DOI:
10.1097/AOG.0000000000001508

Document Version
Peer reviewed version

Diagnostic Biomarkers in Women With Suspected Preeclampsia in a Prospective Multicenter Study

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Sources of funding

Supported by Tommy's Charity (registered charity no 1060508 and SCO39280), the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London, and Alere (San Diego, California). Jenny Myers is supported by Action Medical Research Endowment Fund, the Manchester Biomedical Research Centre and the Greater Manchester Comprehensive Local Research Network. Paul T. Seed's salary is supported by Tommy's Charity. Louise C Kenny is supported by a Science Foundation Ireland Program Grant for INFANT (12/RC/2272).

Financial Disclosure:

Robyn North was principal investigator on a study investigating biomarker prediction of preeclampsia funded by an unrestricted research grant from AlereInc, San Diego, CA (www.alere.com), to the universities in the SCOPE consortium. Robyn North, Jenny Myers, Nigel Simpson, Louise C. Kenny, Christopher W.G. Redman, and Andrew H. Shennan have received honoraria for speaking at an Alere-sponsored symposium at an international conference in 2013. Nigel Simpson and Christopher W.G. Redman have been paid consultants for Alere up to 2013. Louise C. Kenny has a minority shareholding in Metabolomic

Diagnostics, a company with an interest in preeclampsia biomarkers, based on technology developed by her and licensed from University College Cork. Andrew H. Shennan has been a paid consultant for Alere, Roche, and Perkin Elmer up to 2013. The other authors did not report any potential conflicts of interest.

Short title: Diagnostic markers in suspected preeclampsia

Précis: In women with suspected preterm preeclampsia, a single angiogenesis-related biomarker is a useful diagnostic test to determine preeclampsia that requires delivery within 14 days.

Abstract

Objective: To evaluate 47 biomarkers (selected from the current medical literature), in isolation or in combination with placental growth factor (PlGF), to determine the need for delivery within 14 days, in women presenting with suspected preterm preeclampsia.

Methods: In a prospective, multicentre observational study, 47 biomarkers were measured in 423 women presenting with suspected preterm preeclampsia (in two prespecified groups: Group 1 at <35 weeks of gestation and Group 2 presenting between 35^{+0} and 36^{+6} weeks of gestation), to evaluate their ability to determine the primary endpoint: preeclampsia requiring delivery within 14 days. Using factor analysis and stepwise logistic regression, we sought one or more additional biomarkers for optimal determination of the primary endpoint.

Results: In women presenting <35 weeks of gestation (n=286), the best-performing combination of PlGF, podocalyxin, endoglin, procalcitonin (receiver operating curve (ROC) area 0.90; 95% CI 0.86 to 0.93) was not statistically better than PlGF alone (ROC 0.87; 95% CI 0.83 to 0.92; p=0.43) for preeclampsia requiring delivery within 14 days. Two other single markers had test performance that was not significantly different to PlGF (soluble fms-like tyrosine kinase-1 [sflt-1] ROC 0.83; 95% CI 0.78 - 0.88; endoglin ROC 0.83; 95% CI 0.79 - 0.88). Similar findings were found in women presenting between 35^{+0} and 36^{+6} weeks of gestation (n=137): ROC for PlGF alone 0.75 (95%CI 0.67 to 0.83); ROC for PlGF, cystatin, pregnancy-associated plasma protein A (PAPP-A) in combination 0.81 (95% CI 0.74 to 0.88; p=0.40).

Conclusions: This study supports the growing body of evidence that a single angiogenesis-related biomarker (PlGF, sflt-1 or endoglin) alone represents a useful diagnostic test for women presenting with suspected preterm preeclampsia.

Introduction

Preeclampsia is a common disorder affecting between 5-7% of all pregnancies.(1) It remains a major contributor to maternal mortality(1) and accounts for a substantial proportion of low birthweight infants and iatrogenic preterm delivery.(2) Prevalence and morbidity has remained unchanged over the last decade highlighting the need to improve diagnostic(3, 4) and prognostic(5) testing facilitating appropriate resource allocation. Preeclampsia is unique to pregnancy and is characterised by poor placentation(6) and abnormal inflammatory and vascular responses(7) resulting in multi-organ dysfunction.

Presenting symptoms of preeclampsia are often subjective and non-specific with clinical findings based on features of advanced disease or markers of end organ involvement. High blood pressure and urinary protein excretion are typically used to diagnose the disease but both are secondary features of a primary placental problem and subject to measurement error and poor test accuracy.(8) It is currently difficult to distinguish preeclampsia of a severity that requires early delivery from other less serious phenotypes.(9, 10) An accurate biomarker (or panel of biomarkers) to enable prognosis of perinatal complications could have substantial impact on management strategies with the aim of minimising adverse maternal and fetal outcomes.

The aim of this study was to evaluate a wide panel of 47 candidate biomarkers (including those that are currently widely reported and reflect the heterogeneity of the disease) in women presenting preterm with suspected preeclampsia in order to optimise determination of an important clinical outcome, that of preeclampsia requiring delivery within 14 days.

Materials and Methods

A prospective multicentre cohort study was undertaken between January 2011 and February 2012 in seven consultant-led maternity units in the United Kingdom and Ireland.(4) Women were eligible for the study if they had been referred or presented with suspected preeclampsia (i.e. signs or symptoms of preeclampsia), were 20^{+0} to 36^{+6} weeks of gestation with a singleton or twin pregnancy and were aged ≥16 years. Women with confirmed preeclampsia (or with any adverse outcome already present) were not eligible. We undertook a planned analysis reported here on two groups of women: Group 1: presenting prior to 35 weeks of gestation, and Group 2: presenting between 35^{+0} and 36^{+6} weeks of gestation. These gestational age groupings were pre-specified, based on known differences in pathophysiological pathways associated with preterm pre-eclampsia and our prior knowledge of gestational changes of biomarker concentrations related to these pathways. Written informed consent was obtained and baseline demographic and pregnancy-specific information, including blood pressure readings, were entered onto the study database. Blood pressure was taken according to unit guidelines. Blood samples were drawn into ethylenediamine tetra-acetic acid, with consent, at the time of enrolment. The samples were labelled, transported to the laboratory and the plasma was stored until analysis at -80^{o}C. Pregnancy outcomes were determined by case note review with independent adjudication (masked to all biomarker concentrations) for final maternal diagnosis. All hypertensive disorders of pregnancy were defined according to the American College of Obstetricians and Gynaecologists practice bulletin in use at the time of the study.(11) Independent adjudication was undertaken by two senior physicians, masked to biomarker measurements, requiring documentation of end points required to fulfil the diagnostic criteria; disagreement was resolved by a third adjudicator. The predefined adverse maternal outcomes had been

identified for a previous study in preeclampsia by iterative Delphi consensus(10) and have been described in detail elsewhere.(4) All sites managed women (including decision for delivery) in line with the Hypertension in Pregnancy recommendations from the National Institute for Health and Care Excellence.(12)

An initial panel of biomarkers was selected based on either *a priori* knowledge of an association with preeclampsia, a biological role in placentation or a role in cellular mechanisms involved in the pathogenesis of preeclampsia e.g., angiogenesis, inflammation, coagulation. The full list of 47 biomarkers, measured with 57 assays (where potentially biologically important assays of different epitope specificity were available) was generated following a review of the literature, appraisal of selected bibliographies and consultation with medical experts (Appendix 1, available online at http://links.lww.com/xxx).

Plasma samples were tested for Placental Growth Factor (PlGF) using the Triage PlGF Test by trained laboratory staff at the study site where the sample was taken (as previously published). Samples were labelled, and transported to the laboratory where they were spun at 3000 rotations per minute for 10 minutes. The additional 56 biomarker assays were analysed in a central laboratory facility (Alere, San Diego, CA) and full details of assay methods given in Appendix 2, http://links.lww.com/xxx and Appendix 3, http://links.lww.com/xxx. All participants had delivered and pregnancy outcomes recorded before biomarker concentrations were analysed and revealed and all laboratory staff were masked to clinical outcomes.

Standard distributional checks showed high levels of skewness for all 57 assays, consistent with underlying log normal distributions. Logged values of these biomarkers were therefore used. Before considering the pregnancy outcomes, statistical factor analysis of biomarker data was undertaken, reducing the 47 biomarkers into a smaller group of factors. Factor

analysis sorted the biomarkers into a small number of highly correlated groups, without reference to outcome, containing the majority of the information in the full dataset.(13) Factor summary scores were then calculated for all women. Consideration of scree plots and Eigen-values (> two) identified the most important factors for further analysis.(14) These factors were rotated (orthogonal varimax method) so that each factor related strongly (correlation >0.6) to a small number of biomarkers only (factor analysis is displayed in Appendix 4, http://links.lww.com/xxx). Significant factors (and their biomarkers) were identified for further investigation (Appendix 5, http://links.lww.com/xxx). For the multiple logistic regression model, the principal outcome was preeclampsia requiring delivery within 14 days (pre-specified by consensus of clinical investigators). Stepwise logistic regression was used to determine which biomarkers or factors appeared to provide additional information beyond that derived from PlGF and prediction scores were extracted for the best combinations. A comparison of Receiver Operating Curves (ROC) areas of individual biomarkers and combinations was made to see if any of the additional information was both consistent and large enough to be clinically useful. Significance was assessed through use of a non-parametric test which allowed for non-independence of observations on the same participant, with Bonferroni correction for multiple testing.(15)

Some biomarkers, with high uniqueness scores, were not strongly associated with any factor. To investigate whether any of these biomarkers had diagnostic power in addition to that provided by PlGF and biomarkers identified earlier, stepwise logistic regression was undertaken. To avoid excluding a biomarker that may be of potential value, it had to pass a series of tests, so that the chance of a false positive was greatly reduced (rather than using a standard multiple-testing correction to p-values, such as Bonferroni). The biomarker had to be a component of a significant factor, a significant predictor in logistic regression both

alone and after allowing for PlGF and have a ROC area for the combined score significantly greater than PlGF alone. For biomarkers with a substantial proportion of measurements outside the limits of detection, we used a non-parametric test (ROC area) to determine whether the biomarkers had useful predictive power. Where the biomarker measurement (whether due to censoring or lack of predictive ability) was non-informative, it was excluded from further analysis.

Statistical analysis was carried out in the statistical package Stata (version 11.2), College Station Texas, USA. Clinical variables and outcomes were compared using a Wilcoxon rank-sum non-parametric test. The pre-specified sample size was calculated for accurate estimation of the sensitivity (within 10%) and specificity (within 6%) of a biomarker, assumed a sensitivity of 0.90, specificity 0.90, and 95% confidence intervals (2-tailed), for determining the primary endpoint; this required 62 preeclampsia cases and 150 women not meeting the primary endpoint. The study is reported in accordance with STROBE guidelines ().

The study was approved by East London Research Ethics Committee (ref. 10/H0701/117). Participants gave informed consent and the study followed institutional guidelines.

Results

Four hundred twenty three women with enrolment samples and outcome data available were recruited to the study in seven centres across the UK and Ireland between January 2011 and February 2012, 286 women in Group 1 (presenting at 20^{+0} to 34^{+6} weeks of gestation) and 137 women in Group 2 (presenting at 35^{+0} to 36^{+6} weeks of gestation) (Figure 1).

For the 286 women who were enrolled prior to 35^{+0} weeks of gestation, characteristics of the study population at antenatal booking are shown in table 1, subdivided into those that

met the primary outcome (pre-eclampsia requiring delivery within 14 days) and all others. Table 2 shows characteristics of delivery and maternal and neonatal outcome. Table 3 shows the test performance for the most promising individual biomarkers, depicted by ROC areas. PlGF had the highest ROC area (0.87) for determining preeclampsia requiring delivery within 14 days; the ROC areas for sflt-1 (0.83) and endoglin (0.83) were not significantly different to that for PlGF. Addition of further biomarkers to PlGF increased the ROC area by a small, non-significant increment only. The highest test performance for preeclampsia requiring delivery within 14 days was found using a combination of PlGF, podocalyxin, soluble endoglin and procalcitonin, with a ROC area of 0.90, not significantly greater than the ROC area for PlGF alone (0.87; p=0.43). Appendix 6, http://links.lww.com/xxx shows ROC areas for all 47 biomarkers analysed and individual median biomarker concentrations in all women sampled are shown in Appendix 7, http://links.lww.com/xxx. Sensitivity analysis demonstrated that excluding twin pregnancies altered PlGF test performance by <1%.

For women presenting between 35^{+0} and 36^{+6} weeks of gestation (n=137), the characteristics at booking and enrolment are shown in Appendix 8, http://links.lww.com/xxx and those for delivery and pregnancy outcomes in Appendix 9, http://links.lww.com/xxx. ROC areas and individual median biomarker concentrations for the individual biomarkers are given in Appendix 10, http://links.lww.com/xxx and Appendix 11, http://links.lww.com/xxx, respectively. The results follow a similar pattern as for women presenting at earlier gestations. The ROC area for PlGF alone (0.75; 95% CI (0.67 to 0.83)) in determining need for delivery for preeclampsia within 14 days was lower than that achieved in earlier gestations and other angiogenesis-related biomarkers were not significantly different to that for PlGF alone. Integration of soluble fms-like tyrosine kinase-1 (sFlt-1) with PlGF (as a ratio) increased the ROC to 0.77 (95% CI 0.69 to 0.84). The combination of PlGF, pregnancy-

associated plasma protein A and cystatin yielded the highest ROC area of 0.81 (95% CI (0.74 to 0.88) (table 4). Both increments were small and not significant.

Discussion

This prospective multicentre study is a comprehensive direct comparison of diagnostic biomarkers for preeclampsia. The results demonstrate that in women with suspected preeclampsia presenting preterm, use of a single angiogenesis-related biomarker (PlGF, sflt-1 or endoglin) alone represents a useful diagnostic test for determining preeclampsia requiring delivery within 14 days, a relevant endpoint indicating that a clinician has considered that the risks of adverse outcomes associated with ongoing expectant management are outweighed by the risks of delivery.

Suspected hypertensive disorders in pregnancy are the commonest reason for presentation for obstetric assessment in the third trimester of pregnancy. Diagnostic uncertainty is common when women present to obstetric assessment units with one or more signs suggestive of preeclampsia. Women undergo a series of investigations, many of which are poor predictors of the need for delivery or likely adverse outcome. In practice, obstetricians require a test that enables a woman to be triaged, to determine those that require increased surveillance, and those where the likelihood of needing delivery for preeclampsia within fourteen days is very low and outpatient care may be appropriate. Such a test would enable development of safe clinical algorithms and avoid inappropriate intervention or unnecessary maternal anxiety.

PlGF is an angiogenic factor synthesised by the trophoblast, a marker of associated placental dysfunction in preeclampsia, with known low plasma concentrations in the disease.(16) Whilst combining PlGF with some of the other 46 biologically plausible

biomarkers marginally improved the ROC area, the combinations added little to the diagnostic performance of a single biomarker alone. This important negative result demonstrates the diagnostic option of using a single biomarker (over and above a combination of biomarkers) in preterm preeclampsia. These findings are more marked in women presenting prior to 35 weeks of gestation, and are similar, with lesser diagnostic efficacy, in women presenting between 35^{+0} and 36^{+6} weeks of gestation. This probably reflects the inclusion of women who meet the primary outcome definition (preeclampsia with delivery within 14 days) who were delivered routinely at 37 weeks of gestation following national guideline recommendations and not because of a clinician concern over a potential placentally-mediated adverse event.

Strengths of this study include use of seven study sites and a large participant cohort, encompassing a wide demographic and ethnic profile including women with underlying maternal disease. Plasma testing was carried out in a central laboratory ensuring that results were obtained with rigorous quality control. Progressive statistical analysis explored single biomarker predictive power, and compared the impact of combining groups of markers, or using biomarker ratios. A limitation was that test results were not validated in a repeat sample or by comparative testing at a second laboratory.

Previous studies have described other pathophysiologically relevant third trimester markers, including soluble endoglin,(17) or measurement of a ratio such as PlGF/ soluble fms-like tyrosine kinase-1.(3, 5) However, some of these studies have been small or from a single centre, often using a case-control design. Such study design can result in over-fitting and does not provide data indicative of how a biomarker may perform if introduced into clinical practice.

Systematic reviews have indicated that currently utilised tests such as proteinuria,(8) transaminases(18) and uric acid(19) are not good predictors of maternal or fetal complications in women with suspected preeclampsia. The lack of reliable diagnostic tests results in poorly targeted antenatal monitoring and hospitalisation.(20) Development of an improved diagnostic test, using pathophysiologically relevant biomarkers may have advantages over traditional diagnostic measures.(21) A test performed at presentation that enables targeted surveillance for those at increased risk of maternal or fetal complications and provides appropriate reassurance to those who test negative has the potential to assist in the allocation of health resources.(22) Further work is also needed on prognosis of multi-organ maternal complications in established preeclampsia.

Improved detection of placental disease remains a global health priority. Growing evidence suggests the use of angiogenic factors as biomarkers across a range of demographic settings in the prediction of preeclampsia,(4) adverse outcome(23) and placentally related stillbirth.(24) Previous work has shown that women with low or very low PlGF concentrations experienced adverse perinatal outcomes (4) and our findings suggest that increased surveillance should be considered for these women.

We have previously reported that PlGF out-performs disease markers currently in use;(4) this study confirms that use of a single angiogenesis-related biomarker may be clinically useful as a diagnostic test, without the need for combinations (which entail additional cost and complexity).. Biomarkers such as PlGF can be analysed quickly, representing a test that could aid risk stratification of women with suspected preterm preeclampsia. Further research, through randomised controlled trials, is essential to assess how these biomarker measurements can assist in determining (or refuting) diagnosis in preeclampsia, and how

this can improve outcomes for mother and baby through optimal tailored clinical management.

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Table 1: Characteristics of participants at booking and enrolment for women presenting between 20^{+0} and 34^{+6} weeks of gestation (according to diagnosis of preeclampsia). Values given are median (quartiles) or n (%) as appropriate.

Characteristics	**Women with preeclampsia requiring delivery within 14 days n=76**	**All other participants n=210**	**p value**	**All women n=286**
At booking: Age (years)	31.2 (26.8 - 35.6)	32.0 (27.3 - 35.9)	0.84	31.9 (27.0 - 35.8)
Body mass index (kg/m^2)	26.2 (22.8 - 30.1)	29.1 (25.0 - 34.7)	<0.001	28.6 (24.2 - 33.6)
White ethnicity	50 (66)	137 (65)	0.62	187 (65)

Singleton pregnancy	71 (93)	203 (97)	0.27	274 (96)
Highest first trimester systolic BP (mmHg)	120 (110 - 130)	121 (110 - 130)	0.32	120 (110 - 130)
Highest first trimester diastolic BP (mmHg)	70 (65 - 80)	75 (66 - 84)	0.04	74 (66 - 81)
Smoker at booking	11 (15)	42 (21)	0.30	58 (19)
Quit smoking during pregnancy	7 (10)	27 (13)	0.41	34 (12)
Previous medical history:				
Preeclampsia requiring delivery <34 weeks	10 (13)	20 (10)	0.20	30 (11)
Chronic hypertension	7 (10)	38 (19)	0.08	45 (17)
Known SLE or APS	2 (3)	10 (5)	0.44	12 (5)
Pre-existing diabetes mellitus	2 (3)	4 (2)	0.71	6 (2)
Renal disease	5 (7)	14 (7)	0.98	19 (7)
At enrolment:				
Gestational age at sampling (weeks)	32.1 (29.5 - 33.2)	30.9 (26.3 - 33.3)	0.03	31.1 (28.0 - 33.4)
New onset hypertension	53 (70)	101 (48)	<0.001	154 (54)
Worsening of hypertension	14 (18)	42 (20)	0.77	56 (20)
New onset of dipstick	57 (75)	103 (49)	<0.001	160 (56)

proteinuria (1+ or greater)				
Highest systolic BP (mmHg)	150 (140 - 165)	141 (129 - 156)	<0.001	143 (131 - 159)
Highest diastolic BP (mmHg)	97 (88 - 102)	90 (80 - 98)	<0.001	91 (82 - 100)
Alanine transaminase (U/L)	16 (12 - 21)	14 (11 - 19)	0.10	14 (11 - 20)
Creatinine (mg/dl)	0.68 (0.57 – 0.83)	0.55 (0.48 – 0.64)	<0.001	0.58 (0.50 – 0.70)
Uric acid (mg/dl)	5.50 (4.30 - 6.89)	4.03 (3.03 - 4.86)	<0.001	4.32 (3.19 - 5.55)
Platelet count ($x10^9$/l)	221 (179 - 269)	238 (204 - 274)	0.06	234 (197 - 271)

385 BP: blood pressure; SLE: systemic lupus erythematosus; APS: antiphospholipid syndrome.

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Table 2: Characteristics of delivery and maternal and neonatal outcome for women presenting between 20^{+0} and 34^{+6} weeks of gestation. Values given are median (quartiles) or n (%) as appropriate.

Characteristics	Women with preeclampsia requiring delivery within 14 days n=76	All other participants n=210	p value	All women n=286
Onset of labour				
Spontaneous	3 (4)	38 (18)	0.01	41 (14)
Induced	13 (17)	95 (45)	<0.001	108 (38)
Pre-labour caesarean section	59 (78)	75 (36)	<0.001	134 (47)
Mode of delivery				
Spontaneous	3 (4)	67 (32)	<0.001	70 (25)
Assisted vaginal delivery	4 (5)	27 (13)	<0.001	31 (11)
Caesarean section	67 (91)	116 (55)	<0.001	183 (64)
Adverse maternal outcome*	37 (49)	84 (40)	0.11	121 (42)
Gestation at delivery (weeks)	32.9 (30 - 34.4)	37.9 (36 - 39.3)	<0.001	36.9 (33.6 - 38.7)
Enrolment to delivery	6.5 (3.0 – 10.0)	43.5 (25.0 –	<0.001	29.5 (11.0 – 59.0)

interval (days)		74.0)		
Neonatal outcomes	n=71	n=203		n=274
Fetal death	3 (4)	3 (2)	0.19	6 (2)
Neonatal death	2 (3)	0 (0)	<0.001	2 (1)
Birthweight (g)	1460 (1030 - 1740)	2900 (2320 - 3350)	<0.001	2500 (1620 - 3170)
Small for gestational age (<10th birthweight centile)	55 (78)	75 (37)	<0.001	130 (47)
Small for gestational age (<3rd birthweight centile)	49 (69)	47 (23)	<0.001	96 (35)
Small for gestational age (<1st birthweight centile)	38 (54)	30 (15)	<0.001	68 (25)
Adverse perinatal outcome†	34 (48)	26 (13)	<0.001	60 (22)

* Adverse maternal outcome defined as presence of any of the following complications: maternal death, eclampsia, stroke, cortical blindness or retinal detachment, hypertensive encephalopathy, systolic blood pressure ≥160mmHg, myocardial infarction, Intubation (other than for caesarean section), pulmonary oedema, platelets <50×10^{9}/L (without transfusion), disseminated intravascular coagulation, thrombotic thrombocytopenic purpura/ haemolytic uraemic syndrome, hepatic dysfunction (alanine transaminase ≥70IU/L), hepatic haematoma or rupture, acute fatty liver of pregnancy, creatinine >150 µmol/L, renal dialysis, placental abruption, major postpartum haemorrhage, major infection.

† Adverse perinatal outcome defined as presence of any of the following complications: antepartum/ intrapartum fetal or neonatal death, neonatal unit admission for >48 hrs at term, intraventricular haemorrhage, periventricular leucomalacia, seizure, retinopathy of prematurity, respiratory distress syndrome, bronchopulmonary dysplasia or necrotising enterocolitis.

Table 3: ROC areas (95% confidence intervals) for individual biomarkers and combinations (derived from logistic regression) to determine preeclampsia requiring delivery within 14 days of sampling in women presenting for women presenting between 20^{+0} and 34^{+6} weeks of gestation. [ ] indicates low concentration of biomarker/ratio correlated to disease.

Biomarkers or combinations	**ROC areas (95% confidence intervals)**	**P value (vs. PlGF alone)**
[Pregnancy specific plasma protein A] (PAPP-A)	0.65 (0.57 - 0.72)	<0.001
Procalcitonin	0.65 (0.58 - 0.72)	<0.001
Neutrophil gelatinase-associated lipocalin (NGAL)	0.67 (0.61 - 0.74)	<0.001
Cystatin	0.68 (0.61 - 0.75)	<0.001
Brain natriuretic peptide (BNP)	0.75 (0.69 - 0.82)	<0.001
Interleukin-1 receptor-like 1 (ST2)	0.76 (0.85 - 0.93)	<0.001
Endoglin	0.83 (0.79 - 0.88)	0.08
Soluble fms-like tyrosine kinase-1 (sFlt-1)	0.83 (0.78 - 0.88)	0.07
[Placental growth factor] (PlGF)	0.87 (0.83 - 0.92)	-
Combinations		
[PlGF/sFlt-1 ratio]	0.88 (0.83 - 0.91)	>0.99
[PlGF], Tyrosine kinase (C-Met)	0.88 (0.83 - 0.91)	>0.99
[PlGF/endoglin ratio]	0.88 (0.84 - 0.92)	>0.99
[PlGF], endoglin	0.88 (0.84 - 0.92)	>0.99
[PlGF], ST2	0.89 (0.85 - 0.93)	>0.99

[PlGF], procalcitonin	0.89 (0.84 - 0.92)	0.86
[PlGF], Cystatin, PAPP-A	0.89 (0.85 - 0.93)	>0.99
[PlGF], Podocalyxin, BNP, procalcitonin	0.90 (0.86 - 0.93)	0.23
[PlGF], Podocalyxin, endoglin, procalcitonin	0.90 (0.86 - 0.93)	0.43

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414 **Table 4: ROC areas (95% confidence intervals) for individual biomarkers and combinations**

415 **(derived from logistic regression) to determine preeclampsia requiring delivery within 14**

416 **days of sampling in women presenting between 35^{+0} and 36^{+6} weeks of gestation. [ ]**

417 **indicates low concentrations of biomarker correlated to disease.**

Biomarkers or combinations	**ROC areas (95% confidence intervals)**	**P value (vs. PlGF alone)**
Cystatin	0.64 (0.55 - 0.73)	0.11
[Pregnancy specific plasma protein A] (PAPP-A)	0.66 (0.58 - 0.75)	0.12
Neutrophil gelatinase-associated lipocalin (NGAL)	0.67 (0.59 - 0.76)	0.22
Brain natriuretic peptide (BNP)	0.70 (0.61 - 0.78)	0.35
Interleukin-1 receptor-like 1 (ST2)	0.71 (0.63 - 0.79)	0.50
Endoglin	0.71 (0.63 - 0.80)	0.60
Soluble fms-like tyrosine kinase-1 (sFlt-1)	0.75 (0.67 - 0.83)	0.88
[Placental growth factor] (PlGF)	0.75 (0.67 - 0.83)	
Combinations		
[PlGF], procalcitonin	0.73 (0.65 - 0.81)	>0.99
[PlGF], endoglin	0.75 (0.67 - 0.83)	>0.99
[PlGF], Podocalyxin, BNP, procalcitonin	0.76 (0.68 - 0.84)	>0.99
[PlGF], Podocalyxin, sEng, procalcitonin	0.76 (0.68 - 0.83)	>0.99
[PlGF/sFlt-1 ratio]	0.77 (0.69 - 0.84)	>0.99
[PlGF/endoglin ratio]	0.77 (0.66 - 0.82)	>0.99
[PlGF], Cystatin, [PAPP-A]	0.81 (0.74 - 0.88)	0.40

Figure legends

Figure 1: Participant flow diagram