.7–30.7)
MF + MAP	3025	0.742 (0.710–0.773)	70/209	33.5 (27.1–40.3)
MF + UtA-PI	3502	0.689 (0.655–0.723)	71/238	29.8 (24.1–36.1)
MF + PlGF	2635	0.744 (0.708–0.780)	58/175	33.1 (26.2–40.6)
MF + PAPP-A	3724	0.694 (0.661–0.727)	65/241	27.0 (21.5–33.0)
MF + MAP + UtA-PI	3001	0.747 (0.715–0.779)	75/208	36.1 (29.5–43.0)
MF + MAP + PlGF	2398	0.773 (0.739–0.808)	66/164	40.2 (32.7–48.2)
MF + UtA-PI + PlGF	2584	0.748 (0.712–0.784)	59/173	34.1 (27.1–41.7)
MF + MAP + UtA-PI + PlGF	2383	0.776 (0.741–0.811)	67/163	41.1 (33.5–49.1)
MF + MAP + UtA-PI + PlGF + PAPP-A	2383	0.776 (0.741–0.811)	67/163	41.1 (33.5–49.1)
Pre-eclampsia < 32 weeks				
MF	3938	0.702 (0.622–0.782)	11/36	30.6 (16.4–48.1)
MF + MAP	3025	0.838 (0.778–0.897)	17/28	60.7 (40.6–78.5)
MF + UtA-PI	3502	0.847 (0.791–0.904)	18/33	54.5 (36.4–71.9)
MF + PlGF	2635	0.888 (0.830–0.946)	15/23	65.2 (42.7–83.6)
MF + PAPP-A	3724	0.728 (0.652–0.805)	8/33	24.2 (11.1–42.3)
MF + MAP + UtA-PI	3001	0.915 (0.879–0.950)	21/28	75.0 (55.1–89.3)
MF + MAP + PlGF	2398	0.932 (0.902–0.962)	18/22	81.8 (59.7–94.8)
MF + UtA-PI + PlGF	2584	0.915 (0.865–0.966)	18/23	78.3 (56.3–92.5)
MF + MAP + UtA-PI + PlGF	2383	0.950 (0.924–0.976)	19/22	86.4 (65.1–97.1)
MF + MAP + UtA-PI + PlGF + PAPP-A	2383	0.953 (0.930–0.976)	19/22	86.4 (65.1–97.1)

AUC, area under the receiver-operating-characteristics curve.

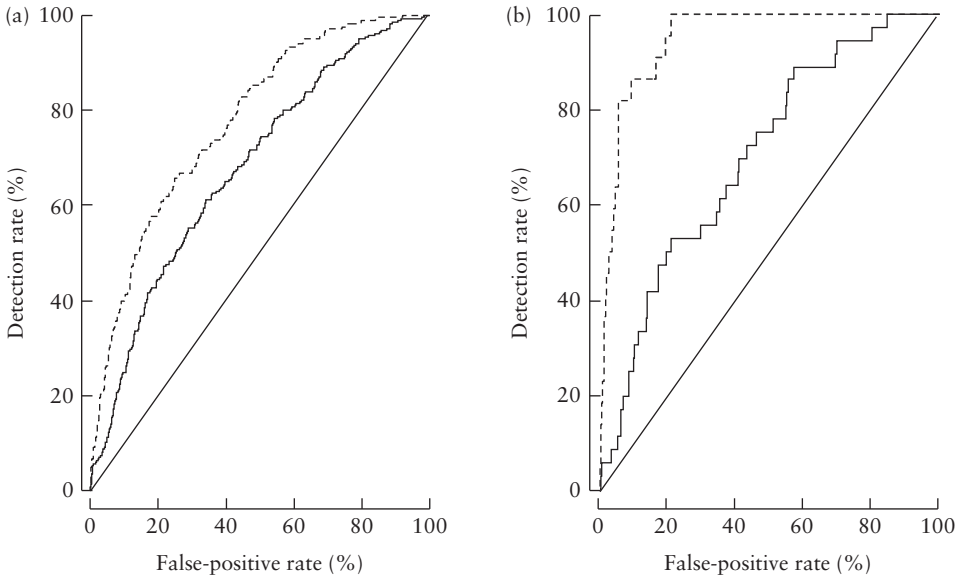

Figure 3 Receiver-operating-characteristics curves for prediction of delivery with pre-eclampsia at < 37 weeks' gestation (a) and < 32 weeks (b) in twin pregnancies by maternal factors (——) and combination of maternal factors, mean arterial pressure, uterine artery pulsatility index and serum placental growth factor (- - - -).

with the fact that twin pregnancies deliver earlier and have a much higher incidence of preterm PE than singletons. Fourth, the best performance of first-trimester screening for PE in twin pregnancy is achieved by a combination of maternal characteristics and medical history, MAP, UtA-PI and PlGF (the triple test) and there is no additional contribution from PAPP-A; this is also the case for first-trimester screening in singleton pregnancy[3,4]. Fifth, in singleton pregnancies, the DR of delivery with PE at < 37 weeks' gestation in first-trimester screening by the triple test is 75% at a 10% FPR[4] and in twin pregnancies the same DR of 75% can be achieved at a FPR of 40%.

Clinical implications

In singleton pregnancy, screening for PE by the triple test at 11–13 weeks' gestation is beneficial because such screening identifies a group with the highest risk of PE, comprising 10% of the total, which contains about 90% of women who will subsequently develop PE at < 32 weeks and 75% of those who will develop PE at < 37 weeks; treatment of this high-risk group with aspirin reduces the respective risks by about 90% and 60%[3,4,19,20]. There is no such clear evidence of benefit in the case of twin pregnancy[21] and we are therefore undertaking a major randomized trial to investigate the possible effect of aspirin in the prevention of preterm PE in twin pregnancy.

In population screening for conditions such as fetal trisomy 21, the same risk cut-off is used to define the high-risk group in both singleton and twin pregnancies. Should this approach of using the same risk cut-off in both singleton and twin pregnancies be adopted when screening for PE, then about 10% of singletons and almost all twins would be classified as screen-positive. Alternatively, if the objective of screening is defined by a desired DR in both singleton and twin pregnancies, the risk cut-off can be set in such a way as to achieve this DR; for example, if the desired DR of preterm PE is 75%, then the risk cut-off and consequent FPR in singleton pregnancies would be about 1 in 70 and 10%, respectively, and the corresponding values in twin pregnancies would be 1 in 15 and 40%.

Strengths and limitations

The strengths of this first-trimester multicenter screening study for PE are, first, examination of a large population of twin pregnancies attending for routine care in a gestational-age range which is used widely for assessment of risk for chromosomal abnormalities, second, recording of data on maternal characteristics and medical history to identify known risk factors associated with PE, third, use of a specific methodology and appropriately trained doctors to measure UtA-PI and MAP and use of automated machines to provide accurate measurement of maternal serum concentration of PlGF and PAPP-A, fourth, expression of the values of the biomarkers as MoMs after adjustment for factors that affect the measurements, and, fifth, use of Bayes' theorem to combine the prior risk from maternal characteristics and medical history with biomarkers to estimate patient-specific risks and the performance of screening for PE with delivery at different stages of pregnancy. A limitation of the study is that the number of twin pregnancies, compared with our population of singleton pregnancies, was relatively small and the model may require further adjustments based on results of future large multicenter studies.

Conclusions

In the assessment of risk for PE in twin pregnancy, we can use the same prior model based on maternal characteristics

and medical history as reported previously[10], but in the calculation of posterior risks it is necessary to use the new distributions of $\log_{10}$ MoM values of UtA-PI, MAP and PlGF according to gestational age at delivery with PE.

ACKNOWLEDGMENTS

This study was supported by grants from The Fetal Medicine Foundation (UK Charity No: 1037116) and Instituto Carlos III, Spain (grant PI18/01027). Reagents and equipment for the measurement of serum PlGF were provided free of charge by Roche Diagnostics, PerkinElmer Life and Analytical Sciences and Thermo Fisher Scientific. These bodies had no involvement in the study design, in the collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the article for publication.

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