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Maternal Serum Placental Growth Factor, Pregnancy-Associated Plasma Protein-A and Free β-Human Chorionic Gonadotrophin at 30–33 Weeks in the Prediction of Pre-Eclampsia

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Key Words
Antenatal care · Free β-human chorionic gonadotrophin · Placental growth factor · Pre-eclampsia · Pregnancy-associated plasma protein-A · Third-trimester screening

Abstract
Objective: To investigate the potential value of maternal serum concentrations of free β-human chorionic gonadotrophin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A) and placental growth factor (PlGF) at 30–33 weeks of gestation in the prediction of pre-eclampsia (PE) developing at or after 34 weeks. ***Methods:*** Serum free β-hCG, PAPP-A and PlGF were measured at 11–13 and at 30–33 weeks of gestation in a case-control study of 50 cases that developed PE at or after 34 weeks and 250 unaffected controls. The measured concentration of metabolites was converted into multiples of the unaffected median (MoM) and the MoM values in the PE and control groups were compared. ***Results:*** At 11–13 weeks, serum PlGF and PAPP-A, but not free β-hCG, were significantly lower in the PE group than in the controls (0.824, 0.748 and 0.857 vs. 1.000 MoM). At 30–33 weeks in the PE group, PlGF was reduced (0.356 MoM), free β-hCG was increased (1.750 MoM), but PAPP-A was not significantly different (0.991 MoM) from control (1.000 MoM). In screening for PE at 30–33 weeks by a combination of maternal characteristics and serum PlGF, the estimated detection rates, at a false-positive rate of 10%, of intermediate PE (requiring delivery at 34–37 weeks) and late PE (with delivery after 37 weeks) were 85.7 and 52.8%, respectively. The performance of screening was not improved by the addition of free β-hCG or the free β-hCG/PlGF ratio. ***Conclusion:*** Screening by maternal characteristics and serum PlGF at 30–33 weeks could identify most pregnancies that will subsequently develop PE.

Introduction

Pre-eclampsia (PE), which affects 2–3% of pregnancies, is a major cause of maternal and perinatal morbidity and mortality [1–3]. The condition has been subdivided into early PE, requiring delivery before 34 weeks, intermediate PE, requiring delivery at 34–37 weeks, and late PE, delivering after 37 weeks [4]. We have recently proposed a two-stage strategy for the identification of pregnancies at risk of PE [5, 6]. The first stage, at 11–13 weeks,

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should be primarily aimed at effective prediction of early PE because the prevalence of this condition can be potentially reduced substantially by the prophylactic use of low-dose aspirin started before 16 weeks of gestation [7, 8]. The second stage, at 30–33 weeks, should be aimed at effective prediction of intermediate and late PE because close monitoring of such pregnancies for earlier diagnosis of the clinical signs of the disease could potentially improve perinatal outcome through such interventions as the administration of antihypertensive medication and early delivery [9].

Several studies have reported that maternal serum concentration of the placental products serum free β-human chorionic gonadotropin (free β-hCG), pregnancy-associated plasma protein-A (PAPP-A) and placental growth factor (PlGF) are altered in pregnancies with established PE. There is also evidence that the altered levels of these metabolites may be apparent from the first trimester of pregnancy. Serum free β-hCG is increased in PE but at 11–13 weeks it is decreased or not altered [4, 10–14], PAPP-A is increased in PE but reduced at 11–13 weeks [4, 14–19] and PlGF is reduced in both established PE and in the first trimester [4, 20–26].

The objective of this case-control study is to investigate the potential value of maternal serum concentrations of free β-hCG, PAPP-A and PlGF at 30–33 weeks of gestation in the prediction of intermediate and late PE.

Methods

Study Population

This was a case-control study drawn from a prospective observational study for adverse pregnancy outcomes in women attending for their routine first- and third-trimester hospital visits in pregnancy at the King's College Hospital London and the Medway Maritime Hospital Kent between May 2011 and March 2012. In the first-trimester visit, at 11^{+0}–13^{+6} weeks of gestation, an ultrasound scan was carried out to firstly confirm gestational age from the measurement of the fetal crown-rump length [27], secondly, diagnose any major fetal abnormalities [28] and thirdly, measure fetal nuchal translucency thickness as part of screening for aneuploidies [5]. In addition, the maternal serum free β-hCG, PAPP-A and PlGF were determined (DELFIA Xpress system; PerkinElmer Life and Analytical Sciences, Waltham, Mass., USA) and the results were combined with fetal nuchal translucency to estimate the patient-specific risk for aneuploidies [29, 30]. The third-trimester visit, at 30^{+0}–33^{+6} weeks of gestation, included ultrasound examination for assessment of fetal growth and well-being. During this visit, maternal blood was collected for research and the serum was stored at –80°C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the Ethics Committee of each participating hospital.

The base cohort study population, wherein the present case-control study was nested, constituted 5,099 singleton pregnancies. We excluded 244 cases because they had missing outcome data (n = 156), they had PE at the time of screening or before 34 weeks (n = 25), the pregnancy resulted in delivery before 34 weeks of gestation (n = 37) or the birth of babies with major defects (n = 26). In the remaining 4,855 cases, there were 145 (3.0%) cases that developed PE, with 37 cases requiring delivery at 34–37 weeks (intermediate PE) and 108 cases delivering at or after 38 weeks (late PE), 161 (3.3%) cases developed gestational hypertension and 4,294 cases were unaffected by PE or gestational hypertension. Stored maternal blood was available from 50 cases that developed PE, and third-trimester maternal serum free β-hCG, PAPP-A and PlGF were measured in these 50 cases (DELFIA Xpress system). Each case of PE was matched with 5 controls who had blood collected on the same day of visit at 30–33 weeks and delivered a phenotypically normal neonate appropriate for gestational age at term and did not develop any hypertensive disorder of pregnancy. None of the samples in the case-control study were previously thawed and refrozen.

Patient Characteristics

Patient characteristics including maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs), cigarette smoking during pregnancy (yes or no), history of chronic hypertension (yes or no), family history of PE in the mother of the patient (yes or no) and obstetric history, including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks) and previous pregnancy with PE (yes or no), maternal weight and height were recorded.

Outcome Measures

The definition of PE was that of the International Society for the Study of Hypertension in Pregnancy [31]. The systolic blood pressure should be 140 mm Hg or more and/or the diastolic blood pressure should be 90 mm Hg or more which develops after 20 weeks of gestation together with significant proteinuria in previously normotensive women. Significant proteinuria is defined by 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-hour 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 (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).

Statistical Analysis

Comparisons of maternal characteristics between outcome groups were by χ^2 or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables.

The measured first-trimester free β-hCG, PAPP-A and PlGF were converted into multiples of expected median (MoM) for gestational age adjusted for maternal weight, racial origin, smoking status, method of conception, parity, pre-gestational diabetes mellitus and machine for the assays [32, 33]. The third-trimester values of maternal serum free β-hCG, PAPP-A and PlGF were $\log_{10}$ transformed to make their distributions gaussian. Multiple regression analysis was used to determine which of the factors

amongst the maternal characteristics and gestation were significant predictors of third-trimester $\log_{10}$ β-hCG, $\log_{10}$ PAPP-A and $\log_{10}$ PlGF in the control group. Gestational age at screening was centred by subtracting 32 from gestational age in weeks in the third trimester. Maternal weight was centred by subtracting 75 kg in the third trimester, and maternal height was centred by subtracting 165 cm.

Each measured value in the outcome groups was expressed as MoM after adjustment for those characteristics found to provide a substantial contribution to the $\log_{10}$-transformed value. The Mann-Whitney U test with Bonferroni correction was used to compare the median value of first- and third-trimester free β-hCG MoM, PAPP-A MoM and PlGF MoM between the cases and controls. Regression analysis was used to determine the significance of associations between the third-trimester $\log_{10}$ β-hCG MoM and $\log_{10}$ PlGF MoM with gestational age at delivery. Likelihood ratios for intermediate and late PE were calculated from the fitted bivariate gaussian distributions for third-trimester free β-hCG and PlGF.

The a priori risks for intermediate and late PE based on maternal characteristics and obstetric history were determined as previously described [6]. Likelihood ratios for intermediate and late PE were calculated from the fitted bivariate gaussian distributions for third-trimester free β-hCG and PlGF and these were combined with the a priori risks to produce a posteriori risks. The performance of screening for intermediate and late PE by maternal characteristics, third-trimester free β-hCG and PlGF and their combination was determined by receiver-operating characteristic curve analysis.

The statistical software package SPSS 20.0 (SPSS Inc., Chicago, Ill., USA) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for all data analyses.

Table 1. Maternal characteristics in the cases and controls

Characteristics	Control (n = 250)	PE (n = 50)
Maternal age, years	31.2 (27.6–34.9)	29.8 (24.2–33.8)
Maternal weight, kg	76.9 (69.6–85.6)	77.2 (67.9–90.1)
Maternal height, cm	165 (161–169)	163 (158–166)
Racial origin		
Caucasian	123 (49.2)	24 (48.0)
Afro-Caribbean	100 (40.0)	20 (40.0)
South Asian	9 (3.6)	1 (2.0)
East Asian	10 (4.0)	2 (4.0)
Mixed	8 (3.2)	3 (6.0)
Parity		
Nulliparous	128 (51.2)	30 (60.0)
Parous with no previous PE	119 (47.6)	16 (32.0)*
Parous with previous PE	3 (1.2)	4 (8.0)*
Cigarette smoker	16 (6.4)	4 (8.0)
Family history of PE	10 (4.0)	5 (10.0)
Conception		
Spontaneous	246 (98.4)	48 (96.0)
Assisted	4 (1.6)	2 (4.0)
History of chronic hypertension	0	5 (10.0)*

Values are given as medians (interquartile ranges) or n (%). Comparisons between each outcome group with controls: χ^2 test and Fisher's exact test for categorical variables and Mann-Whitney U test: * $p < 0.05$.

Results

The maternal characteristics of the study population are given in table 1. In the PE group, compared to the control group, there was a higher prevalence of women with personal history of PE and chronic hypertension.

Unaffected Group

Multiple regression analysis in the unaffected group demonstrated that for $\log_{10}$ β-hCG at 30–33 weeks significant independent contributions were provided by maternal weight and parity but not by maternal age (p = 0.671), height (p = 0.865), racial origin (p = 0.809), smoking (p = 0.880), conception (p = 0.321) or gestational age (p = 0.544):

$\log_{10}$ expected free β-hCG at 30–33 weeks = 0.79024 – 0.0050279 × (weight – 75 kg) + (0.13544 if nulliparous; 0 if parous); $R^2 = 0.077$, $p < 0.0001$.

Multiple regression analysis in the unaffected group demonstrated that for $\log_{10}$ PAPP-A at 30–33 weeks significant independent contributions were provided by Afro-Caribbean racial origin but not by maternal age (p = 0.596), weight (p = 0.566), height (p = 0.715), smoking (p = 0.179), parity (p = 0.633), conception (p = 0.296) or gestational age (p = 0.196):

$\log_{10}$ expected PAPP-A at 30–33 weeks = 1.36548 + (0.053744 if Afro-Caribbean racial origin; 0 if other racial origin); $R^2 = 0.039$, $p = 0.002$.

Multiple regression analysis in the unaffected group demonstrated that for $\log_{10}$ PlGF at 30–33 weeks significant independent contributions were provided by Afro-Caribbean racial origin but not by maternal age (p = 0.501), weight (p = 0.113), height (p = 0.572), parity (p = 0.098), smoking (p = 0.246), conception (p = 0.431) or gestational age (p = 0.391):

$\log_{10}$ expected PlGF at 30–33 weeks = 2.62536 + (0.090812 if Afro-Caribbean racial origin; 0 if other racial origin); $R^2 = 0.019$, $p = 0.028$.

Table 2. Median serum PlGF, PAPP-A and free β-hCG (interquartile ranges) in the outcome groups

Outcome group	PlGF		PAPP-A		Free β-hCG	
	pg/ml	MoM	mIU/ml	MoM	IU/ml	MoM
11–13 weeks						
Control (n = 250)	34.71 (25.31–46.75)	1.000 (0.773–1.288)	3.27 (2.02–5.28)	1.000 (0.686–1.375)	42.12 (27.56–59.22)	1.000 (0.683–1.421)
Preeclampsia						
All (n = 50)	25.08 (18.56–37.19)	0.824 (0.592–1.148)*	1.979 (1.505–3.355)	0.748 (0.533–1.160)*	35.22 (20.55–74.13)	0.857 (0.560–1.448)
Intermediate (n = 14)	20.50 (14.47–27.32)	0.713 (0.527–1.007)**	1.759 (1.157–2.521)	0.610 (0.409–0.819)**	31.68 (24.10–62.94)	0.891 (0.591–1.319)
Late (n = 36)	27.22 (19.63–42.25)	0.870 (0.611–1.192)	2.093 (1.662–4.060)	0.787 (0.571–1.222)	36.70 (19.63–77.99)	0.857 (0.481–1.652)
30–33 weeks						
Control (n = 250)	475.25 (286.38–791.88)	1.000 (0.560–1.581)	26.10 (21.18–29.90)	1.000 (0.822–1.133)	6.85 (3.90–12.30)	1.000 (0.614–1.792)
Preeclampsia						
All (n = 50)	178.10 (98.35–351.96)	0.356 (0.205–0.679)*	25.75 (20.90–28.70)	0.991 (0.802–1.136)	11.05 (4.58–20.98)	1.750 (0.647–3.215)*
Intermediate (n = 14)	87.90 (60.78–194.65)	0.172 (0.132–0.384)**	24.25 (19.63–28.10)	0.863 (0.786–1.125)	17.50 (8.43–30.73)	2.743 (0.995–4.645)**
Late (n = 36)	209.30 (126.48–486.97)	0.412 (0.261–0.965)**	25.95 (21.95–29.40)	1.002 (0.815–1.136)	9.45 (3.93–18.53)	1.514 (0.545-2.397)

Comparisons between PE and controls by Mann-Whitney U test (* $p < 0.05$), and between intermediate PE, late PE and controls by Mann-Whitney U test with post hoc Bonferroni correction (** $p < 0.025$).

In each patient we used these formulae to derive the expected $\log_{10}$ free β-hCG, $\log_{10}$ PAPP-A and $\log_{10}$ PlGF at 30–33 weeks, and then expressed the observed values as MoM of the expected (table 2).

PE Group

In the PE group, compared to controls, the median MoM free β-hCG at 11–13 weeks was not significantly different, but at 30–33 weeks it was higher (table 2). In the PE group, there was a significant association between $\log_{10}$-MoM free β-hCG at 30–33 weeks with gestational age at delivery ($r = -0.287$, $p = 0.043$; fig. 1).

In the PE group, compared to controls, the median MoM PAPP-A was lower at 11–13 weeks, but it was not significantly different at 30–33 weeks (table 2).

In the PE group, compared to controls, the median MoM PlGF was lower both at 11–13 and at 30–33 weeks (table 2), but the value was significantly lower at 30–33 weeks than at 11–13 weeks (Wilcoxon signed rank test: $p < 0.0001$). There was a significant association between $\log_{10}$ PlGF MoM at 11–13 weeks with $\log_{10}$ PlGF MoM at 30–33 weeks ($r = 0.459$, $p = 0.001$). There was a significant association between $\log_{10}$-MoM PlGF at 30–33 weeks with gestational age at delivery ($r = 0.338$, $p = 0.016$; fig. 2).

At 11–13 weeks, there was a significant association between $\log_{10}$-MoM PlGF and $\log_{10}$-MoM PAPP-A ($r = 0.331$, $p = 0.019$) and between $\log_{10}$-MoM PlGF and $\log_{10}$-MoM free β-hCG ($r = 0.303$, $p = 0.032$), but not between $\log_{10}$-MoM PAPP-A and $\log_{10}$-MoM free β-hCG ($r = 0.148$, $p = 0.304$). At 30–33 weeks, there was no significant association between $\log_{10}$-MoM PlGF and $\log_{10}$-MoM PAPP-A ($r = 0.019$, $p = 0.896$), between $\log_{10}$-MoM PlGF and $\log_{10}$-MoM free β-hCG ($r = -0.221$, $p = 0.123$) or between $\log_{10}$-MoM PAPP-A and $\log_{10}$-MoM free β-hCG ($r = -0.047$, $p = 0.745$).

In the PE group, compared to controls, the median free β-hCG MoM/PlGF MoM ratio at 30–33 weeks was higher ($p < 0.0001$), and there was a significant association between the $\log_{10}$ value of the ratio and gestational age at delivery ($r = -0.398$, $p = 0.004$; fig. 3).

The overlapping gaussian distributions of $\log_{10}$ values of the free β-hCG MoM and PlGF MoM and free β-hCG MoM to PlGF MoM ratio at 30–33 weeks in the normal group and the intermediate- and late-PE groups were used to calculate likelihood ratios for intermediate and late PE (table 3). The a posteriori risks for intermediate and late PE were derived by multiplying the a priori risks [6] by the likelihood ratios for free β-hCG, PlGF and the free β-hCG/PlGF ratio.

Performance of PE Screening

In screening for PE at 30–33 weeks by a combination of maternal characteristics and serum PlGF, the estimated detection rates, at a false-positive rate of 10%, of intermediate PE (requiring delivery at 34–37 weeks) and late PE (with delivery after 37 weeks) were 85.7 and 52.8%,

Fig. 1. Free β-hCG (MoM) at 30–33 weeks with gestational age at delivery in PE-complicated pregnancies plotted on the 10th, 50th and 90th percentiles of the normal range (horizontal lines and shaded area).

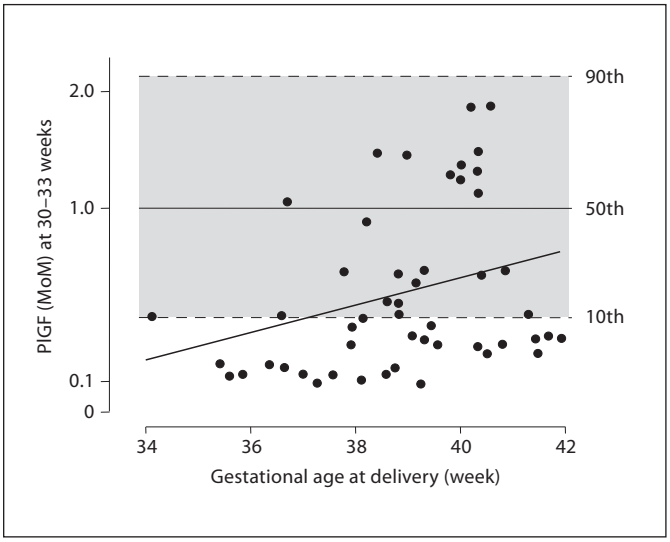

Fig. 2. PlGF (MoM) at 30–33 weeks with gestational age at delivery in PE-complicated pregnancies plotted on the 10th, 50th and 90th percentiles of the normal range (horizontal lines and shaded area).

Fig. 3. Free β-hCG (MoM) to PlGF (MoM) ratio at 30–33 weeks with gestational age at delivery in PE-complicated pregnancies plotted on the 10th, 50th and 90th percentiles of the normal range (horizontal lines and shaded area).

respectively. The performance of screening was not improved by the addition of free β-hCG or the free β-hCG/PlGF ratio (table 4; fig. 4).

Discussion

This study has demonstrated that at 30–33 weeks of gestation in pregnancies which subsequently develop PE, compared to normal pregnancies, maternal serum level of free β-hCG is higher, PlGF is lower, and PAPP-A is not significantly different. The increase in serum free β-hCG and decrease in PlGF is related to the severity of PE reflected in the gestational age at delivery. In screening for PE at 30–33 weeks by a combination of maternal characteristics and serum PlGF, the estimated detection rates, at a false-positive rate of 10%, of intermediate and late PE were 86 and 53%, respectively. The performance of screening was not improved by the addition of free β-hCG or the free β-hCG/PlGF ratio.

At 11–13 weeks of gestation in pregnancies which subsequently developed PE both serum PAPP-A and PlGF were lower, but free β-hCG was not significantly different from normal pregnancies. These findings are compatible with the results of several previous studies [4, 18, 19, 23–26]. The decrease in serum PAPP-A and PlGF is likely to be the consequence of impaired trophoblastic invasion of

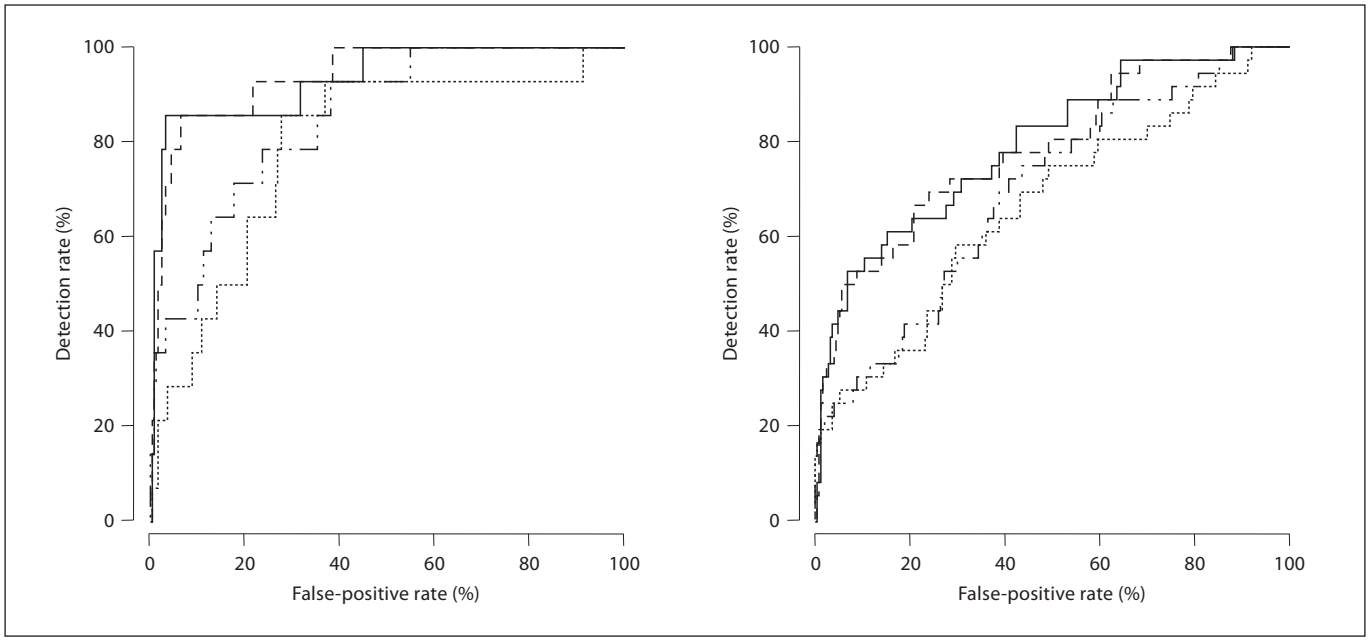

Fig. 4. Receiver-operating characteristic curves of maternal characteristics only (·····), maternal characteristics with PlGF (– – –), maternal characteristics with free β-hCG (— · · —) and their combination (————) in the prediction of intermediate (left) and late PE (right).

Table 3. Likelihood ratios for PE from free β-hCG and PlGF MoM at 30–33 weeks of gestation

Free β-hCG			PlGF		
MoM	likelihood ratio (95% CI)		MoM	likelihood ratio (95% CI)	
	intermediate PE	late PE		intermediate PE	late PE
0.5	0.35 (0.33–0.38)	0.79 (0.79–0.79)	0.1	19.26 (10.05–36.52)	7.57 (4.96–11.86)
1.0	0.66 (0.61–0.67)	0.85 (0.84–0.86)	0.2	4.90 (3.40–7.70)	3.22 (2.62–4.21)
1.5	0.97 (0.96–1.01)	0.96 (0.96–0.97)	0.3	1.77 (1.54–2.51)	1.86 (1.73–2.48)
2.0	1.34 (1.31–1.37)	1.09 (1.08–1.10)	0.4	1.02 (0.81–1.43)	1.42 (1.28–1.67)
2.5	1.85 (1.74–1.87)	1.27 (1.23–1.27)	0.5	0.60 (0.49–0.74)	1.12 (1.02–1.23)
3.0	2.19 (2.17–2.22)	1.38 (1.37–1.39)	0.6	0.39 (0.31–0.46)	0.93 (0.85–0.99)
3.5	2.72 (2.69–2.75)	1.55 (1.54–1.56)	0.7	0.26 (0.22–0.28)	0.79 (0.74–0.81)
4.0	3.21 (3.21–3.26)	1.70 (1.70–1.72)	0.8	0.17 (0.16–0.18)	0.67 (0.66–0.69)
5.0	4.38 (4.37–4.40)	2.05 (2.04–2.06)	1.0	0.10 (0.09–0.11)	0.56 (0.53–0.57)

the spiral arteries and their conversion from high-impedance narrow vessels to wide non-muscular channels, which is thought to be the underlying cause of PE [34, 35]. Supportive evidence is provided by the previously reported inverse association between serum PAPP-A and PlGF with the Doppler measurement of the uterine artery pulsatility index, which provides an indirect measure of impaired placental perfusion [19, 24]. It is uncertain whether PAPP-A and PlGF are implicated in the pathogenesis of PE or their reduced levels merely act as markers of impaired placental function. However, if the latter was true there is no obvious explanation for the normal levels of free β-hCG which is also produced by the placenta.

In the pathogenesis of PE, impaired trophoblastic invasion of the spiral arteries leads to placental hypoxia and the release of pro-inflammatory cytokines which cause

Table 4. Performance of screening for intermediate and late PE by maternal characteristics, PlGF, free β-hCG and their combination

Screening test	Area under the receiver-operating curve (95% CI)			
	intermediate PE		late PE	
Maternal history	0.793 (0.739–0.840)		0.663 (0.604–0.717)	
PlGF	0.907 (0.866–0.940)		0.734 (0.678–0.787)	
Free β-hCG	0.749 (0.692–0.800)		0.583 (0.523–0.641)	
PlGF and free β-hCG	0.904 (0.862–0.936)		0.743 (0.688–0.792)	
β-hCG/PlGF ratio	0.873 (0.827–0.911)		0.696 (0.639–0.749)	
Maternal history plus				
PlGF	0.939 (0.903–0.965)		0.783 (0.731–0.829)	
Free β-hCG	0.822 (0.771–0.866)		0.665 (0.608–0.720)	
PlGF and free β-hCG	0.904 (0.862–0.936)		0.778 (0.725–0.825)	
β-hCG/PlGF ratio	0.900 (0.858–0.934)		0.763 (0.709–0.811)	
	Detection rate (95% CI) for fixed false-positive rate of 5 and 10%			
	5%	10%	5%	10%
Maternal history	21.4 (4.9–50.8)	35.7 (12.9–64.8)	27.8 (14.2–45.2)	30.6 (16.4–48.1)
PlGF	64.3 (35.2–87.1)	71.4 (41.9–91.4)	22.2 (10.1–39.2)	44.4 (27.9–61.9)
Free β-hCG	42.9 (17.8–71.1)	50.0 (23.1–76.9)	13.9 (4.7–29.5)	19.4 (8.2–36.0)
PlGF and free β-hCG	71.4 (41.9–91.4)	85.7 (57.2–97.8)	27.8 (14.2–45.2)	47.2 (30.4–64.5)
Free β-hCG/PlGF ratio	71.4 (41.9–91.4)	78.6 (49.2–95.1)	25.0 (12.1–42.2)	36.1 (20.8–53.8)
Maternal history plus				
PlGF	78.6 (49.2–95.1)	85.7 (57.2–97.8)	41.7 (25.5–59.2)	52.8 (35.5–69.6)
Free β-hCG	42.9 (17.8–71.1)	64.3 (35.2–87.1)	25.0 (12.1–42.2)	33.3 (18.6–51.0)
PlGF and free β-hCG	85.7 (57.2–97.8)	85.7 (57.2–97.8)	41.7 (25.5–59.2)	52.8 (35.5–69.6)
Free β-hCG/PlGF ratio	78.6 (49.2–95.1)	85.7 (57.2–97.8)	33.3 (18.6–51.0)	50.0 (32.9–67.1)

widespread activation and dysfunction of the vascular endothelium and development of the clinical signs of PE [36–38]. The increase in serum free β-hCG, which has vasodilatory properties, observed during and in the few weeks prior to the development of the clinical signs of PE, has been attributed to hypoxia-induced hypersecretion to counteract the generalized vasoconstriction which characterizes PE [10–13, 39, 40]. The reported paradoxical behaviour of serum PAPP-A in PE with decrease at 11–13 weeks and increase at the time of PE [4, 14–19] could be the consequence of impaired placental function for the former and global activation of inflammation for the latter. Our finding that serum PAPP-A at 30–33 weeks is normal suggests that the increase in this metabolite does not precede but coincides with the clinical onset of PE. This finding is consistent with the results of a previous study in which serum PAPP-A at 17 weeks of gestation was reduced in pregnancies that subsequently developed PE but the levels at 25 and 33 weeks were not significantly different from controls [41].

In contrast to PAPP-A, in pregnancies that develop PE, the levels of PlGF were reduced at 11–13 weeks and even more so at 30–33 weeks. The underlying cause for this decrease has been attributed to early and persisting placental hypoxia [41]. In cultured trophoblast cells, the production of PlGF mRNA is inhibited under experimental hypoxia [42]. The further deviation from normal observed nearer the clinical onset of the disease has been suggested to be the consequence of an exacerbation of the physiological anti-angiogenic state and deteriorating placental growth and function at term [43]. In normal pregnancy, serum PlGF increases with gestation to reach a peak at around 30 weeks and decreases thereafter until delivery. This late decrease coincides with an increase in the serum level of the anti-angiogenic factor sFlt-1, and in women developing PE the levels of sFlt-1 increase steeply about 5 weeks before the clinical onset of the disease [43].

In our cross-sectional study, the selected gestational ages for investigation were 11–13 and 30–33 weeks because these are widely used for assessment of risk for an-

euploidies and fetal growth and well-being, respectively. The aim of the assessment at 11–13 weeks is to effectively identify pregnancies that subsequently develop early PE for early prophylactic administration of low-dose aspirin to potentially reduce the prevalence of the disease [4, 7, 8]. The objective of the 30- to 33-week assessment is to identify pregnancies that would develop intermediate and late PE and improve perinatal outcome in such pregnancies by close surveillance and early delivery [9]. As shown in this study, a high proportion of cases developing intermediate and late PE could be identified by a combination of maternal factors and measurement of serum PlGF. Although the addition of free β-hCG or the free β-hCG/PlGF ratio did not improve the performance of screening, this area merits further investigation by examining a larger number of patients. Similarly, further studies will investigate the potential improvement of screening by inclusion of additional biochemical and biophysical markers.

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