The final version of this paper was published in *Pregnancy Hypertension* 2013;3:215-221

First trimester screening of serum soluble fms-like tyrosine kinase-1 and placental growth factor predicting hypertensive disorders of pregnancy

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ABSTRACT

Objective: To assess the accuracy of first trimester soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) in predicting pregnancy hypertension and pre-eclampsia; and compare with the accuracy of routinely collected maternal and clinical risk factors.

Study design: In this population-based cohort study, serum sFlt-1 and PlGF levels were measured in first trimester in 2,681 women with singleton pregnancies in New South Wales, Australia.

Main outcome measures: Prediction of pregnancy hypertension and pre-eclampsia.

Results: There were 213 (7.9%) women with pregnancy hypertension, including 68 (2.5%) with pre-eclampsia. The area under the curve (AUC) for both sFlt-1 and PlGF was not different from chance, but combined was 0.55 (P=0.005). Parity and previous diagnosed hypertension had better predictive accuracy than serum biomarkers (AUC=0.64, P<0.001) and the predictive accuracy for all maternal and clinical information was fair (AUC=0.70, P<0.001 for pregnancy hypertension and AUC=0.74, P<0.001 for pre-eclampsia). Adding sFlt-1 and PlGF to maternal risk factors did not improve the ability of the models to predict pregnancy hypertension or pre-eclampsia.

Conclusions: Maternal first trimester serum concentrations of sFlt-1 and PlGF do not predict hypertensive disorders in pregnancy any better than routinely collected clinical and maternal risk factor information. Screening for sFlt-1 and PlGF levels in early pregnancy would not identify those pregnancies at-risk.

Key words: fms-like tyrosine kinase-1, placental growth factor, first trimester, pregnancy hypertension, pre-eclampsia, predictive accuracy

INTRODUCTION

Hypertensive disorders of pregnancy have a major impact on maternal health and are responsible for 9% - 25% of deaths worldwide [1] and encompass two different conditions. Chronic hypertension has onset prior to pregnancy or is diagnosed prior to 20 weeks gestation. Pregnancy hypertension has onset from 20 weeks gestation and ranges from hypertension alone (gestational hypertension) through proteinuria and multi-organ dysfunction (pre-eclampsia) to seizures (eclampsia). While these broad classifications are widely accepted, the diagnostic criteria for each subgroup vary internationally. Pregnancy hypertension and pre-eclampsia are of particular interest because if women at risk can be identified early in pregnancy this would allow ample time for monitoring and implementing preventive strategies.

The pathogenesis of pre-eclampsia involves inadequate remodelling of spiral arteries during placental development [2], influenced by imbalances in expression of pro-angiogenic factors such as placental growth factor (PlGF) and anti-angiogenic fms-like tyrosine kinase-1 (sFlt-1) receptor [3]. Serum concentrations of sFlt-1, PlGF and other biomarkers of placental development have been suggested to have predictive value along with several maternal and clinical risk factors that can also help identify women at-risk [4, 5]. However, results have come mostly from small studies [6], and have been inconsistent and not reliable enough for implementation in routine clinical practice. Evaluation of biomarkers for their clinical utility needs to assess the added benefit they offer to risks that can be ascertained from an antenatal booking history. The aim of this study was to assess the accuracy of first trimester fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), both alone and in combination, in predicting pregnancy hypertension and pre-eclampsia in a population-based cohort; and compare them with the accuracy of routinely collected maternal and clinical risk factors.

MATERIALS AND METHODS

Study population and sample testing

The study population included pregnant women attending first trimester Down syndrome screening between July and October 2006 in New South Wales (NSW), Australia. Serum samples were collected by the Pacific Laboratory Medicine Services (PaLMs), and then archived and stored at -80 degrees Celsius. During this period this was the state's only public screening service and received samples from throughout NSW.

Serum samples for this study were thawed and serum levels of sFlt-1 were measured using a commercially available Quantikine ELISA kit (R&D Systems, Minneapolis, USA) while PlGF was measured by an automated immunoassay using commercially available kits (AutoDELFIA PerkinElmer Inc. Turku, Finland). Intra-assay and inter-assay coefficients of variation were <12% and the reported analytic sensitivity of the assay was 7.7 - 1980 pg/ml for sFlt-1 and 0.7 – 168 pg/ml for PlGF. Laboratory scientists were blinded to pregnancy outcomes.

Data sources

The laboratory database contained maternal information for those with archived serum samples and women's corresponding pregnancy and birth outcomes were ascertained from the Perinatal Data Collection (PDC) and the Admitted Patient Data Collection (APDC), all three sources were then combined via record linkage. The PDC is a statutory surveillance system of all births in NSW of at least 400 grams birth weight, or at least 20 weeks gestation and includes demographic, medical and obstetric information on the mother, labour, delivery and birth outcomes. The APDC is a census of all patient hospital admissions from NSW public and private hospitals, with records for both mothers and liveborn infants. It holds demographic, clinical and health services information for each admission. Relevant diagnoses and procedures are also recorded for each hospital admission

and coded according to the International Classification of Diseases version 10 – Australian Modification (ICD10-AM) and Australian Classification of Healthcare Interventions, respectively.

In Australia unit record data from multiple datasets cannot be produced because unique identifiers are not available for record linkage. Therefore, probabilistic linkage methods are used [7, 8]. This involves a complex process of blocking and matching combinations of selected variables (such as name, date of birth, address and hospital) using record-linkage software [9]. The validity of the probabilistic record linkage is extremely high with less than 1% of records having an incorrect match [8, 9]. Record linkage was conducted by The NSW Centre for Health Record Linkage (CHeReL) and identifying information are removed before the data are sent to researchers. The CHeReL assesses the linkage quality for each study, and for this study there were <5/1000 missed links and <2/1000 false positive links. The study was approved by the NSW Population and Health Services Research Ethics Committee.

Study outcomes included were pregnancy hypertension and pre-eclampsia and to maximize ascertainment, information was identified from both the APDC and PDC data [10, 11]. Pre-eclampsia (regardless of severity) and any pregnancy hypertension (pre-eclampsia or gestational hypertension) were determined either if 'Yes' was recorded in response to the relevant questions (proteinuric or non proteinuric hypertension with onset >20 weeks) in the PDC record, or if any APDC record had a diagnosis of gestational hypertension (ICD10-AM: O13 and O16), pre-eclampsia (O11 and O14) or eclampsia (O15) [11]. The key maternal and clinical risk factors used in this analysis included maternal age and weight (kilograms) ascertained at the time of first trimester screening, parity (nulliparous/multiparous), smoking during pregnancy, any previously diagnosed hypertension (chronic or pregnancy) or high blood pressure, any previously diagnosed diabetes (pre gestational or gestational), country of birth and socio-economic disadvantage quintile. Socio-economic disadvantage was determined using the Socio-Economic Indexes for Areas

(SEIFA) relative disadvantage scores developed by the Australian Bureau of Statistics (ABS) [12]. Information on Pregnancy Associated Plasma Protein A (PAPP-A) from laboratory data (used for Down syndrome screening) was also available for analysis. Only factors that are well and accurately reported were included in the analyses [13]. Maternal weight was missing in 570 (21%) of the records. Multiple imputation was used to account for the missing maternal weight, a technique that predicts missing values using existing values from other variables [14]. Other missing data were infrequent: there were no records with missing maternal age, parity, country of birth or socio-economic disadvantage. Smoking was missing in 29 records (1.1%) and there were 5 missing records for PAPP-A (0.2%) which were excluded from the analysis.

Statistical analysis

Comparison of maternal characteristics and concentrations of sFlt-1 and PlGF between women with and without each clinical outcome was performed using contingency tables, student's *t*-test or Wilcoxon-rank sum test for categorical, normal or non-normally distributed data, respectively. As sFlt-1 and PlGF varied by gestational age, weight and smoking status, levels were standardized using multiple of the median (MoM) as described by Cuckle and Wald [15]. Logarithmic transformation of sFlt-1 and PlGF MoM was used to produce Gaussian distributions.

Univariate logistic regression analysis was performed to assess associations between serum biomarkers and adverse pregnancy outcomes. Multivariate logistic regression analysis was then conducted to evaluate serum biomarkers taking into account maternal factors identified in the literature to be associated with the outcomes of interest (parity, weight, previous diagnosis of any hypertension). Separate models were conducted, firstly evaluating just serum biomarkers alone, then serum biomarkers combined, then maternal and clinical factors only (excluding biomarkers) and finally a combined model including both serum biomarkers and maternal risk factors. Each of these models were compared to determine whether serum biomarker levels provided any additional

information to maternal and clinical risk factors in predicting pregnancy hypertension and pre-eclampsia, by evaluating the differences in maximum likelihood estimates from each model using the likelihood ratio test (X^2).

The diagnostic performance of the models was determined by examining the area under the Receiver Operating Characteristic (ROC) curves (AUC). A standardized scale was then used to assess the AUC results [16], where an AUC of 1 represents a perfect test, 0.9 – <1 an excellent test, 0.8 – <0.9 a good test, 0.7 – <0.8 a fair test, 0.6 – <0.7 a poor test and 0.5 – <0.6 a worthless test. AUC results were also examined to determine whether models performed better than chance (0.5). Finally, estimates of predictive accuracy at a fixed 5% false positive rate were calculated including sensitivity, specificity, positive (PPV), negative predictive values (NPV) and positive likelihood ratio with exact binominal confidence intervals. Models and predictive accuracy were examined among all women and for a sub-group of nulliparous women that have increased risk of pregnancy hypertension or pre-eclampsia [2, 5]. A *P*-value of <0.05 was considered to be statistically significant and analyses performed using SAS software 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 2,973 serum samples were tested for sFlt-1 and PlGF; with health information relevant to the pregnancy available for 2,782 (93.6%) samples. We excluded 101 women whose blood sample was taken before 10 or after 14 weeks gestation, had a medical abortion, had a twin pregnancy or had an infant with a major congenital anomaly. A total of 2,681 women were included in the analysis. Levels of sFlt-1 and PlGF were outside the limits of assay detection for 40 and 6 women, respectively. Table 1 presents the maternal characteristics and biomarker levels by pregnancy outcome. The mean (SD) maternal age was 32.8 (4.6) years, mean maternal weight was 66.9 (12.7) kg, 1,182 (44.8%) women were nulliparous and 162 (6.1%) smoked during pregnancy. Compared to unaffected pregnancies, women with pregnancy hypertension or pre-eclampsia were heavier,

were more likely to be having their first baby and to have been previously diagnosed with hypertension.

Median [inter quartile range (IQR)] serum levels of sFlt-1 and PlGF for the total cohort were 286.4 (167.1 – 466.8) pg/ml and 23.9 (18.1 – 31.5) pg/ml, respectively. There were 213 (7.9%) women diagnosed with pregnancy hypertension, including 68 (2.5%) with pre-eclampsia. Compared with unaffected pregnancies (median PlGF: 24.1, IQR: 18.3 – 31.7 pg/ml), median levels of PlGF in first trimester were significantly lower for women subsequently diagnosed with pregnancy hypertension (median PlGF 21.3, IQR: 16.9 - 28.0 pg/ml; P<0.001). Compared with unaffected pregnancies, women with pregnancy hypertension and pre-eclampsia had a tendency to have lower sFlt-1 levels, but differences were not significant (Table1).

Figure 1 presents the distribution of log sflt-1 (MoM) and log PlGF (MoM) for women with and women without pregnancy hypertension and pre-eclampsia. There was no difference in the distribution of log PlGF and log sFlt-1 comparing women with and without pregnancy hypertension and pre-eclampsia. Table 2 presents the predictive accuracy results for pregnancy hypertension and pre-eclampsia for all women. The area under the curve (AUC) for univariate models evaluating individual biomarkers was no different to chance, but for all three biomarkers combined, AUC was 0.55 (P=0.005). Parity and previous diagnosed hypertension had better predictive accuracy than serum biomarkers (AUC=0.64, P<0.001) and predictive accuracy for all maternal and clinical information was fair (AUC=0.70, P<0.001 for pregnancy hypertension and AUC=0.74, P<0.001 for pre-eclampsia). Adding serum biomarkers to maternal risk factors did not improve the ability of the models to predict pregnancy hypertension or pre-eclampsia (X^2=2.70, P=0.10 for GH; and X^2=1.24, P=0.27 for pre-eclampsia).

Table 3 presents the predictive accuracy results for pregnancy hypertension and pre-eclampsia for nulliparous women only. The AUC results for individual biomarkers were similar compared to those for all women. Maternal weight had better predictive accuracy than serum biomarkers (AUC=0.61, P<0.001) and predictive accuracy of all maternal and clinical information was similar to all women (Table 3). Including serum biomarkers with maternal risk factors did not improve the ability of the models to predict pregnancy hypertension or pre-eclampsia (X^2=3.00, P=0.08 for pregnancy hypertension; and X^2=0.97, P=0.32 for pre-eclampsia) (Table 3). In analyses for all and for nulliparous women, the positive likelihood ratio results for maternal and clinical risk factors were superior, ranging between 4.09 and 5.25 (Tables 2 and 3).

DISCUSSION

This is one of the largest studies to investigate the accuracy of sFlt-1 and PlGF in early pregnancy in predicting pregnancy hypertension and pre-eclampsia. It also provides an important comparison of the utility of serum biomarkers with maternal and clinical risk factors. Although sFlt-1 and PlGF levels were generally lower among women subsequently diagnosed with pregnancy hypertension and pre-eclampsia, our results indicate that the predictive accuracies of first trimester serum concentrations of sFlt-1 and PlGF were insufficient in predicting these outcomes. Clinical and maternal risk factors had fair predictive accuracy and outperformed a combination of these first trimester serum biomarkers. Adding serum sFlt-1, PlGF and PAPP-A levels to risk factors did not improve the accuracy of models in predicting pregnancy hypertension and pre-eclampsia, even when limiting the analysis to nulliparous women.

Consistent with most previous studies we found little or no difference in sFlt-1 or PlGF levels in first trimester between women with and without subsequent pre-eclampsia [3, 17-22]; and poor accuracy in predicting any pre-eclampsia or pre-eclampsia >34 weeks [23-25]. This includes a large population-based prospective cohort study of 7,519 women, highlighting little association and no

potential predictive ability between sFlt-1 or PlGF and pre-eclampsia [3]. In contrast, three studies reported a potential utility of sFlt-1 or PlGF levels in first trimester for predicting any pre-eclampsia or pre-eclampsia <34 weeks (based on a 10% fixed false positive rate, sensitivity ranging between 0.33 and 0.58 and AUC between 0.65 and 0.83, respectively) [26-28]. Promising results have been also reported for PlGF in predicting early (<34 weeks) or severe pre-eclampsia (based on a 5% fixed false positive rate, sensitivity: 0.28 - 0.30; AUC: 0.75 - 0.80, respectively) [26, 29, 30], however, we could not assess the accuracy for this outcome due to the low number of cases in our cohort. The accuracy of sFlt-1 and PlGF alone in predicting pre-eclampsia in nulliparous women has been investigated by three other studies [30-32], in addition to ours. In all studies, results for sFlt-1 were comparable with ours, but in other studies PlGF performed better in predicting pre-eclampsia in this sub-group of women. The studies reported an AUC for PlGF in predicting any pre-eclampsia ranging between 0.61 and 0.65 [30, 31] and an AUC of 0.77 for pre-eclampsia <37 weeks [32]. Factors that may influence variation in predictive accuracy results and biomarker concentrations include the timing of the sampling, timing of the onset of disease and whether levels were standardized or not to MoM values.

The main issue attributed to the lack of predictive ability of sFlt-1 and PlGF in early pregnancy is that imbalances between pro and anti angiogenic factors involved in the pathogenesis of pre-eclampsia, may not be expressed until later in pregnancy. Longitudinal studies of serum sFlt-1 and PlGF [3, 20, 22, 24] have demonstrated that the association of levels with pre-eclampsia strengthens with the course of pregnancy, but that these would not be clinically useful in predicting pre-eclampsia until third trimester [24]. Furthermore, better predictive accuracy has been reported with testing in second trimester [33], but screening at 2nd or 3rd trimester may be too late for preventive interventions to be effective. The potential advantages of first trimester screening include the opportunity to incorporate an additional test into existing, routine antenatal testing for identification of at-risk pregnancies for closer surveillance. In addition, early implementation of dietary and

lifestyle interventions [34] or low-dose aspirin [35] in these pregnancies, may reduce the risk of pre-eclampsia.

Compared with serum biomarker information alone, we found maternal and clinical risk factors, specifically parity, previously diagnosed hypertension and maternal weight provide greater predictive value. And, when sFlt-1 or PlGF information is added to these combined, neither biomarker provided any additional predictive information. In other studies, the addition of PlGF to maternal risk factors improves the predictive accuracy, but the significance of this was not reported (relative increase in AUC ranging between 5% and 16%) [26, 30-32]. Although, our LR results for clinical risk factors revealed these would be three to five times more likely to be present in women with, as opposed to women without, pregnancy hypertension or pre-eclampsia, these LR values are still only considered to be indicative of a relatively small likelihood of disease [36]. A systematic review of risk factors for pre-eclampsia revealed that there are a broad range of other important risk factors that are also important to be taken into account, but we did not have information on, such as the presence of antiphospholipid antibodies or family history of hypertension [5]. Overall, our results highlight that complete maternal risk factor information compared with any serum biomarker tested in early pregnancy would potentially provide much better information in predicting hypertensive disorders in pregnancy.

Some of the potential limitations of the study include the lower prevalence of pre-eclampsia (2.5%) compared with the maternity population in NSW (3.1%) [37] which may be due to a healthier and more affluent cohort. Maternal weight was missing in 21% of the women, although this was addressed by applying multiple imputations, which has shown to be a robust and valid technique for dealing with missing data [14]. Despite these, strengths of this study were the assessment of an unselected consecutive cohort of women attending first trimester screening. Record linkage of laboratory to birth and hospital data also ensured follow up and ascertainment of pregnancy

outcomes with only minimal missing information. Missing health and pregnancy information was mostly attributable to women giving birth in hospitals out of state, although, these women had similar characteristics compared with those included in the study.

In conclusion, our findings suggest that maternal first trimester serum concentrations of sFlt-1 and PlGF do not predict pregnancy hypertension and pre-eclampsia any better than routinely assessed clinical and maternal risk factor information. Screening for sFlt-1 and PlGF levels in early pregnancy would not predict those pregnancies at-risk.

Contributors

FJS, NN, VT, AWA, JMM and CLR conceived the study. NN, JMM and CLR obtained the funding. NN, VT, JMM and CLR acquired the data. FJS conducted the statistical analysis. FJS and NN drafted the manuscript which was approved by all authors. All authors critically reviewed the manuscript for important intellectual content. FJS takes responsibility for the integrity of the data and the accuracy of the data analyses.

Acknowledgements

We thank the NSW Department of Health and the NSW Centre for Health Record Linkage for record linkage, and for access to the population health data**.** We thank Samantha Lain for preparation of data for linkage. There is no conflict of interest to declare.

Financial Disclosure

This work was funded by a National Health and Medical Research Council (NHMRC) Project Grant (#632653) and a Centre for Research Excellence Grant (#1001066). CLR is supported by a NHMRC Senior Research Fellowship (#1021025) and NN by a NHMRC Career Development

Fellowship (#632955). The NHMRC had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Table 1: Demographic characteristics and serum levels of PlGF, sFlt-1 and PAPP-A of the study population by pregnancy outcome

Variable	Unaffected women n=2,468	Pregnancy Hypertension n=213	Pre-eclampsia n=68
Age (SD)	32.8 (4.7)	32.6 (4.3)	32.1 (4.1)
Maternal Weight (SD)	66.3 (13.7)	74.4 (18.0) [b]	72.8 (16.8)[b]
Smoking (%)	150 (6.2)	12 (5.6)	1 (1.5)
Nulliparous (%)	1064 (43.9)	118 (55.7) [b]	44 (65.7)[b]
Country of Birth (%)			
Australia & New Zealand	1641 (66.5)	180 (84.5) [b]	50 (73.5)
Asian countries	322 (13.1)	11 (5.2) [b]	6 (8.8)
Other countries	505 (20.5)	22 (10.3) [b]	12 (17.7)
Previously diagnosed hypertension (%)	139 (15.6)	40 (18.8) [b]	11 (16.2) [b]
Previously diagnosed diabetes (%)	61 (2.5)	11 (5.2) [a]	4 (5.9)
PlGF pg/ml (IQR)	24.1 (18.3, 31.7)	21.3 (16.9, 28.0) [b]	20.7 (17.2, 32.6)
sFlt-1 pg/ml (IQR)	286.8 (167.1, 472.1)	272 (169.6, 441.7)	268.1 (164.8, 390.5)
PAPP-A pg/ml (IQR)	1.71 (1.06, 2.79)	1.41 (0.80, 2.14) [b]	1.34 (0.76, 2.4) [a]
PlGF MoM (IQR)	1.01 (0.77, 1.31)	0.92 (0.71, 1.24) [b]	0.92 (0.73, 1.31)
sFlt-1 MoM (IQR)	1.01 (0.60, 1.67)	1.01 (0.62, 1.56)	0.82 (0.53, 1.46)
PAPP-A MoM (IQR)	0.98 (0.66, 1.46)	0.94 (0.62, 1.37)	0.83 (0.57, 1.32)

a P<0.05; b P<0.001; SD: Standard deviation; MoM: Multiple of the median; IQR: Interquartile range

Table 2: Accuracy of models using serum biomarkers levels and maternal and clinical information in early pregnancy to predict pregnancy hypertension and pre-eclampsia based on a 5% false positive rate in all women

Variable	AUC (95%CI)	P-value	Sensitivity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	LR (+)
(N=2,681)						
Pregnancy hypertension (n=213)						
PlGF MoM	0.56 (0.52, 0.60)	0.005	6.1 (3.3, 10.3)	9.6 (5.2, 15.8)	92.2 (91.0, 93.2)	1.23
sFlt-1 MoM	0.52 (0.48, 0.55)	0.4	5.8 (3.0, 9.9)	9.0 (4.7, 15.2)	92.2 (91.1, 93.2)	1.17
PAPP-A MoM	0.53 (0.49, 0.57)	0.15	7.5 (4.4, 11.9)	11.8 (6.9, 18.4)	92.2 (91.1, 93.3)	1.54
Serum Biomarkers only	0.55 (0.51, 0.58)	0.005	6.3 (3.4, 10.5)	9.7 (5.3, 16.0)	92.3 (91.2, 93.3)	1.26
Previously diagnosed hypertension + Parity	0.64 (0.60, 0.67)	<0.0001	18.0 (13.0, 23.9)	21.5 (15.6, 28.4)	93.0 (91.9, 94.0)	3.17
All maternal and clinical information*	0.70 (0.67, 0.74)	<0.0001	26.2 (20.3, 32.8)	31.2 (24.4, 38.7)	93.7 (92.7, 94.6)	5.25
Combined – biomarkers + maternal and clinical information	0.70 (0.67, 0.74)	<0.0001	24.8 (19.0, 31.2)	30.0 (23.2, 37.5)	93.6 (92.5, 94.5)	4.96
Pre-eclampsia (n=68)						
PlGF MoM	0.52 (0.45, 0.60)	0.5	7.4 (2.4, 17.3)	3.7 (1.2, 8.4)	97.5 (96.8, 98.1)	1.47
sFlt-1 MoM	0.56 (0.49, 0.62)	0.1	5.9 (1.6, 14.4)	3.0 (0.8, 7.6)	97.5 (96.8, 98.0)	1.18
PAPP-A MoM	0.57 (0.49, 0.64)	0.07	8.8 (3.3, 18.2)	4.4 (1.6, 9.4)	97.6 (96.9, 98.1)	1.77
Serum Biomarkers only	0.57 (0.50, 0.65)	0.04	7.4 (2.4, 16.3)	3.8 (1.2, 8.6)	97.5 (96.8, 98.1)	1.77
Previously diagnosed hypertension + Parity	0.66 (0.60, 0.72)	<0.0001	16.2 (8.4, 27.1)	6.4 (3.2, 11.2)	97.6 (97.0, 98.2)	2.53
All maternal and clinical information*	0.74 (0.68, 0.80)	<0.0001	25.0 (15.3, 37.0)	12.0 (7.1, 18.5)	97.9 (97.3, 98.4)	5.03
Combined – biomarkers + maternal and clinical information	0.76 (0.70, 0.82)	<0.0001	25.0 (15.3, 37.0)	12.0 (7.1, 18.5)	97.9 (97.3, 98.4)	5.03

*Including: Maternal weight, smoking during pregnancy, parity, previously diagnosed hypertension, previously diagnosed diabetes, high blood pressure recorded during pregnancy and country of birth; PPV: Positive likelihood ratio; NPV: Negative predictive value; LR: Likelihood ratio; MoM: Multiple of the median

Table 3: Accuracy of models using serum biomarkers levels and maternal and clinical information in early pregnancy to predict pregnancy hypertension based on a 5% false positive rate in nulliparous women

Variable	AUC (95%CI)	P-value	Sensitivity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	LR (+)
(N=1,182)						
Pregnancy hypertension (n=118)						
PlGF MoM	0.57 (0.51, 0.63)	0.02	6.0 (2.4, 11.9)	11.5 (4.4, 22.2)	90.3 (88.5, 92.0)	1.20
sFlt-1 MoM	0.52 (0.46, 0.57)	0.5	5.3 (2.0, 11.2)	10.2 (3.8, 20.8)	90.5 (88.6, 92.1)	1.07
PAPP-A MoM	0.54 (0.49, 0.60)	0.11	6.8 (3.0, 12.9)	12.1 (5.4, 22.5)	90.3 (88.4, 92.0)	1.26
Serum Biomarkers only	0.57 (0.52, 0.63)	0.01	4.4 (1.5, 10.0)	8.5 (2.8, 18.7)	90.4 (88.5, 92.0)	0.87
Maternal weight	0.61 (0.55, 0.67)	0.0001	19.5 (12.8, 27.8)	29.9 (20.0, 41.4)	91.6 (89.8, 93.1)	3.92
All maternal and clinical information only*	0.68 (0.63, 0.74)	<0.0001	25.6 (18.0, 34.5)	36.1 (25.9, 47.4)	92.1 (90.3, 93.6)	5.16
Combined – biomarkers + maternal and clinical information	0.70 (0.65, 0.75)	<0.0001	26.8 (18.9, 36.0)	36.1 (25.9, 47.4)	92.4 (90.7, 93.9)	5.31
Pre-eclampsia (n=44)						
PlGF MoM	0.49 (0.39, 0.59)	0.9	9.1 (2.5, 21.7)	6.5 (1.8, 15.7)	96.5 (95.2, 97.5)	1.81
sFlt-1 MoM	0.58 (0.49, 0.66)	0.07	9.1 (2.5, 21.7)	6.6 (1.8, 15.9)	96.4 (95.2, 97.4)	1.82
PAPP-A MoM	0.54 (0.45, 0.63)	0.3	11.4 (3.8, 24.6)	7.6 (2.5, 16.8)	96.6 (95.3, 97.5)	2.15
Serum Biomarkers only	0.58 (0.49, 0.67)	0.07	11.4 (3.8, 24.6)	8.1 (2.7, 17.8)	96.5 (95.3, 97.5)	2.26
Maternal weight	0.63 (0.55, 0.71)	0.002	18.2 (8.2, 32.7)	12.1 (5.4, 22.5)	96.8 (95.6, 97.8)	3.63
All maternal and clinical information only*	0.71 (0.64, 0.78)	<0.0001	20.5 (9.8, 35.3)	13.6 (6.4, 24.3)	96.9 (95.7, 97.8)	4.09
Combined – biomarkers + maternal and clinical information	0.74 (0.66, 0.81)	<0.0001	25.0 (13.2, 40.3)	16.4 (8.5, 27.5)	97.0 (95.8, 97.9)	4.99

*Including: Maternal weight, smoking during pregnancy, parity, previously diagnosed hypertension, previously diagnosed diabetes, high blood pressure

recorded during pregnancy and country of birth; PPV: Positive likelihood ratio; NPV: Negative predictive value; LR: Likelihood ratio; MoM: Multiple of the median

Figure 1: Comparison of log sFlt-1 MoM and log PlGF MoM distributions in affected and unaffected pregnancies for pregnancy hypertension and pre-eclampsia