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Prediction of early and late pre-eclampsia from maternal characteristics, uterine artery Doppler and markers of vasculogenesis during first trimester of pregnancy

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KEYWORDS: biochemical markers; first-trimester screening; placental growth factor; pre-eclampsia; uterine artery Doppler

ABSTRACT

Objective *To develop a predictive model for pre-eclampsia using clinical, biochemical and ultrasound markers during the first trimester of pregnancy.*

Methods *This was a nested case–control study within a pre-eclampsia screening project that involved 5367 asymptomatic pregnant women undergoing routine transvaginal uterine artery (UtA) Doppler at 11 + 0 to 13 + 6 weeks. Following exclusions, there were 70 pregnant women who later developed pre-eclampsia and 289 control patients enrolled during the first trimester who had serum or plasma samples taken at enrolment available for the purposes of this study. Of these, 17 pregnancies were diagnosed with early-onset (delivery < 34 weeks) pre-eclampsia and 53 with late-onset (delivery ≥ 34 weeks) pre-eclampsia. The lowest, highest and mean of left and right UtA pulsatility indices (PI) were calculated. Blood samples were stored at −84 °C until biochemical analysis for markers of vasculogenesis was performed. The distributions of the lowest UtA-PI and the biochemical markers were adjusted for maternal characteristics, expressed as multiples of the median (MoM), and compared between groups. Logistic regression analysis was used to evaluate if any variable was significantly associated with pre-eclampsia.*

Results *Pregnancies that later developed pre-eclampsia were associated with higher maternal prepregnancy body mass index. An increased lowest UtA-PI was significantly associated with both early- and late-onset disease. Placental growth factor (PlGF) MoM was significantly reduced in women who later developed early- or late-onset pre-eclampsia compared with controls (median (interquartile range), 0.69 (0.33–1.46) and 1.10 (0.39–1.56), respectively,* vs *1.19 (0.65–1.84),* $P < 0.05$*). Different combined models were generated by logistic regression analysis, and the detection rate with a fixed 10% false-positive rate was 47% and 29% for early- and late-onset pre-eclampsia, respectively.*

Conclusion *Pregnancies that later developed early or late pre-eclampsia were characterized by impaired placentation and an anti-angiogenic state during the first trimester of pregnancy. Regression models which include maternal characteristics, UtA Doppler and PlGF can apparently predict approximately half of pregnancies that will be complicated by early-onset pre-eclampsia. We believe more research in several areas is needed to aid in the creation of a better and more population-specific screening test for pre-eclampsia during the first trimester of pregnancy.* Copyright © 2012 ISUOG. Published by John Wiley & Sons Ltd.

INTRODUCTION

Pre-eclampsia is a systemic disorder associated with high maternal and neonatal morbidity and mortality[1–3]. Its diagnosis is based on clinical features, such as high blood pressure and proteinuria, which are the terminal events of a cascade of phenomena that are likely initiated during placental formation and development in the late first trimester of gestation[3]. Increasingly, early-onset pre-eclampsia is considered to be a more severe form of the disease than is the late-onset condition[4]. Studies have reported that the frequency of placental lesions observed histologically is correlated negatively with gestational viability[5] and that the early-onset condition is associated with an even greater risk of maternal and perinatal mortality and morbidity[6–8].

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Many groups have focused on methods to predict pre-eclampsia in order to identify accurately pregnant women who are at risk[9]. Maternal uterine artery (UtA) Doppler at 22–25 weeks of gestation has become the most reliable tool for prediction of pre-eclampsia; this diagnostic test has the ability to predict nearly 50% of instances of any form of the disease and approximately 85% of cases of severe or early-onset disease[10,11]. However, UtA Doppler performed in the final weeks of the first trimester of gestation (11 + 0 to 13 + 6 weeks) varies much more in its predictive ability for early-onset pre-eclampsia than it does at the second-trimester scan[12–14].

There are also new data concerning the role of biochemical markers in predicting pre-eclampsia, especially the early-onset form, during the first trimester of pregnancy[4,15,16]. Although, low-risk first-trimester pregnancies that later develop pre-eclampsia have been characterized by altered angiogenic factors, including increased soluble VEGF (vascular endothelial growth factor) receptor 1 (soluble human fms-like tyrosine kinase 1) (sFlt-1) and soluble endoglin (sEng, a receptor for members of the TGFβ (transforming growth factor beta) superfamily), and reduced placental growth factor (PlGF), there are still some discrepancies regarding both the most appropriate cut-off values of these markers for pre-eclampsia screening purposes in early pregnancy and the influence of different population characteristics on their performance[17].

The aim of this study was to determine in a Chilean population the usefulness as a screening method for early- and late-onset pre-eclampsia of a combination of a series of measurements, including maternal characteristics, biochemical markers of vasculogenesis and UtA Doppler measured at 11 + 0 to 13 + 6 weeks of gestation.

METHODS

This was a nested case–control study within a Chilean-funded project for pre-eclampsia screening. The study involved 5367 asymptomatic pregnant women who underwent a routine scan, including UtA Doppler evaluation, at 11 + 0 to 13 + 6 weeks of gestation between April 2002 and July 2010. Written informed consent was obtained from patients agreeing to take part in the study, which was approved by the institution's ethics committee, and blood samples were drawn from 2955 women. To select the subject and control groups for UtA Doppler and biochemical studies, we excluded 34 pregnancies that ended before 22 weeks' gestation, 47 that were associated with pregnancy-induced hypertension, 52 that were complicated by major fetal abnormalities, 152 that were small-for-gestational age (SGA), 16 that underwent intrauterine death or placental abruption and 35 in mothers with various chronic diseases, leaving 2619 women. Among these pregnancies, 2536 remained normotensive throughout pregnancy, while 83 women went on to develop pre-eclampsia. Among these, only 70 women had frozen serum or plasma samples available for this study. To select a control group, we reviewed first-trimester scans and blood samples of all 2536 eligible patients. For each woman who eventually developed pre-eclampsia, four controls were selected retrospectively, being the next four successive pregnancies with first-trimester ultrasound results and blood samples who did not develop pre-eclampsia. This method provided 289 control women for biochemical and UtA Doppler assessment (Figure 1).

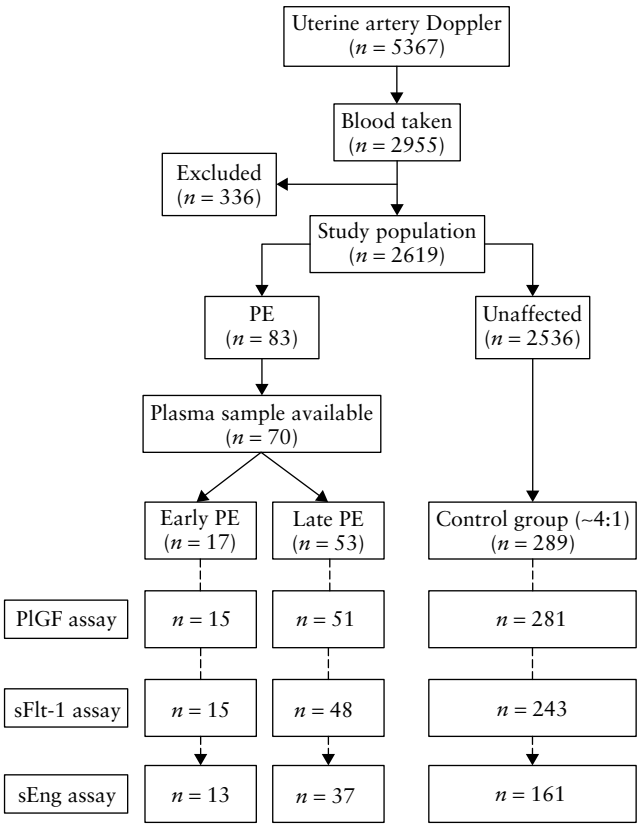

Figure 1 Flow diagram showing study design and analyses. PE, pre-eclampsia; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine 1; sEng, soluble endoglin.

All women were interviewed immediately before the ultrasound examination to obtain demographic data on maternal age, smoking habit during pregnancy, alcohol intake during pregnancy, drug abuse, medical history, medication, parity, obstetric history and body mass index (BMI) calculated from maternal weight and height before pregnancy; these data were input into the computer database.

Transvaginal UtA Doppler was performed at 11 + 0 to 13 + 6 weeks as described in previous publications[12,18]. Briefly, measurements were performed using an Aloka 4000 (Aloka, Tokyo, Japan) ultrasound machine equipped with a 5-MHz probe. Women were placed in the lithotomy position, and the transducer was inserted into the vagina and placed in the anterior fornix. At the time of cervical length measurement, the probe was gently tilted laterally at the level of the internal cervical os, and the UtAs were identified using color Doppler. After ensuring an angle of insonation < 30°, pulsed wave

Doppler was used to obtain three consecutive waveforms. Both UtA pulsatility indices (PI) were recorded, and the presence or absence of early diastolic notches was noted. All operators in our fetal medicine unit have The Fetal Medicine Foundation (FMF) certification to perform the 11 + 0 to 13 + 6-week scan, including UtA Doppler measurement.

Blood samples were collected in plastic tubes containing EDTA (ethylenediaminetetraacetic acid) at the time of the routine first-trimester ultrasound exam for measurement of vasculogenesis parameters. After centrifugation, the plasma was stored in a freezer at −84 °C. Determination of circulating soluble human fms-like tyrosine kinase 1 (sFlt-1), human PlGF and human sEng was performed using commercial enzyme-linked immunosorbent assays (ELISA, R&D Systems, Milford, IA, USA)[19].

Outcome measures

Control pregnancies were defined as those in women with normal blood pressure (≤ 140/90 mmHg), no proteinuria and no medical complications. Pre-eclampsia was defined as maternal blood pressure ≥ 140/90 mmHg with proteinuria > 300 mg/24 h and resolution of hypertension and proteinuria following delivery[20]. Pre-eclampsia was subclassified based on gestational age at delivery as either early-onset (delivery < 34 weeks gestation) or late-onset (delivery ≥ 34 weeks) pre-eclampsia. SGA was defined as birth weight < 10th percentile for our population[21].

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normality of continuous data. First, as previously described by The FMF[22], biochemical markers (sFlt-1, PlGF, sEng) and UtA Doppler (lowest, highest and mean of left and right PIs) measurements were log transformed and converted into multiples of the expected normal median (MoM) of the control group. Secondly, multiple linear regression was used to determine which maternal and pregnancy characteristics were significant predictors of each of the log-transformed biochemical markers and Doppler indices in the unaffected group. The resulting equations were used to derive the MoM values used in the assessment of screening performance Finally, the performance of screening was assessed by receiver–operating characteristics (ROC) curves.

Comparisons between groups were performed using the Kruskal–Wallis test and, in the case of significant differences between groups, we used the Mann–Whitney *U*-test to compare the pre-eclamptic groups and controls. Categorical variables were compared using the χ^2 test. A difference was considered statistically significant when $P < 0.05$.

RESULTS

Patient characteristics

The pre-eclamptic group (early plus late pre-eclamptic groups) was characterized by higher maternal BMI, earlier delivery, lower birth weight and birth-weight percentile and higher Cesarean section rate compared with the control group. Moreover, there were also significant differences between the two pre-eclamptic groups in birth-weight percentile, gestational age at delivery and Cesarean section rate (Table 1). In addition, the percentage of obese women in the early- (17.6%) and late-onset (20.8%) pre-eclamptic groups was significantly higher compared to that in the control group (10.7%).

Assessment in the control group

Multiple regression analyses in the control group revealed nulliparity to be a significant and independent predictor for sFlt-1 plasma level, crown–rump length (CRL) to be a predictor for PlGF and BMI to be a predictor for sEng. With regards to UtA Doppler, the significant predictors for the lowest UtA-PI in all unaffected patients were maternal age and CRL. These results were used to derive the MoM values of each screening marker in each woman adjusted for maternal and pregnancy characteristics.

Table 1 Baseline characteristics in groups who later developed early- or late-onset pre-eclampsia (PE) and in controls

Characteristic	*Controls (n = 289)*	*Early PE (n = 17)*	*Late PE (n = 53)*
Maternal age (years)	29.1 ± 0.4	29.7 ± 1.8	30.4 ± 0.8
Smoker	18 (6.2)	3 (17.6)	2 (3.8)
Prepregnancy BMI (kg/m^2)	23.9 (22.0–26.9)	26.7 (22.6–29.7)	26.4 (24.4–29.8)*
Nulliparous	136 (47.1)	8 (47.1)	28 (52.8)
CRL at 11–13-week scan (mm)	66.0 (57.0–74.0)	64.0 (51.0–79.5)	65.0 (59.0–72.0)
GA at delivery (weeks)	39.0 (38.1–39.9)	30.5 (28.5–31.8)*†	37.6 (36.5–38.9)*
Birth weight (g)	3419.2 ± 28.9	1167.0 ± 98.5*†	3055.2 ± 77.1*
Delivery at < 37 weeks	22 (7.6)	17 (100)*†	17 (32.1)*
Small-for-gestational age (<10th centile)	—	10 (58.8)*†	11 (20.8)*
Birth weight percentile	54.6 (31.5–76.0)	8.1 (5.3–21.0)*†	37.2 (16.7–71.8)*
Cesarean section	142 (49.1)	16 (94.1)*†	35 (66.0)*

Data are presented as mean ± SEM, *n* (%) or median (interquartile range). *$P < 0.05$ comparing controls with each PE group. †$P < 0.05$ comparing early-PE with late-PE groups. CRL, crown–rump length; GA, gestational age.

Table 2 Biochemical markers of vasculogenesis and lowest uterine artery (UtA) pulsatility index (PI) multiples of the median (MoM) at 11 + 0 to 13 + 6 weeks of gestation in women who later developed early-onset or late-onset pre-eclampsia (PE) and in controls

	Controls (n = 289)	*Early PE (n = 17)*	*Late PE (n = 53)*
UtA-PI MoM	1.02 (0.83–1.31)	1.39* (1.14–1.76)	1.14* (0.97–1.43)
PlGF MoM	1.19 (0.65–1.84)	0.69* (0.33–1.46)	1.10* (0.39–1.56)
sFlt-1 MoM	1.25 (0.62–1.80)	0.88 (0.45–1.52)	0.98 (0.65–1.66)
sEng MoM	0.91 (0.80–1.80)	0.98 (0.76–1.16)	1.04 (0.87–1.67)

See Figure 1 for number of patients in each group and assessment data. Data are presented as median ± interquartile range. *$P < 0.05$ compared with controls. PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine 1; sEng, soluble endoglin.

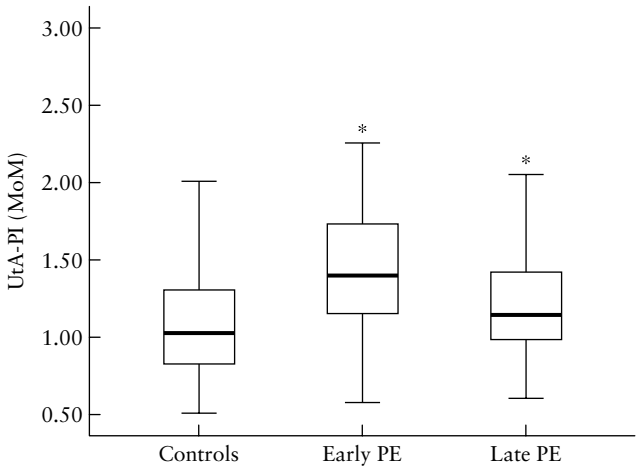

Figure 2 Box-and-whisker plot (median, interquartile range and range) of lowest uterine artery pulsatility index (UtA-PI) multiples of the median (MoM) at 11 + 0 to 13 + 6 weeks of gestation in women who subsequently developed early-onset ($n = 17$) or late-onset ($n = 53$) pre-eclampsia and in controls ($n = 289$). *$P < 0.05$ *vs* controls.

Uterine artery Doppler and biochemical markers in pre-eclamptic groups

We found that the lowest UtA-PI, expressed as MoM of the unaffected control group, was significantly higher than controls in both early-onset and late-onset pre-eclamptic groups (Table 2 and Figure 2). Moreover, PlGF levels, also expressed as MoM of the control group, were significantly lower in the early- and late-onset pre-eclamptic groups compared with the controls (Table 2, Figure 3). In contrast, there were no significant differences between the pre-eclamptic groups and controls in the other antiangiogenic factors, sFlt-1 MoM and sEng MoM (Table 2).

Assessment of predictors of early- and late-onset pre-eclampsia

The logistic regression analysis demonstrated that significant contributions for the detection of early-onset and late-onset pre-eclampsia were made by two maternal characteristics, PlGF and lowest UtA-PI:

$$\text{Early pre-eclampsia} = -6.942 + (0.074 \times \text{BMI}) + (1.878 \text{ if smoker}) + (2.1116 \times \log \text{lowest UtA-PI MoM}) - (0.671 \times \log \text{PlGF MoM}),\ r = 0.45,\ P < 0.001;$$

$$\text{Late pre-eclampsia} = -5.584 + (0.137 \times \text{BMI}) + (0.822 \times \log \text{lowest UtA-PI MoM}) - (0.533 \times \log \text{PlGF MoM}),\ r = 0.38,\ P < 0.001.$$

The performance of screening was assessed by ROC curves, and the detection rate (DR) for early-onset and late-onset pre-eclampsia for different false-positive rates (FPR) of screening, based on maternal factors, UtA Doppler, PlGF or a combined assessment, are shown in Table 3 and Figure 4. The numbers of cases that were used to build the regression models for early and late pre-eclampsia is documented in Table 4.

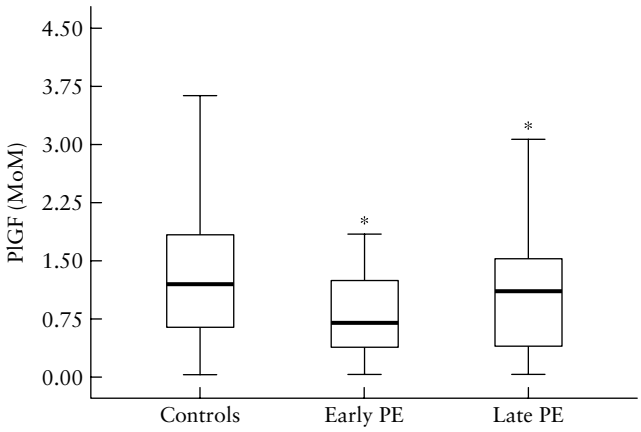

Figure 3 Box-and-whisker plot (median, interquartile range and range) of placental growth factor (PlGF) multiples of the median (MoM) at 11 + 0 to 13 + 6 weeks of gestation in women who subsequently developed early-onset ($n = 17$) or late-onset ($n = 53$) pre-eclampsia and in controls ($n = 289$). *$P < 0.05$ *vs* controls.

Table 3 Detection rates of early-onset ($n = 17$) and late-onset ($n = 53$) pre-eclampsia (PE) at 11 + 0 to 13 + 6 weeks of gestation in women with blood samples available, using different models with fixed false-positive rates (FPR)

	Detection rate (%)			
	Early PE		*Late PE*	
Model	*5% FPR*	*10% FPR*	*5% FPR*	*10% FPR*
History alone	11.8	29.4	17.0	20.8
History + UtA-PI	37.5	43.8	20.8	28.3
History + UtA-PI + PlGF	33.3	46.7	19.6	29.4

History included smoking and body mass index (BMI) for early-PE group and BMI for late-PE group. PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

DISCUSSION

This study confirms previous reports that women who later develop pre-eclampsia have increased vascular

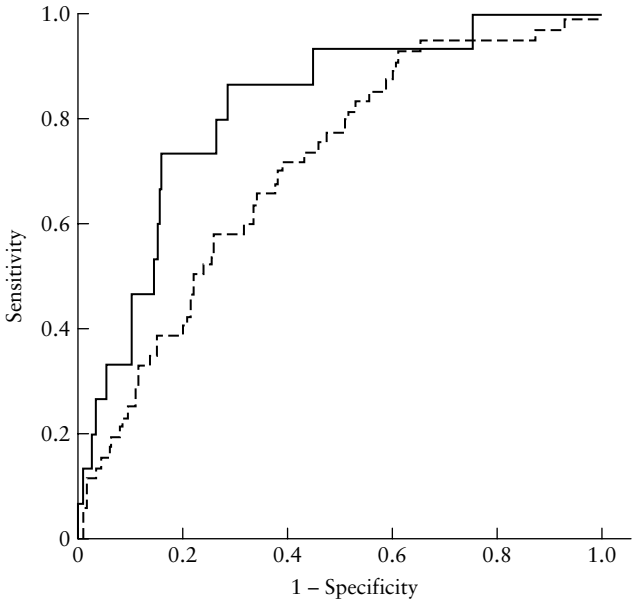

Figure 4 Receiver–operating characteristics curves for combined screening model for early-onset (——) and late-onset (– – –) pre-eclampsia at 11 + 0 to 13 + 6 weeks. Screening model for early-onset PE included maternal factors (smoking habits and body mass index (BMI)), uterine artery (UtA) Doppler and placental growth factor (PlGF); model for late-onset PE included maternal factors (BMI), UtA Doppler and PlGF.

Table 4 Numbers of patients who were included in each regression model for early and late pre-eclampsia (PE)

Group/Model	*PE*	*Controls*	*Total*
Early PE			
History alone	17	279	296
History + UtA-PI	16	281	297
History + UtA-PI + PlGF	15	273	288
Late PE			
History alone	53	284	337
History + UtA-PI	53	281	334
History + UtA-PI + PlGF	51	273	324

Data are presented as *n*. History included smoking and body mass index (BMI) for early-PE group and BMI for late-PE group. PlGF, placental growth factor; UtA-PI, uterine artery pulsatility index.

placental resistance that can be detected by UtA Doppler and reduced maternal plasma PlGF levels during the first half of pregnancy[13,23]. Another relevant finding of this study is that a combined model, taking into account clinical characteristics, UtA Doppler and biochemical markers during the first trimester of pregnancy significantly improved the prediction of early- and late-onset pre-eclampsia.

The finding in this study that higher UtA Doppler impedance at 11 + 0 to 13 + 6 weeks' gestation, reflecting incomplete physiological spiral artery remodeling, was observed in those women who subsequently developed pre-eclampsia is in agreement with previous publications showing altered UtA Doppler measurements during the second and, more recently, the first trimester of pregnancy[10,12,13,24,25]. However, the second-trimester UtA Doppler DR for early and late pre-eclampsia is substantially higher than is the first trimester DR, which might be explained by the physiological process of UtA vessel modeling that occurs during the first half of pregnancy[26]. Our data are also in agreement with the findings of Poon *et al.*[22], who stated that the lowest UtA-PI provided the best performance in screening for pre-eclampsia. This finding also agrees with the concept that pre-eclampsia is related to an effect of placental side (i.e. left or right placenta) on UtA remodeling, which is expected to be much more marked in placental vessels that are contiguous to the placental insertion site[27]. On the contrary, Napolitano *et al.*[28] did not find any difference between lower, mean and higher UtA-PIs and they considered that any variation between these values would be unlikely to have any impact on screening sensitivities.

There is a consensus that pre-eclampsia is characterized by an imbalance between angiogenic and anti-angiogenic factors, and this has been clearly demonstrated in the second trimester of pregnancy by several authors[10,17,23,29–32]. Although there was initially some disagreement regarding the influence of first-trimester pro- or anti-angiogenic markers in women who eventually developed pre-eclampsia[23,33], there have been recent publications that clearly support the role of PlGF in the pathogenesis of pre-eclampsia and as a biochemical marker for pre-eclampsia screening, especially for early-onset cases[34,35]. However, the other angiogenic markers, sEng and sFlt-1, do not appear to play an important role in the early detection of high-risk pregnant women[34,36,37].

As explained above, we, like others, are interested mainly in screening for severe or early-onset pre-eclampsia. Our study, although in disagreement with recent publications[4,14,16], showed that using a combined model, including clinical, biochemical and biophysical characteristics, can detect approximately half of the women who will eventually develop early-onset pre-eclampsia, for only a 10% FPR.

The FMF group has already published several papers evaluating different biochemical markers for the prediction of pre-eclampsia, especially for early-onset disease. A recent prospective study by Akolekar *et al.*[37] evaluated singleton pregnancies that had undergone a screening examination at 11 + 0 to 13 + 6 weeks' gestation, including 32 850 unaffected patients and 752 women who subsequently developed pre-eclampsia. They conducted an initial screening study, investigating maternal characteristics, medical history, mean UtA-PI, blood pressure and serum pregnancy-associated plasma protein-A (PAPP-A), and a case–control study, with individuals drawn from the screening study population on the basis of availability of stored serum, investigating measurement of PlGF, inhibin-A, activin-A, placental protein-13 (PP13), sEng, pentraxin-3 and P-selectin, and concluded that a combination of these maternal characteristics and biophysical and biochemical markers can detect

approximately 91% of early-onset pre-eclampsia with a 5% FPR.

However, other groups, including other populations, have shown different performances of various parameters in screening for pre-eclampsia. Audibert *et al.*[38] recently carried out a cohort study in 893 pregnant women who underwent UtA Doppler and a series of biochemical tests during the first trimester of pregnancy. However, among this cohort, there were only nine women who later developed early-onset pre-eclampsia. They concluded that only a combination of clinical characteristics and biochemical markers, such as PAPP-A and PlGF, can detect 75% of early-onset pre-eclampsia at a fixed FPR of 10%. Wortelboer *et al.*[39], after performing a nested case–control study during the first trimester of pregnancy, concluded that a combination of PlGF and PP13 can only detect 54% of early-onset pre-eclampsia at a fixed FPR of 10%; their study population included 88 women who later developed either pre-eclampsia or HELLP syndrome before 34 weeks and 480 controls. Finally, Odibo *et al.*[40] performed a case–control study during the first trimester of pregnancy in 42 women who subsequently developed pre-eclampsia and 410 controls. They concluded that UtA Doppler, PP13 and PAPP-A are reasonable predictors of pre-eclampsia, though combining these markers did not improve the DR. They also concluded that the best screening test for early-onset pre-eclampsia was the plasma level of PP13, which has a sensitivity of 79% for a 20% FPR.

In summary, this report generally agrees with the concepts described in other publications[17,32,41] regarding the fact that the best model to predict pre-eclampsia includes a combination of maternal factors, biochemical markers and UtA Doppler. A multivariate algorithm is then generated that allows stratification of patients at risk for this disease. The differences observed in DR in different publications could be explained by the different sizes of the studies, but it is also possible that maternal characteristics, especially ethnicity, may play a role in these diverse results[32]. Although it has not yet been proven, early, intensive surveillance of women at high risk for pre-eclampsia could substantially improve their pregnancy outcome, and this may also allow for future studies investigating the role of pharmacological supplementation or treatment during the first trimester of pregnancy.

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