**The prognostic utility of soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) biomarkers for predicting preeclampsia: A secondary analysis of data from the INSPIRE trial**

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**Running title**: Prognostic utility of biomarkers for Preeclampsia

**Abstract**

**Objective:** To compare the prognostic performance of biomarkers soluble fms-like tyrosine kinase-1 (sFlt-1), Placental Growth Factor (PIGF), and sFlt-1/PIGF ratio as continuous values or as a binary cut-off of 38 for predicting preeclampsia (PE) within 7 days.

**Design**: Secondary analysis of a randomised clinical trial.

**Setting**: Oxford University Hospitals, Oxford, United Kingdom (UK).

**Population**: Pregnant women between 24+0 to 37+0 weeks of gestation with a clinical suspicion of preeclampsia.

**Main outcome**: Onset of preeclampsia within seven days of the initial biomarker test.

**Methods:** Logistic regression model for onset of preeclampsia using: (i) sFlt-1 (ii) PlGF, (iii) sFlt-1/PlGF ratio (continuous), and (iv) sFlt-1/PlGF ratio as a cut-off above or below 38.

**Results:** Of the total 370 women, 42 (11.3%) developed PE within seven days of screening. Models with sFlt-1 and sFlt-1/PIGF ratio (continuous) had greater overall performance than models with PIGF or with sFlt-1/PlGF ratio as a cut-off at 38 (R2: sFlt-1=55%, PIGF=38%, sFlt-1/PIGF ratio=57%, sFlt-1/PlGF ratio as cut-off at 38 model=46%). The discrimination performance was highest in the models with sFlt-1 (c-statistic=0.94) and sFlt-1/PIGF ratio (continuous) (c-statistic=0.94) compared to PIGF model (c-statistic=0.87) or sFlt-1/PlGF ratio cut-off at 38 (c-statistic=0.88).

**Conclusion**: Models using continuous values of sFlt-1/PIGF ratio or sFlt-1 only had better predictive performance compared to a PIGF only model or the model with sFlt-1/PlGF ratio as a cut-off at 38. Further studies based on a larger sample size are warranted to substantiate this finding.

**Keywords**: Prognostic model, preeclampsia, sFlt-1, PlGF, INSPIRE trial

**Introduction**

Preeclampsia (PE), defined as a new onset of hypertension and proteinuria usually occurring after 20 weeks of gestation up to and after delivery 1, confers a significant burden on maternal and fetal health outcomes. Globally, 2 to 5% of pregnancies suffer from PE leading to 76,000 maternal and more than 500,000 fetal deaths annually 2. In the United Kingdom (UK), the incidence of mild PE remains very low (6 per 1,000 pregnancies) while severe PE occurs in around 1 to 2 % of pregnancies 3 4.

To date, supportive management remains the current standard of care for treating PE as there are no therapeutic interventions other than delivery. The pathophysiological mechanisms underlying PE highlight an angiogenic imbalance, reflected by elevated placenta-derived soluble fms-like [tyrosine](https://www.sciencedirect.com/topics/medicine-and-dentistry/tyrosine) kinase-1 (sFlt-1) and decreased [placental growth factor](https://www.sciencedirect.com/topics/medicine-and-dentistry/placental-growth-factor) (PlGF) levels in the maternal circulation 5–7. These biomarkers have been successfully used for diagnosis, prediction of the disease 6–9 and clinical decision making with a combination of other clinical parameters such as high blood pressure and proteinuria 3. Nevertheless, latest evidence indicates that the ratio of the biomarkers is elevated but its predictive value in women with suspected preeclampsia is unclear 5.

Prognostic models based solely on the classical clinical risk factors (high blood pressure and proteinuria) have been reported to have poorer predictive ability compared to the models developed using sFlt-1 and PIGF biomarkers 10 11. However, most of the prognostic models built using sFlt-1, PIGF, or their ratio are based on dichotomising the continuous measurements of these markers using threshold values 4 5 12–15. Moreover, only a few studies have modelled or compared sFlt-1, PIGF, or their ratio on a continuous scale 16–18.

Binary thresholds, whilst simpler for clinical application 19, may lead to a loss in statistical power and lead to models with poor predictive performance 20. In addition, an explicit comparison of the predictive performance of continuous values of sFlt-1, PlGF biomarkers or their ratio against cut-off-based models for predicting PE currently remains uncertain. This study aimed to bridge these gaps by applying a probabilistic approach that uses the sFlt-1 and PlGF biomarkers as continuous as opposed to a simplistic discrete (rule-in/rule-out) approach based on a defined threshold obtained from the mathematical ratio of sFlt-1 and PlGF. Specifically, we aimed to compare the prognostic utility of models using the continuous values of sFlt-1, PlGF, or sFlt-1/PlGF ratio for predicting PE within 7 days of screening among those with suspected PE compared to the recommended cut-off-based value of the ratio of sFlt-1/PlGF of 38.

**Methodology**

**Data source: The INSPIRE trial**

Data from the prospective, parallel-group, randomized interventional study evaluating the short-term prediction of preeclampsia/eclampsia (INSPIRE) trial was used for the purpose of this research 21. The INSPIRE trial was conducted in the UK that aimed to evaluate the use of sFlt-1/PlGF ratio in women presenting with suspected preeclampsia and its effect on PE-related hospitalisation within 24 hours of the test, within 7 days, or by delivery as the primary outcome. The study was conducted from June 2015 to April 2017 at the John Radcliffe Hospital, Oxford, UK - a tertiary referral centre with a preeclampsia prevalence of 2.9 %.

The study enrolled 370 pregnant women (186 reveal trial arm versus 184 non-reveal trial arm) aged 18 years or above, with singleton pregnancies between 24+0 and 37+0 weeks of gestation with a clinical suspicion of preeclampsia. Women with pre-existing diagnosed preeclampsia/eclampsia were excluded from the trial. Suspicion of preeclampsia was defined by a new onset elevated blood pressure or worsening of pre-existing hypertension or new-onset proteinuria or worsening of pre-existing proteinuria or new-onset headache, visual disturbance, oedema or right upper quadrant pain, or any other clinical suspicion of preeclampsia 21.

Overall, there were 85 women with PE until delivery and 42 had PE within 7 days of screening. The study found that there was no difference in preeclampsia-related admissions within 24 hours of the test between trial arms (sixty patients were admitted in the intervention group (reveal trial arm: standard clinical management plus revealing biomarker results) and 48 in the comparator group (non-reveal trial arm: standard clinical management).

**Primary outcome and candidate predictors**

The primary outcome in this study was the onset of PE within seven days of the initial biomarker test as defined in the INSPIRE trial 21. After informed consent, study participants had standard clinical assessment and additional blood sample for biomarker measurement were collected and centrifuged within one hour of collection. The sFlt-1 and PIGF values were then measured using the fully automated methods (Elecsys® sFlt-1/PlGF) using the Roche e411 analyzer (Roche Diagnostics Limited, Burgess Hill, United Kingdom) 24.

A logistic regression model for predicting the onset of PE within 7 days of screening was done using biomarkers sFlt-1 (continuous values), PlGF (continuous values), sFlt-1/PIGF ratio (continuous values), or sFlt-1/PlGF ratio as a binary cut-off of 38.

**Sample size assessment for the development of a prognostic model**

The adequacy of sample size was assessed using the ***pmsampsize*** library in R program as recommended by Riley et al. 22. The minimum events per parameter required for reliably developing a new model that achieved the desired shrinkage factor, R2, and margin of difference was ~10 events per variable. Therefore, with 42 outcome events, ~3-4 variables could be used for model development.

**Multivariable model building**

Four multivariable logistic regression models were constructed for the three biomarkers (sFlt-1, PlGF, sFlt-1/PlGF ratio as continuous, and sFlt-1/PlGF ratio cut off at 38). All four models were adjusted for trial arm 23. Natural log transformation was carried out for continuous values of sFlt-1, PlGF and sFlt-1/PlGF ratio. The sFlt-1/PlGF cut-off used was 38 since it is commonly used by many studies including the INSPIRE trial 24. The multivariable logistic regression model assumed the log-odds of PE were linearly associated with the biomarkers. This assumption was formally tested using fractional polynomial transformations. Models were compared using the likelihood ratio test and Bayesian Information Criterion.

**Model performance and internal validation**

The predictive performances of the four developed models were assessed using calibration and discrimination. Model calibration was assessed by calibration-in-the-large (CITL) and calibration slope whereas model discrimination was assessed by Harrell’s concordance statistic (c-statistic) 25. Overall model fit was assessed using Pseudo (Nagelkerke's) R2 and Bayesian Information Criterion (BIC) 25. The apparent performance measures (model fit performance in the development data) were adjusted for optimism using bootstrapping by drawing 1,000 resamples from the original dataset and calculating adjusted measures of concordance statistic (calculated from the Somers' D rank correlation value) 22, calibration slope and calibration-in-the-large. Adjusted coefficients were corrected for optimism using the uniform shrinkage factor i.e., the calibration slope obtained from bootstrapping. The intercept of the optimism-adjusted model was also re-estimated to maintain the overall calibration. The transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) guideline was used for model development and reporting 26 27.

**Results**

**Baseline characteristics of the study population**

There were 370 study participants of whom 42 (11.3%) were diagnosed with preeclampsia within 7 days of taking the screening test. Baseline characteristics for women with and without PE were similar except for gestational age at recruitment and parity (Table 1). The distributions for PIGF, sFlt-1, and the ratio sFlt-1/PIGF were skewed and therefore were transformed using natural logarithms.

The median ln(PIGF) was higher among non-preeclamptic women (median: 2.40 pg/mL; interquartile range (IQR): 2.17 pg/mL–2.73 pg/mL) compared to preeclamptic women (median: 1.87 pg/mL; IQR: 1.72 pg/mL – 2.07 pg/mL). The median ln(sFlt-1) for women without PE was 3.38 pg/mL (IQR: 3.18 pg/mL – 3.61 pg/mL) compared to 4.03 pg/mL (IQR: 3.38 pg/mL – 4.15 pg/mL) for preeclamptic women. Similarly, for ln(sFlt-1/PIGF) ratio, the median was 0.89 (IQR: 0.56 – 1.44) among non-preeclamptic women compared to 2.11 (IQR: 1.82 – 2.35) for preeclamptic women. The sFlt-1/PIGF ratio values ≤ 38 were present in 78% of women without PE while 21.9 % of women without PE had sFlt-1/PIGF cut-off values > 38 compared to 97.6 % in women with PE (Table 1). The sFlt-1/PIGF ratio values >85 were present in 24 (7.3%) women without PE compared to 29 (69%) women with PE. Considering sFlt-1/PlGF ratio values between 38 to 85, 12 (28.6%) women had PE compared to 48 (14.6%) women without PE (data not shown).

|  |  |  |
| --- | --- | --- |
| Characteristics | No preeclampsia within 7 days (n=328) | Preeclampsia within 7 days (n=42) |
| **Maternal age at recruitment** (in years), Mean (SD) | 31.2 (6.1) | 31.2 (5.8) |
| **Gestational age** (in weeks), Median (IQR) | 34.1 (31.1 - 35.9) | 35.3 (33.4 - 36.1) |
| **BMI** (in kg/m²), Median (IQR) | 27.3 (23.9 - 32) | 27.3 (24.5 - 31.7) |
| **Parity** |  |  |
| Nulliparous | 145 (44.2%) | 35 (83.3%) |
| Multiparous | 183(55.8%) | 7 (16.7%) |
| **Smoking status** |  |  |
| Current smoker | 31 (9.4%) | 2 (4.8%) |
| Never smoker | 201 (61.3%) | 24 (57.1%) |
| Previous smoker | 96 (29.3%) | 16 (38.1%) |
| **Ethnicity** |  |  |
| White British | 295 (89.9%) | 37 (88.1%) |
| Other | 33 (10.1%) | 5 (11.9%) |
| \***sFlt-1**, Median (IQR) | 3.38 (3.18 – 3.61) | 4.03 (3.88 - 4.15) |
| \***PlGF**, Median (IQR) | 2.40 (2.17 -2.73) | 1.87 (1.72 – 2.07) |
| \***sFlt-1/PlGF ratio**, Median (IQR) | 0.89 (0.56 – 1.44) | 2.11 (1.82 – 2.35 |
| **sFlt-1/PlGF cut off** |  |  |
| Ratio ≤ 38 | 256 (78.1%) | 1 (2.4%) |
| Ratio >38 | 72 (21.9%) | 41 (97.6%) |

**Table 1. Baseline characteristics of study population (n=370)**

SD-standard deviation, PIGF - Placental growth factor; sFlt-1 - Soluble fms-like tyrosine kinase – 1, IQR-interquartile range, BMI- Body mass index, BP-blood pressure, \* sFlt-1, PIGF and the sFlt-1/PIGF ratio reported on natural log scale

**Model development and performance**

The fitted models are presented in table 2 and the performance measures are presented in table 3. The sFlt-1 (R2=55%, BIC=144) and sFlt-1/PIGF ratio models (R2=57%,BIC=139) showed higher overall model fit than PIGF model (R2=38%, BIC=184) or sFlt-1/PlGF ratio using a binary cut-off of 38 model (R2=46%, BIC=166).

The mean predictions (CITL) for PE were close to zero for all models. (Table 3). The sFlt-1, PlGF and sFlt-1/PlGF ratio (continuous) models all had calibration slope close to 1 (slope>=0.98) with the exception of sFlt-1/PlGF ratio using a binary cut-off of 38 model having the lowest value (slope=0.84) (Figure 1 and Table 3). Model discrimination was ≥0.87 across all models (c-statistic for: sFlt-1 =0.94, PIGF=0.87, sFlt-1/PIGF ratio=0.94, sFlt-1/PlGF ratio using a binary cut-off of 38 model=0.88) (Table 3).

Chart, line chart

Description automatically generated

A=0.98

B=0.94

A=0.99

B=0.87

A=0.99

B=0.94

A=0.84

B=0.88

Legend:

Text

Description automatically generated

A= calibration slope, B= c-statistic

**Figure 1**. Assessment of model calibration for sFlt, PIGF, sFlt/PIGF ratio and sFlt/PIGF cut-off-based models. sFlt, PIGF and sFlt/PIGF ratio values are in log scales. Estimated probabilities are plotted in ten risk groups. The plot for sFlt/PIGF cut-off-based model is based on the log odds of predictions of the 0/1 outcome.

|  |  |
| --- | --- |
| **Models** | **Model form** |
| Model 1 |  |
| Model 2 |  |
| Model 3 |  |
| Model 4 |  |

PE= Pre-eclampsia (1=Yes, 0=No); PIGF = Placental growth factor; sFlt-1 - Soluble fms-like tyrosine kinase – 1; log indicates natural logarithmic transformation;

**Table 2. Details of the four multivariable logistic regression models fitted**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Model | Regression model output | | Model performance corrected for optimism | | | | |
|  | Coefficient | Intercept | Calibration  slope | CITL | C-index | R2 | BIC |
| Model 1: sFlt-1 only + | 7.37 | -29.92 | 0.98 | 0 | 0.94 | 0.55 | 144 |
| Model 2: PIGF only + | -4.77 | 8.08 | 0.99 | 0 | 0.87 | 0.38 | 184 |
| Model 3: sFlt-1/PIGF ratio + | -9.47 | 4.19 | 0.99 | 0 | 0.94 | 0.57 | 139 |
| Model 4: sFlt-1/PIGF cut-off at 38 ++ | 4.37 | -5.81 | 0.84 | 0 | 0.88 | 0.46 | 165 |

**Table 3. Performance measures of biomarkers in identifying preeclampsia.**

PIGF = Placental growth factor; sFlt-1 - Soluble fms-like tyrosine kinase – 1; CITL – calibration-in-the-large; BIC = Bayesian information criteria (the lowest BIC values indicating better model performance); C-index – concordance index

+ Predictor variables are continuous values included in the model equation of biomarker/s as every unit increases in log-scale. All model results are optimism corrected and adjusted for the trial arm. ++ No log transformation applied for the cut off predictor variable.

**Discussion**

**Main findings**

This study compared the discriminative ability of sFlt-1, PlGF, sFlt-1/PlGF ratio and sFlt-1/PlGF ratio using a binary cut-off of 38 for identifying preeclampsia within 7 days of screening. The continuous values of biomarkers sFlt-1 alone and sFlt-1/PIGF ratio had comparable predictive performance with similar discrimination ability to identify PE cases. These performed better than PlGF alone. Interestingly, in our dataset, the commonly used sFlt-1/PlGF ratio using a binary cut-off of 38 models had poorer predictive performance relative to other models considered. This finding was in line with the fact that biomarkers perform better as continuous variables than as dichotomous cut-offs as it is more biologically plausible because the disease is a continuum 17, and in particular in preeclampsia, where the biomarkers are related to its pathogenesis 28. This finding highlights the need for further studies to evaluate approaches for handling biomarkers (continuous versus cut off) for identifying PE.

**Interpretation**

Studies that utilise continuous values of biomarkers for the prediction of preeclampsia are limited as many biomarker-based prediction studies often employ cut off values 10 or predict maternal outcomes after the diagnosis of preeclampsia 29. A systematic review and meta-analysis of the sFlt-1/PIGF ratio cut off for prediction of PE pointed out that the ratio-based model has a good predictive potential but conclusive evidence is lacking because of the differences in the choice of cut-offs used, timing and frequency of testing, and due to heterogeneity in the target population 10. Saleh et al compared the continuous versus cut off based biomarker-based prediction of pregnancy complications including preeclampsia 17 and found that continuous value of sFlt-1/PlGF ratio had high discrimination performance with sFlt-1 and PlGF cut off values having lower predictive ability than the continuous biomarker values. Perry et al. 30 also showed that continuous values of sFlt-1/PlGF ratio performed better than cut off based predictions with an additive value from baseline clinical predictors. Our findings were consistent with the findings of Saleh et al and Perry et al. However, these previous studies had a target population of pregnancies already complicated with preeclampsia, gestational hypertension, or chronic hypertension; making direct comparisons difficult because the study participants included in the INSPIRE trial were only women suspected with preeclampsia 21.

In our study, models for sFlt-1 or sFlt-1/PIGF ratio had similar performance in terms of calibration and discrimination. This finding concurs with the results reported in the preeclampsia prediction literature 10 16. For instance, Anderson et al. found that PIGF and sFlt-1/PlGF ratio values had a good predictive performance for early-onset and severe preeclampsia cases with PIGF having slightly better performance but with no significant difference between the tests 16.

In our study, the PIGF model had lower overall predictive and discriminative performance than sFlt-1 and sFlt-1/PIGF ratio models. A systematic review on the value of PIGF as a prognostic tool found that PlGF had moderate-to-high evidence for identifying women at the highest risk of preterm delivery or neonatal outcomes but showed no clinically useful performance for the prediction of adverse maternal outcomes 8. This might be physiologically plausible as PIGF levels are considered to be associated with other non-placental factors and intrauterine growth restriction; in contrast to sFlt-1 levels which have a strong correlation with the placenta 31. Furthermore, free PlGF levels seem to be decreased in preeclampsia mostly as a consequence of sFlt-1 binding 32. Nevertheless, Ukah et al. did recognise that it is not clear if PIGF performs better alone or in combination, and more studies are needed to assess this 8. In real-world clinical practice, the use of PIGF is reported to have a predictive potential for delivery of small for gestational age infants 33 and lower time for preeclampsia diagnosis by clinicians 9.

**Strengths and limitations**

Our study has several strengths and limitations. The predictive ability of the biomarkers was assessed as continuous variables as recommended in prognostic model development 19. Prior to starting modelling, the recommended test of sample size adequacy was performed and only parameters with the minimum events were included, indicating the relevance of the parameters used for model building. Adjustment of model performance parameters by bootstrapping also increased the likelihood of having realistic model performance measures.

However, the INSPIRE trial was a single centre study and the institution-specific level practices and overall context might affect the generalisability of the study findings. Finally, the small number of events in our study limited the scope of constructing a multivariable model with other potentially important parameters such as age, parity, and BMI.

**Conclusion**

Using continuous values of either sFlt-1/PIGF ratio or sFlt-1 alone had better predictive performance over models based only on continuous values of PIGF only and sFlt-1/PlGF cut-off at 38. There was no obvious incremental value in using sFlt-1 only or sFlt-1/PIGF ratio values as they both had comparable predictive performance. More studies with larger numbers of patients and sufficient outcome variables are warranted to evaluate whether the ratio cut-off of 38 or an alternative that is based on a continuous value of a single biomarker or ratio can perform better in predicting PE.

**Declarations**

**List of abbreviations**

CITL – Calibration-in-the large

c-statistic - Concordance statistic

INSPIRE- Interventional Study Evaluating the Short-Term Prediction of Preeclampsia/

IQR – Interquartile range

PE - Preeclampsia

PIGF - Placental growth factor

RMSE –Root mean square error

SD – Standard deviation

sFlt-1 - Soluble fms-like tyrosine kinase - 1

TRIPOD - Transparent Reporting of a multivariable prediction model for Individual

**Ethics approval and consent to participate**

Ethical approval of the secondary data analysis of the INSPIRE clinical trial was secured from the Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK. The original clinical trial was performed in accordance with the Declaration of Helsinki and is registered at - [http://www.isrctn.com](http://www.isrctn.com/). Unique identifier: ISRCTN87470468. Informed consent was secured from all study participants by filing a written consent form after being briefed about the study information and consented to participate in the study.

**Consent for publication**

Not applicable (NA)

**Availability of data and materials**

The datasets used and analysed during the current study are not publicly available due to data privacy for identifiable patient information and ethical concerns but are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions:**

Conceptualization : MMK, PD, EOO

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