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1 **Diagnostic Biomarkers in Women With Suspected Preeclampsia in a Prospective**

2 **Multicenter Study**

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51 *report any potential conflicts of interest.*

52 **Short title: Diagnostic markers in suspected preeclampsia**

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55 **Précis:** In women with suspected preterm preeclampsia, a single angiogenesis-related

56 biomarker is a useful diagnostic test to determine preeclampsia that requires delivery within

57 14 days.

58

59

60 **Abstract**

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

64

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

72

73 **Results:** In women presenting <35 weeks of gestation (n=286), the best-performing  
74 combination of PIGF, podocalyxin, endoglin, procalcitonin (receiver operating curve (ROC)  
75 area 0.90; 95% CI 0.86 to 0.93) was not statistically better than PIGF alone (ROC 0.87; 95% CI  
76 0.83 to 0.92; p=0.43) for preeclampsia requiring delivery within 14 days. Two other single  
77 markers had test performance that was not significantly different to PIGF (soluble fms-like  
78 tyrosine kinase-1 [sflt-1] ROC 0.83; 95% CI 0.78 - 0.88; endoglin ROC 0.83; 95% CI 0.79 -  
79 0.88). Similar findings were found in women presenting between 35<sup>+0</sup> and 36<sup>+6</sup> weeks of  
80 gestation (n=137): ROC for PIGF alone 0.75 (95%CI 0.67 to 0.83); ROC for PIGF, cystatin,  
81 pregnancy-associated plasma protein A (PAPP-A) in combination 0.81 (95% CI 0.74 to 0.88;  
82 p=0.40).

83

84 **Conclusions:** This study supports the growing body of evidence that a single angiogenesis-

85 related biomarker (PlGF, sFlt-1 or endoglin) alone represents a useful diagnostic test for

86 women presenting with suspected preterm preeclampsia.

87

88

89 **Introduction**

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

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

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

111



112 **Materials and Methods**

113 A prospective multicentre cohort study was undertaken between January 2011 and February  
114 2012 in seven consultant-led maternity units in the United Kingdom and Ireland.(4) Women  
115 were eligible for the study if they had been referred or presented with suspected  
116 preeclampsia (i.e. signs or symptoms of preeclampsia), were 20<sup>+0</sup> to 36<sup>+6</sup> weeks of gestation  
117 with a singleton or twin pregnancy and were aged  $\geq 16$  years. Women with confirmed  
118 preeclampsia (or with any adverse outcome already present) were not eligible. We  
119 undertook a planned analysis reported here on two groups of women: Group 1: presenting  
120 prior to 35 weeks of gestation, and Group 2: presenting between 35<sup>+0</sup> and 36<sup>+6</sup> weeks of  
121 gestation. These gestational age groupings were pre-specified, based on known differences  
122 in pathophysiological pathways associated with preterm pre-eclampsia and our prior  
123 knowledge of gestational changes of biomarker concentrations related to these pathways.  
124 Written informed consent was obtained and baseline demographic and pregnancy-specific  
125 information, including blood pressure readings, were entered onto the study database.  
126 Blood pressure was taken according to unit guidelines. Blood samples were drawn into  
127 ethylenediamine tetra-acetic acid, with consent, at the time of enrolment. The samples were  
128 labelled, transported to the laboratory and the plasma was stored until analysis at  $-80^{\circ}\text{C}$ .  
129 Pregnancy outcomes were determined by case note review with independent adjudication  
130 (masked to all biomarker concentrations) for final maternal diagnosis. All hypertensive  
131 disorders of pregnancy were defined according to the American College of Obstetricians and  
132 Gynaecologists practice bulletin in use at the time of the study.(11) Independent  
133 adjudication was undertaken by two senior physicians, masked to biomarker measurements,  
134 requiring documentation of end points required to fulfil the diagnostic criteria; disagreement  
135 was resolved by a third adjudicator. The predefined adverse maternal outcomes had been

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

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

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

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

160 analysis sorted the biomarkers into a small number of highly correlated groups, without  
161 reference to outcome, containing the majority of the information in the full dataset.(13)  
162 Factor summary scores were then calculated for all women. Consideration of scree plots and  
163 Eigen-values (> two) identified the most important factors for further analysis.(14) These  
164 factors were rotated (orthogonal varimax method) so that each factor related strongly  
165 (correlation >0.6) to a small number of biomarkers only (factor analysis is displayed in  
166 Appendix 4, <http://links.lww.com/xxx>). Significant factors (and their biomarkers) were  
167 identified for further investigation (Appendix 5, <http://links.lww.com/xxx>). For the multiple  
168 logistic regression model, the principal outcome was preeclampsia requiring delivery within  
169 14 days (pre-specified by consensus of clinical investigators). Stepwise logistic regression  
170 was used to determine which biomarkers or factors appeared to provide additional  
171 information beyond that derived from PIGF and prediction scores were extracted for the  
172 best combinations. A comparison of Receiver Operating Curves (ROC) areas of individual  
173 biomarkers and combinations was made to see if any of the additional information was both  
174 consistent and large enough to be clinically useful. Significance was assessed through use of  
175 a non-parametric test which allowed for non-independence of observations on the same  
176 participant, with Bonferroni correction for multiple testing.(15)

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

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

190         Statistical analysis was carried out in the statistical package Stata (version 11.2),  
191 College Station Texas, USA. Clinical variables and outcomes were compared using a Wilcoxon  
192 rank-sum non-parametric test. The pre-specified sample size was calculated for accurate  
193 estimation of the sensitivity (within 10%) and specificity (within 6%) of a biomarker, assumed  
194 a sensitivity of 0.90, specificity 0.90, and 95% confidence intervals (2-tailed), for determining  
195 the primary endpoint; this required 62 preeclampsia cases and 150 women not meeting the  
196 primary endpoint. The study is reported in accordance with STROBE guidelines ().  
197 The study was approved by East London Research Ethics Committee (ref. 10/H0701/117).  
198 Participants gave informed consent and the study followed institutional guidelines.

199

## 200 **Results**

201 Four hundred twenty three women with enrolment samples and outcome data available  
202 were recruited to the study in seven centres across the UK and Ireland between January  
203 2011 and February 2012, 286 women in Group 1 (presenting at 20<sup>+0</sup> to 34<sup>+6</sup> weeks of  
204 gestation) and 137 women in Group 2 (presenting at 35<sup>+0</sup> to 36<sup>+6</sup> weeks of gestation) (Figure  
205 1).

206 For the 286 women who were enrolled prior to 35<sup>+0</sup> weeks of gestation, characteristics of  
207 the study population at antenatal booking are shown in table 1, subdivided into those that

208 met the primary outcome (pre-eclampsia requiring delivery within 14 days) and all others.

209 Table 2 shows characteristics of delivery and maternal and neonatal outcome. Table 3 shows

210 the test performance for the most promising individual biomarkers, depicted by ROC areas.

211 PIGF had the highest ROC area (0.87) for determining preeclampsia requiring delivery within

212 14 days; the ROC areas for sflt-1 (0.83) and endoglin (0.83) were not significantly different to

213 that for PIGF. Addition of further biomarkers to PIGF increased the ROC area by a small, non-

214 significant increment only. The highest test performance for preeclampsia requiring delivery

215 within 14 days was found using a combination of PIGF, podocalyxin, soluble endoglin and

216 procalcitonin, with a ROC area of 0.90, not significantly greater than the ROC area for PIGF

217 alone (0.87;  $p=0.43$ ). Appendix 6, <http://links.lww.com/xxx> shows ROC areas for all 47

218 biomarkers analysed and individual median biomarker concentrations in all women sampled

219 are shown in Appendix 7, <http://links.lww.com/xxx>. Sensitivity analysis demonstrated that

220 excluding twin pregnancies altered PIGF test performance by <1%.

221 For women presenting between 35<sup>+0</sup> and 36<sup>+6</sup> weeks of gestation (n=137), the characteristics

222 at booking and enrolment are shown in Appendix 8, <http://links.lww.com/xxx> and those for

223 delivery and pregnancy outcomes in Appendix 9, <http://links.lww.com/xxx>. ROC areas and

224 individual median biomarker concentrations for the individual biomarkers are given in

225 Appendix 10, <http://links.lww.com/xxx> and Appendix 11, <http://links.lww.com/xxx>,

226 respectively. The results follow a similar pattern as for women presenting at earlier

227 gestations. The ROC area for PIGF alone (0.75; 95% CI (0.67 to 0.83)) in determining need for

228 delivery for preeclampsia within 14 days was lower than that achieved in earlier gestations

229 and other angiogenesis-related biomarkers were not significantly different to that for PIGF

230 alone. Integration of soluble fms-like tyrosine kinase-1 (sFlt-1) with PIGF (as a ratio)

231 increased the ROC to 0.77 (95% CI 0.69 to 0.84). The combination of PIGF, pregnancy-

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

234

## 235 **Discussion**

236 This prospective multicentre study is a comprehensive direct comparison of diagnostic  
237 biomarkers for preeclampsia. The results demonstrate that in women with suspected  
238 preeclampsia presenting preterm, use of a single angiogenesis-related biomarker (PIGF, sflt-  
239 1 or endoglin) alone represents a useful diagnostic test for determining preeclampsia  
240 requiring delivery within 14 days, a relevant endpoint indicating that a clinician has  
241 considered that the risks of adverse outcomes associated with ongoing expectant  
242 management are outweighed by the risks of delivery.

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

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

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

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

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

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

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

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



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

304

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382 **Table 1: Characteristics of participants at booking and enrolment for women presenting**  
 383 **between 20<sup>+0</sup> and 34<sup>+6</sup> weeks of gestation (according to diagnosis of preeclampsia). Values**  
 384 **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
<b>At booking:</b>				
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 <sup>2</sup> )	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)
<b>Previous medical history:</b>				
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)
<b>At enrolment:</b>				
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 <sup>9</sup> /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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388 **Table 2: Characteristics of delivery and maternal and neonatal outcome for women**

389 **presenting between 20<sup>+0</sup> and 34<sup>+6</sup> weeks of gestation. Values given are median (quartiles)**

390 **or n (%) as appropriate.**

<b>Characteristics</b>	<b>Women with preeclampsia requiring delivery within 14 days n=76</b>	<b>All other participants n=210</b>	<b>p value</b>	<b>All women n=286</b>
<b>Onset of labour</b>				
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)
<b>Mode of delivery</b>				
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)		
<b>Neonatal outcomes</b>	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 (<10 <sup>th</sup> birthweight centile)	55 (78)	75 (37)	<0.001	130 (47)
Small for gestational age (<3 <sup>rd</sup> birthweight centile)	49 (69)	47 (23)	<0.001	96 (35)
Small for gestational age (<1 <sup>st</sup> birthweight centile)	38 (54)	30 (15)	<0.001	68 (25)
Adverse perinatal outcome†	34 (48)	26 (13)	<0.001	60 (22)

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



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

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

<b>Biomarkers or combinations</b>	<b>ROC areas (95% confidence intervals)</b>	<b>P value (vs. PIGF alone)</b>
[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] (PIGF)	0.87 (0.83 - 0.92)	-
<b>Combinations</b>		
[PIGF/sFlt-1 ratio]	0.88 (0.83 - 0.91)	>0.99
[PIGF], Tyrosine kinase (C-Met )	0.88 (0.83 - 0.91)	>0.99
[PIGF/endoglin ratio]	0.88 (0.84 - 0.92)	>0.99
[PIGF], endoglin	0.88 (0.84 - 0.92)	>0.99
[PIGF], ST2	0.89 (0.85 - 0.93)	>0.99

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[PIGF], procalcitonin	0.89 (0.84 - 0.92)	0.86
[PIGF], Cystatin, PAPP-A	0.89 (0.85 - 0.93)	>0.99
[PIGF], Podocalyxin, BNP, procalcitonin	0.90 (0.86 - 0.93)	0.23
[PIGF], 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<sup>+0</sup> and 36<sup>+6</sup> weeks of gestation. [ ]**

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

<b>Biomarkers or combinations</b>	<b>ROC areas (95% confidence intervals)</b>	<b>P value (vs. PIGF alone)</b>
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] (PIGF)	0.75 (0.67 - 0.83)	
<b>Combinations</b>		
[PIGF], procalcitonin	0.73 (0.65 - 0.81)	>0.99
[PIGF], endoglin	0.75 (0.67 - 0.83)	>0.99
[PIGF], Podocalyxin, BNP, procalcitonin	0.76 (0.68 - 0.84)	>0.99
[PIGF], Podocalyxin, sEng, procalcitonin	0.76 (0.68 - 0.83)	>0.99
[PIGF/sFlt-1 ratio]	0.77 (0.69 - 0.84)	>0.99
[PIGF/endoglin ratio]	0.77 (0.66 - 0.82)	>0.99
[PIGF], Cystatin, [PAPP-A]	0.81 (0.74 - 0.88)	0.40

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419 **Figure legends**

420 Figure 1: Participant flow diagram