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Angiogenic factors combined with clinical risk factors to predict preterm pre-eclampsia in nulliparous women: a predictive test accuracy study

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Objectives To assess the performance of clinical risk factors, uterine artery Doppler and angiogenic markers to predict preterm pre-eclampsia in nulliparous women.

Design Predictive test accuracy study.

Setting Prospective multicentre cohort study Screening for Pregnancy Endpoints (SCOPE).

Methods Low-risk nulliparous women with a singleton pregnancy were recruited. Clinical risk factor data were obtained and plasma placental growth factor (PlGF), soluble endoglin and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured at 14–16 weeks of gestation. Prediction models were developed using multivariable stepwise logistic regression.

Main outcome measure Preterm pre-eclampsia (delivered before 37^{+0} weeks of gestation).

Results Of the 3529 women recruited, 187 (5.3%) developed pre-eclampsia of whom 47 (1.3%) delivered preterm. Controls (n = 188) were randomly selected from women without preterm pre-eclampsia and included women who developed other pregnancy complications. An area under a receiver operating characteristic curve (AUC) of 0.76 (95% CI 0.67–0.84) was observed using previously reported clinical risk variables. The AUC improved following the addition of PlGF measured at 14–16 weeks (0.84; 95% CI 0.77–0.91), but no further improvement was observed with the addition of uterine artery Doppler or the other angiogenic markers. A sensitivity of 45% (95% CI 0.31–0.59) (5% false-positive rate) and post-test probability of 11% (95% CI 9–13) were observed using clinical risk variables and PlGF measurement.

Conclusions Addition of plasma PlGF at 14–16 weeks of gestation to clinical risk assessment improved the identification of nulliparous women at increased risk of developing preterm pre-eclampsia, but the performance is not sufficient to warrant introduction as a clinical screening test. These findings are marker dependent, not assay dependent; additional markers are needed to achieve clinical utility.

Keywords Angiogenic markers, placental growth factor, pre-eclampsia, sensitivity, specificity.

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Introduction

The early detection of pre-eclampsia remains one of the major focuses of antenatal care in the developed world. Recent guidelines produced by the National Institute of Clinical Excellence (NICE) recommend routine screening in the first trimester for specific risk factors for pre-eclampsia and treatment with low-dose aspirin for those identified to reduce the risk of pre-eclampsia.[1,2] As the strongest of these risk factors (chronic hypertension and previous pre-

eclampsia) are not applicable to low-risk nulliparous women, stratification of this group to high-risk and low-risk care is particularly challenging. Application of NICE criteria (older age, high body mass index, family history of pre-eclampsia) to nulliparous women will result in the detection of approximately a third of cases of preterm pre-eclampsia.[3] Therefore, there is an urgent need to characterise the screening performance of existing and novel prediction tests in low-risk populations[4] so as to facilitate the design of future intervention and cost-effectiveness studies.[5] Several angiogenic markers, including placental growth factor (PlGF), endoglin and soluble fms-like tyrosine kinase 1 (sFlt-1) have been proposed to be useful in the prediction of pre-eclampsia.[6–9]

The predictive performance of these angiogenic markers varies depending on the combination of variables used and the population investigated.[10–14] The majority of these studies have included women with a history of previous pre-eclampsia or significant underlying medical disease, which has resulted in higher areas under the curve (AUCs).[8] Few studies have investigated a low-risk nulliparous population in isolation.[14] Furthermore, nested case–control studies, which include only women with uncomplicated pregnancies as the comparator, will also lead to over-optimistic AUC values.[10]

We hypothesise that a combination of clinical risk factors together with biochemical markers and Doppler indices improves the prediction of preterm pre-eclampsia in women compared with clinical risk factors alone. The objective of the study was to determine the performance of plasma angiogenic markers measured at 14–16 and 19–21 weeks of gestation, alone and in combination with a set of clinical risk variables, to predict preterm pre-eclampsia in a low-risk nulliparous population.

Methods

Between November 2004 and August 2008, nulliparous women with a singleton pregnancy were recruited into the SCOPE study (Screening for Pregnancy Endpoints, www.scopestudy.net): a prospective, multicentre (Auckland, New Zealand; Adelaide, Australia; London and Manchester, UK; and Cork, Ireland), cohort study of nulliparous women with the primary aim of developing screening tests to predict pre-eclampsia, infants who are small for gestational age and spontaneous preterm birth[3] (Australian, New Zealand Clinical Trials Registry ACTRN12607000551493).[15] Exclusion criteria included underlying medical disease, previous cervical knife cone biopsy, three or more early pregnancy losses, known fetal abnormality or abnormal karyotype at recruitment, or intervention which could modify the pregnancy outcome (such as aspirin or cervical suture). A predictive test accuracy study using a reversed flow design was performed,[16] with cases comprising preterm pre-eclampsia and controls randomly selected from all women without preterm pre-eclampsia at a ratio of four controls for each case, stratified by SCOPE centre.

The SCOPE study design has been described in detail previously.[3] In brief, a research midwife interviewed participants at 14–16 weeks (classified as 15 weeks) and 19–21 weeks (classified as 20 weeks) of gestation. Detailed clinical data and examination findings were entered on a web-accessed database with a complete audit trail (MedSciNet, Stockholm, Sweden[AB]). Clinical data included demographic data, previous miscarriage, abortion or ectopic pregnancies, history of infertility, medical history and family history including pre-eclampsia affecting mothers or sisters. Information about the index pregnancy included use of folate, multivitamins, cigarettes, alcohol and recreational drugs before conception, in the first trimester and at 15 weeks. Two consecutive manual blood pressure measurements (mercury or aneroid sphygmomanometer) were recorded, along with maternal height and weight. Proteinuria was assessed by dipstick or protein:creatinine ratio in a midstream sample. Ultrasound examination at 19–21 weeks of gestation included fetal biometry and Doppler studies of both the umbilical and uterine arteries. Mean uterine resistance index (RI) was calculated from the average of the left and right RIs; if only one reading was available, this was used as the mean RI; an abnormal Doppler was defined as mean RI >90th centile. The presence or absence of notching in each uterine artery waveform was recorded.

Women were followed prospectively and research midwives collected data on pregnancy outcome and measurements of the baby. Estimated date of delivery was calculated by the date of last menstrual period where this was known. The estimated date of delivery was adjusted only if a scan at <16 weeks of gestation identified a discrepancy of seven or more days or a scan at 19–21 weeks identified a discrepancy of ten or more days. If the last menstrual period was uncertain, scan dates were used to calculate the estimated date of delivery.

Cases were defined as women who developed pre-eclampsia requiring delivery before 37 weeks of gestation. Pre-eclampsia was defined as systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg, or both, on at least two occasions 4 hours apart after 20 weeks of gestation, but before onset of labour, or postpartum, with either proteinuria (24-hour protein >300 mg or spot protein:creatinine ratio $\geq$30 mg/mmol or urine dipstick $\geq$++) or any multiorgan complication of pre-eclampsia.[15,17] Multiorgan complications included any of acute renal insufficiency (new increase in serum creatinine $\geq$100 μmol/l antepartum or >130 μmol/l postpartum); liver involvement (aspartate transaminase or alanine trans-

aminase >45 IU/l and or severe right upper quadrant pain or liver rupture); eclampsia, imminent eclampsia (severe headache with hyper-reflexia and persistent visual disturbance); cerebral haemorrhage or haematological abnormalities (platelets $<100 \times 10^9$/l, disseminated intravascular coagulation or haemolysis).

Clinical variable selection

Two data sets were used to construct the predictive models. The first comprised clinical variables obtained at 15 weeks of gestation. The second comprised 15-week clinical data and ultrasound data collected at 20 weeks. The selection of variables available to the models was based on clinical risk factors used in other screening studies for pre-eclampsia and includes the risk factors identified in the NICE guidelines.[8,9] Given the difficulties defining ethnicity and that almost 90% of our study population was Caucasian, ethnicity was not included in the modelling. Clinical and ultrasound variables included age, gravidity, use of fertility treatment, a history of a previous miscarriage with the same partner, a family history of pre-eclampsia (mother/sisters), smoking status at 15 weeks, recreational drugs in the first trimester, mean arterial pressure and BMI measured at 15 weeks, mean uterine artery RI and bilateral uterine artery diastolic notching. Details of the variables available to the models are summarised in Table S1. Data were complete in 12 of the 13 clinical and ultrasound variables. There were missing data for mean uterine artery resistance index ($n = 5$); these women were removed from models containing this variable.

Biomarker measurements

Biomarkers were quantified in EDTA plasma samples at 15 weeks and 20 weeks of gestation. Blood specimen collection and preparation of EDTA plasma were performed using standard operating procedures, with samples centrifuged and stored at −80°C within 4 hours of collection. Laboratory personnel were blinded to pregnancy outcome status.

Plasma PlGF was measured by Triage® (Alere, San Diego, CA, USA). The test procedure involves the addition of 250 μl EDTA plasma to the sample port on the Triage Test Device™ (Alere, San Diego, CA, USA). The test reports concentration in the range 12–3000 pg/ml with an overall coefficient of variation (CV) of 12.8–13.2%. For comparison, PlGF was also quantified by the Delfia® (Perkin Elmer, Turku, Finland) in a paired serum sample. The test reports PlGF concentration in the range 7–4000 pg/ml with an overall CV of <10%.

Plasma sFlt-1 was measured using multiplexed bead-based immunoassays in microtitre plates (Alere). The primary antibody was conjugated to modified paramagnetic beads (Radix Biosolutions, Georgetown, TX, USA). An eight-point calibration curve was made gravimetrically by spiking each antigen into the calibration matrix. Following incubation with biotinylated secondary antibodies, the assay mixtures were labelled with Streptavidin-R–Phycoerythrin Prozyme, washed, and read using a Luminex LX200 reader (Luminex, Austin, TX, USA). The antigen concentrations were calculated using a standard curve determined by fitting a five-parameter logistic function to the signals obtained for the eight-point calibration curves. CV across the concentration range (94–37 000 pg/ml) was 7.1–16.7%.

Plasma endoglin was measured using immunoassay (Alere). Plates were washed three times with borate-buffered saline containing 0.02% Tween-20 (BBS-Tween). Eight-point calibration curves were prepared gravimetrically in plasma from healthy donors. The plates were read by a fluorometer (Tecan Spectrafluor; Tecan, Männedorf, Switzerland). The calibration curve was calculated using a five-parameter logistic fit and sample concentration was determined. The CV across the concentration range (1.02–650 ng/ml) was 8.2–16.1%.

Statistical analysis

Clinical characteristics in preterm pre-eclampsia and controls were compared using either a Student's *t* test or if categorical data, chi-square or Fisher's exact test. Wilcoxon rank sum was used to compare plasma biomarker concentrations at 15 and 20 weeks of gestation in women who later developed preterm pre-eclampsia and control women who did not develop preterm pre-eclampsia. A P value <0.05 was considered significant. The effect of gestation at sampling on biomarker levels was investigated using the Pearson correlation coefficient.

A series of stepwise logistic regressions were performed, with the order of variable selection determined by the chi-square statistic for each potential variable. The forward selection step could be followed by removal of variables in one or more backward elimination steps. The AUCs were calculated to determine the performance of the different models to predict preterm pre-eclampsia. Participants with missing data were excluded. As a 15-week PlGF result was unavailable for seven controls, these women were excluded from all multivariable analyses.

The risk assessment tool recommended by the NICE guidelines[1] was applied to the SCOPE cohort[3] to provide a comparison for the performance of the variable combinations tested in this study, using a theoretical population of 1000 low-risk women (13 of whom would develop preterm pre-eclampsia).

Power calculation

The sample size was determined by the number of samples/cases of preterm pre-eclampsia available at the time of the study. From previous studies, the minimum expected sensitivity for the detection of preterm pre-eclampsia using clin-

ical risk factors at 95% specificity would be around 28%.[8] A total sample of 229 (1:4 cases to controls) would give an accuracy of ±13% for a sensitivity of 28% and ±5% for a specificity of 95%.[18] Ten-fold cross-validation was used for internal validation of the AUC estimate. The dataset was split into ten equal subgroups, stratified for cases and controls. Using nine-tenths of the data (training dataset), backwards stepwise selection was used to fit a logistic regression model to the clinical variables. This model was applied to the remaining tenth of the data (test dataset) to generate prediction for each individual. This process was repeated ten times, with a different tenth held out as the test dataset, to determine a prediction estimate for each woman in the study. These predicted values were then used to calculate the cross-validated AUC. This process was repeated with PlGF, measured at 15 weeks, added to the model. Internal validation analysis was carried out using version 2.15 of the R statistical analysis software.[19] The ROCR package[20] was used within R for calculation of AUC values.

Results

Of the 3780 nulliparous women who agreed to participate, 3572 women were recruited and outcome data were available in 99% ($n = 3529$) (Figure 1). Forty-seven women (1.3%) developed preterm pre-eclampsia. Controls ($n = 188$) included women with pre-eclampsia with delivery $\geq$37 weeks ($n = 8$, 4%), gestational hypertension ($n = 16$, 9%), spontaneous preterm birth ($n = 7$, 4%), small for gestational age infants ($n = 16$, 9%) and a range of other pregnancy complications including antepartum haemorrhage and cholestasis of pregnancy. Of the 188 controls, only 119 (63%) had a completely uncomplicated pregnancy. Maternal characteristics at recruitment and pregnancy outcomes are summarised in Tables 1 and 2. Among the 47 women with preterm pre-eclampsia, 60% developed secondary multi-organ complications.

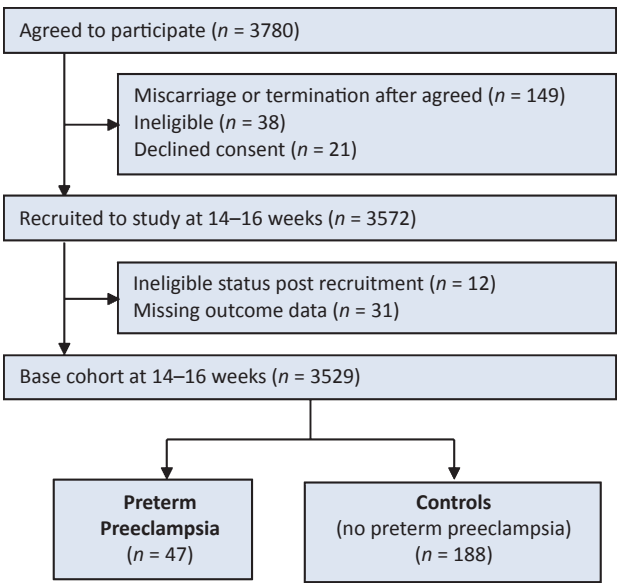

Figure 1. Flow chart of participants in the study.

Univariate biomarker analysis

The univariate biomarker data are shown in Table 3. PlGF and endoglin were significantly different between cases and controls at 15 and 20 weeks, whereas sFlt-1 was not different at either gestation (Table 3). Within the gestational age range of sample collection, neither endoglin ($r = 0.02$) nor sFlt-1 ($r = -0.01$) changed, whereas PlGF increased with increasing gestation ($r = 0.29$, $P < 0.0001$). PlGF was, therefore, transformed in multiples of the median (MoM) before performing logistic regression. The median (interquartile range) for PlGF MoM at 15 weeks was 0.60 (0.33–0.99) and 1.13 (0.65–1.79) for cases and controls, respectively ($P < 0.0001$). The AUC [95% confidence interval] for PlGF MoM, endoglin and sFlt-1 measured at 15 and 20 weeks are shown in Table 4 and Figure 2. The AUC using the Delfia assay PlGF measurement at 15 weeks was not significantly different (0.80 [0.74–0.87]) to the Triage assay.

Multivariate analysis

In combination, the AUC for PlGF MoM and sEng measured at 15 weeks was 0.76 [0.68–0.83] and at 20 weeks was 0.79 [0.70–0.87]. Neither the addition of sFLt-1 nor inclusion of the change in angiogenic marker levels between the two time-points improved the prediction of preterm pre-eclampsia (data not shown).

The AUC for 15-week clinical risk factors was 0.76 [0.67–0.84], Figure 3. The variables included in this model were mean arterial pressure (OR 1.67 [1.33–2.1] per 5 mmHg increase), a sister with a history of pre-eclampsia (OR 4.95 [1.25–19.6]) and a history of previous fertility treatment (OR 6.09 [1.46–25.41]). Ten-fold cross validation of this model produced an average AUC of 0.74 (range 0.735–0.744). The addition of PlGF (OR 0.65 [0.53–0.80] per increase in 0.25 MoM) to clinical risk factors improved prediction performance (AUC 0.84 [0.77–0.91]), Tables 4 and Table S2. Ten-fold cross validation of this model produced an AUC of 0.81 (range 0.808–0.816). The performance was unchanged using Delfia PlGF measurements (0.85 [0.78–0.92]). Neither the addition of sEng or sFlt-1 to clinical risk factors alone or in combination with PlGF further improved prediction of preterm pre-eclampsia (data not shown). Neither uterine artery Doppler at 20 weeks (AUC 0.85 [0.79–0.92]), nor uterine artery Doppler with 20-week endoglin (AUC 0.84 [0.77–0.91]) added to the

Table 1. Maternal characteristics

	No preterm pre-eclampsia (n = 188)		Preterm pre-eclampsia (n = 47)		P value
Maternal age (years)	27.8	(5.2)	28.2	(6.0)	0.62
Caucasian ethnicity	167	89%	41	87%	0.80
Married/de facto	177	94%	42	89%	0.24
Socio-economic Index	41	(18)	36	(15)	0.08
Education <12 years schooling	90	48%	26	55%	0.36
Tertiary education	149	79%	35	75%	0.48
Full/part-time work	162	86%	38	81%	0.36
Primigravid	143	76%	31	66%	0.16
Previous miscarriage	27	14%	8	17%	0.65
Previous miscarriage ≤10 weeks to same partner	18	9%	5	11%	0.89
Previous termination	25	13%	9	19%	0.31
Fertility treatment to conceive	5	3%	7	15%	0.0009
Family history of pre-eclampsia					
Mother	11	6%	6	13%	0.12
Sisters	4	2%	6	13%	0.002
Smoking status					
Nonsmoker	135	72%	36	77%	0.84
Ceased smoking in pregnancy	24	13%	5	10%	
Current smoker	28	15%	6	13%	
Body mass index (kg/m^2)					
<20.0	3	2%	0		0.1
20.0–24.9	96	51%	18	38%	
25.0–29.9	60	32%	15	32%	
≥30	29	15%	14	30%	
Systolic blood pressure (mmHg)	109	(10)	117	(13)	<0.0001
Diastolic blood pressure (mmHg)	65	(8)	72	(10)	<0.0001
Gestational age at sampling 15 weeks	15.5	(0.8)	15.5	(0.7)	0.86
Gestational age at sampling 20 weeks	20.1	(0.7)	20.3	(0.7)	0.53

Data shown as mean (SD) or number (%).
Student's t test was used to compare continuous data and chi-square or Fisher's exact test were used to compare categorical data.

Table 2. Maternal and fetal outcome

Pregnancy outcome	No preterm pre-eclampsia (n = 188)		Preterm pre-eclampsia (n = 47)		P value
Maternal					
Systolic blood pressure (mmHg)	125	(16)	172	(19)	<0.0001
Diastolic blood pressure (mmHg)	76	(12)	110	(12)	<0.0001
Proteinuria*	7	4%	43	92%	<0.0001
Gestational diabetes mellitus	3	2%	5	11%	0.002
Fetal					
Fetal outcome					
Alive	187	99.5%	46	98%	0.12
Termination ≥ 20 weeks	1	0.5%	0		<0.0001
Stillbirth	0		1	2%	
Delivery gestation (weeks)	39.6	(2.1)	34.2	(2.5)	
Preterm birth**					
<37 weeks	10	5%	47	100%	<0.0001
<34 weeks	3	2%	19	40%	
Birthweight (g)	3408	(541)	2026	(685)	
SGA*** (<10th customised centile)	16	9%	27	58%	<0.0001
Admission to neonatal unit	22	12%	36	77%	<0.0001

Data shown as mean (SD) or number %. Student's t test was used to compare continuous data and chi-square or Fisher's Exact test to compare categorical data.
*Four preterm pre-eclampsia and one term pre-eclampsia (control) were diagnosed on the basis of gestational hypertension with multiorgan complications without proteinuria.
**Includes spontaneous and iatrogenic preterm birth.
***Small for gestational age was defined as a birthweight below the tenth customised centile (adjusted for maternal height, booking weight, ethnicity and delivery gestation and infant's gender).[22]

predictive performance of the combination of 15-week clinical risk factors and PlGF levels (Table 4).

For a specificity of 95%, the sensitivity, positive likelihood ratios and post-test probability values are shown in Table 4. The algorithm formulae are also described with Table 4. The prevalence of preterm pre-eclampsia in the nulliparous women recruited to the SCOPE study was 1.3%. After screening with clinical risk factors combined with PlGF, the probability of preterm pre-eclampsia in women with a positive test was 11% compared with 0.6% among those with a negative test.

The translation of the predictive algorithm (clinical risk factors with 15-week PlGF) to clinical practice in relation to low-dose aspirin prophylaxis is shown in Table 5. In a theoretical population of 1000 low-risk nulliparous women, 13/1000 would develop preterm pre-eclampsia. Fifty-five women would have a positive screening test and be treated with aspirin. Assuming aspirin prevents 17% of

Table 3. Comparison of plasma angiogenic markers in preterm pre-eclampsia with controls

	No preterm pre-eclampsia (n = 188)		Preterm pre-eclampsia (n = 47)		*P*-value
14–16 weeks					
PlGF* (pg/ml)	55	(33–90)	25	(17–48)	<0.0001
sEng (ng/ml)	62	(54–72)	71	(56–83)	0.005
sFlt1 (pg/ml)	450	(223–848)	543	(253–1134)	0.20
19–21 weeks					
PlGF* (pg/ml)	134	(82–217)	73	(40–137)	<0.0001
sEng (ng/ml)	61	(51–69)	74	(59–87)	<0.0001
sFlt-1 (pg/ml)	426	(227–732)	371	(202–749)	0.92

Data shown as median (interquartile range); Wilcoxon Rank Sum was used to compare cases and controls.
Missing data due to technical reasons in controls,cases: PlGF 14–16 weeks n = 7,0; PlGF 19–21 weeks n = 9,5; Endoglin 19–21 weeks n = 1,0; sFlt-1 19–21 weeks n = 1,0.
*PlGF measured using Triage® – no significant difference in absolute measurements to Delfia® assay (data not shown).

pre-eclampsia cases,[2] then one case would be prevented for every 55 women treated.

Discussion

Main findings

The aim of this study was to assess whether previously implicated clinical risk factors can be combined with the concentration of plasma angiogenic growth factors measured at 14–16 weeks of gestation to provide a potentially useful test for prediction of preterm pre-eclampsia in low-risk nulliparous women. In this study, the best prediction of preterm pre-eclampsia was observed using a combination of PlGF, measured at 15 weeks, with a selection of easily attainable clinical risk variables, blood pressure, a family history of pre-eclampsia and a history or fertility treatment. The combination of uterine artery Doppler (20 weeks), PlGF (15 weeks) and endoglin (20 weeks) was not associated with significantly improved prediction over the combination of PlGF and clinical variables alone. As the SCOPE study did not include an earlier uterine artery Doppler assessment, it is not possible to make a direct comparison with other studies that have used first-trimester Doppler as part of a screening algorithm.[8]

Strengths and weaknesses

Preterm pre-eclampsia was chosen as the outcome in this study because previous studies have shown little association between first-trimester angiogenic marker levels and term pre-eclampsia.[8,10] The small sample size of preterm pre-eclampsia cases is balanced by the certainty of the diagnosis given the depth and detail associated with the data collection and pregnancy tracking. It is inevitable that the models presented will be over fitted to the SCOPE population, especially given the number of cases. The AUC values derived by this analysis are, therefore, likely to represent the highest achievable performance of these combinations in a nulliparous population and ongoing studies will aim to confirm these findings in the entire SCOPE cohort. The study is strengthened by the use of multicentre recruitment

Table 4. Summary of clinical risk factors, angiogenic biomarkers and uterine artery Doppler to predict preterm pre-eclampsia

	ROC AUC	Sensitivity*	Positive likelihood ratio	Post-test probability
PlGF 15 weeks	0.77 (0.69–0.84)	0.22 (0.12–0.35)	4.3 (1.8–9.9)	5 (4–7)
sEng 20 weeks	0.71 (0.62–0.80)	0.28 (0.17–0.43)	5.7 (2.6–12.5)	7 (5–9)
Clinical risk	0.76 (0.67–0.84)	0.34 (0.22–0.48)	6.8 (3.2–14.5)	8 (6–11)
Clinical risk + 15 weeks PlGF	0.84 (0.77–0.91)	0.45 (0.31–0.59)	9.0 (4.4–18.3)	11 (9–13)
Clinical risk + 20 weeks uterine Doppler	0.81 (0.74–0.88)	0.49 (0.35–0.63)	8.9 (4.5–17.3)	11 (9–13)
Clinical risk + 15 weeks PlGF + 20 weeks uterine Doppler	0.85 (0.79–0.92)	0.47 (0.33–0.61)	9.4 (4.6–9.1)	11 (9–14)
Clinical risk + 15 weeks PlGF + 20 weeks uterine Doppler + 20 weeks sEng	0.84 (0.77–0.91)	0.52 (0.38–0.66)	10.5 (5.2–21)	12 (10–15)

MAP, mean arterial pressure taken at 15 weeks.
PlGF was transformed to MoMs for all models; all data are shown with 95% confidence intervals.
*Based on 95% specificity.
Clinical risk factors: −8.4093 + 0.9037 × fertility treatment + 0.7999 × any sister with pre-eclampsia + 0.1030 × MAP; Clinical risk + 15 week PlGF MoM: −7.7769 + 0.7307 × fertility treatment + 0.1047 × MAP − 1.7269 × PlGF; Clinical risk + 20 week uterine Doppler: −13.5946 + 0.8402 × fertility treatment + 0.1039 × MAP + 7.0938 × 20 week mean uterine artery RI; Clinical risk + 15 week PlGF MoM + uterine Doppler 20 week: −12.5382 + 0.1078 × MAP − 1.5658 × PlGF + 6.1087 × 20 week mean uterine artery RI; Clinical risk + 15 week PlGF MoM + 20 week uterine Doppler + 20 week endoglin: −10.4272 + 0.0994 × MAP − 1.1787 × PlGF + 0.0344 × endoglin 20 week + 0.5285 × 20 week bilateral notches of uterine arteries.

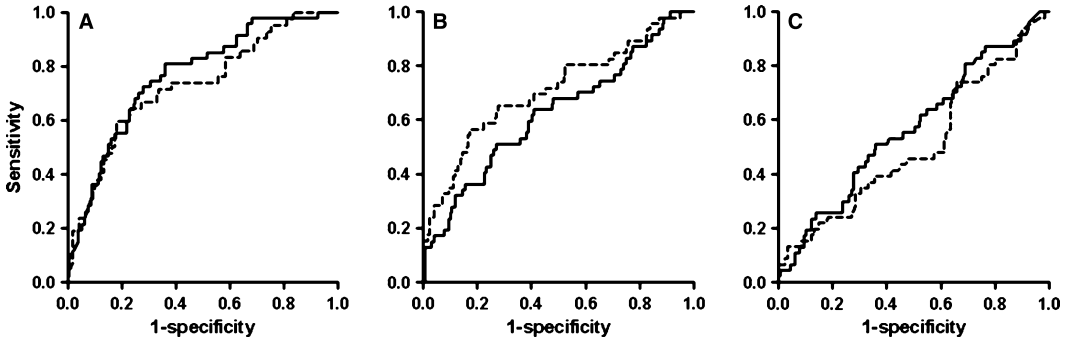

Figure 2. AUC curves for the prediction of preterm pre-eclampsia at 15 weeks (solid line) and 20 weeks (dashed line) of gestation with (A) PlGF (15 weeks AUC 0.77 [0.69–0.84]; 20 weeks AUC 0.73 [0.64–0.81]), (B) endoglin (AUC 0.63 [0.53–0.72]; 0.71 [0.62–0.80]) and (C) sFlt-1 (AUC 0.57 [0.47–0.66]; 0.50 [0.40–0.60]).

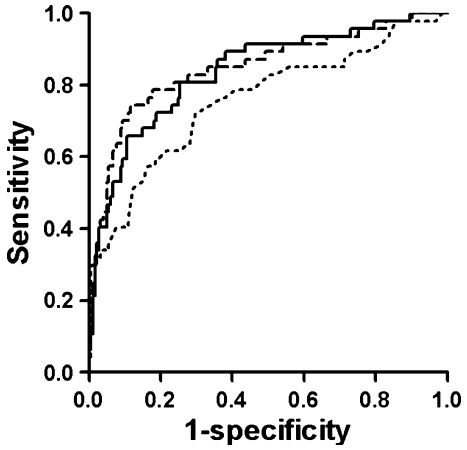

Figure 3. AUC curves for the prediction of preterm pre-eclampsia at 15 weeks using clinical risk variables (AUC 0.76 [0.67–0.84]; dotted line), clinical risk in combination with PlGF at 15 weeks (AUC 0.84 [0.77–0.91]; solid line) and clinical risk in combination with PlGF 15 weeks and uterine artery Doppler mean resistance index at 20 weeks (AUC 0.85 [0.79–0.92]; dashed line).

Table 5. Screening performance in a theoretical population of low-risk nulliparous women (n = 1000) based on the algorithm of clinical risk factors and 15-week PlGF in comparison to application of the existing NICE criteria

	Clinical risk factors and 15-week PlGF	Existing NICE model*
Preterm pre-eclampsia	**Population screened n = 1000**	
Number who develop preterm pre-eclampsia	13	13
Number with positive screening test, treated with aspirin	55	120
Number of true-positive screening tests	6	4
Number of false-positive screening tests	49	116
Number of cases missed	7	9
Number need to treat to prevent one case**	54	196

*Applied to a low-risk nulliparous population.
**Calculated based on a 17% reduction in the risk of pre-eclampsia with low-dose aspirin.[2]

across two continents and the use of a case-cohort design. Inclusion of women with other complications of pregnancy in the 'control' group is extremely important in the assessment of screening tests. Comparison to a control group with no other complications of pregnancy is not applicable to clinical translation as pregnancy outcome is unknown at the time of screening and use of 'normal controls' will artificially inflate the specificity of the test.

In this study, PlGF was measured using the Alere Triage® system. This method is available as a self-contained analysis method that does not require access to clinical laboratory equipment. For comparison purposes, PlGF was also quantified using the Delfia® assay in paired serum samples, to exclude the possibility that the two commonly applied assays were providing different results. There was no significant difference between the two assays, confirming that the performance is marker dependent rather than assay dependent. In contrast to some previous studies,[21] our study quantified PlGF at 14–16 weeks rather than 11–13 weeks. Although this limits an exact comparison of measurements with previous first-trimester reports, the MoM changes between cases and controls were very similar in this study to those shown previously.[21]

Interpretation

Previous studies investigating combinations of clinical risk factors, uterine artery Doppler and PlGF have reported sensitivities of 79–91%, given a specificity of 95%, for the detection of early onset pre-eclampsia (<34 weeks of gestation) and intermediate pre-eclampsia (delivery at 34–36 weeks).[8,9] These studies were performed in a 'general

population' that included women with recognised risk factors, such as a history of pre-existing medical conditions and a history of pre-eclampsia. The presence of these risk factors, known to be strongly associated with pre-eclampsia, may have contributed to the higher AUCs reported. In addition, in multiparous women, the absence of pre-eclampsia in a previous pregnancy is associated with a very high negative predictive value. Furthermore, in keeping with our study, a recent large study of nulliparous women has reported a less optimistic prediction using first-trimester screening.[14] As history of previous pregnancies is not available for women in their first pregnancy, it seems reasonable to target biomarker prediction efforts towards nulliparous women where the burden of disease is greater and prediction using clinical risk parameters is not sufficiently accurate.[3]

Although there is obviously significant room for improvement in screening performance with the addition of further biochemical markers, this model compares favourably with the current risk stratification advocated by NICE.[1] Using current NICE criteria for the identification of women at high risk of developing pre-eclampsia, with a screen positive rate of around 12% in first pregnancies, 28% of the women who developed preterm pre-eclampsia in the SCOPE cohort would have been identified to be at risk in early pregnancy. Using the performance of the clinical risk model presented here, in conjunction with PlGF measurement at 15 weeks gestation, 45% of the preterm pre-eclampsia cases would have been identified. This test also compares favourably with the NICE criteria with a lower screen positive rate of 5% and a higher post-test probability of preterm pre-eclampsia (11% versus 3%).

As randomised studies of over 37 000 women have demonstrated the safety and benefit of prophylactic aspirin if started in the first half of pregnancy for the prevention of pre-eclampsia,[2] current NICE guidance recommends that women identified as being at increased risk of pre-eclampsia are prescribed low-dose aspirin. Given the low prevalence of preterm pre-eclampsia in nulliparous (approximately 1.3%)[3] or general obstetric (<1%) populations,[21] it will require a highly specific test to reduce the number of false-positive tests to a level that the number needed to treat to prevent one case of preterm pre-eclampsia is acceptable. Based on our algorithm incorporating clinical risk factors combined with 15-week PlGF, 54 women would need to be treated to prevent one case of preterm pre-eclampsia.

Conclusion

This study suggests that the addition of PlGF testing at 15 weeks of gestation to clinical risk factor screening could improve our ability to predict preterm pre-eclampsia in this low-risk group. Given the rarity of preterm pre-eclampsia, the number needed to treat with low-dose aspirin to prevent one case of preterm pre-eclampsia was 54. Before clinical implementation, the addition of novel biochemical markers to combinations of clinical risk factors and PlGF will be required to improve screening performance such that the necessary health and economic benefits are achieved.

Disclosure of interests

JM, RN, GD, LP, LK, LM and NS have received consultancy fees (payable to institution) and research expenses as part of an ongoing research collaboration with Alere.

Contribution to authorship

The study was conceived by RN and JM, data analysis was performed by JM, EC and RN. JM, RN, GD, LP, LK, LM and NS contributed to the recruitment of women and outcome data collection. All authors have seen and approved a final version of the manuscript.

Details of ethics approval

Ethics approval was obtained from local ethics committees [New Zealand AKX/02/00/364, Australia REC 1712/5/2008, London and Manchester 06/MRE01/98 and Cork ECM5 (10) 05/02/08].

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical variables available for the multivariate analysis.

Table S2. Adjusted odds ratios for clinical risk factors in the clinical risk and PlGF model. ■

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