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Prognostic value of angiogenic markers in pregnancy with fetal growth restriction

P. PALMRICH[1], E. KALAFAT[2], P. PATEISKY[1], N. SCHIRWANI-HARTL[1], C. HABERL[1], C. HERRMANN[1], A. KHALIL[3,4] and J. BINDER[1]

[1]*Department of Obstetrics and Feto-Maternal Medicine, Medical University of Vienna, Vienna, Austria;* [2]*Department of Obstetrics and Gynecology, School of Medicine, Koc University, Istanbul, Turkey;* [3]*Fetal Medicine Unit, St George's University Hospitals NHS Foundation Trust, University of London, London, UK;* [4]*Vascular Biology Research Centre, Molecular and Clinical Sciences Research Institute, St George's University of London, London, UK*

KEYWORDS: adverse maternal outcome; angiogenic marker; fetal growth restriction; placental growth factor; placental insufficiency; pre-eclampsia; sFlt-1/PlGF ratio; soluble fms-like tyrosine kinase-1

CONTRIBUTION

What are the novel findings of this work?
This study documents the prognostic potential of the angiogenic biomarkers soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), and their ratio, for predicting the short-term development of pre-eclampsia in pregnancies with fetal growth restriction (FGR) and suspected pre-eclampsia.

What are the clinical implications of this work?
This work highlights the potential of the sFlt-1/PlGF ratio to improve the identification of pregnancies with FGR that are at increased risk of developing pre-eclampsia.

ABSTRACT

Objective *Pregnancies with fetal growth restriction (FGR) are at increased risk for pre-eclampsia. Angiogenic markers including soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are altered in pregnancies complicated by FGR, but their utility for predicting pre-eclampsia in growth-restricted pregnancies is uncertain. This study aimed to evaluate the prognostic value of angiogenic markers for predicting the development of pre-eclampsia in pregnancies with FGR and suspected pre-eclampsia.*

Methods *This was a retrospective study of singleton pregnancies with FGR, defined according to Delphi consensus criteria, which underwent sampling of sFlt-1 and PlGF for suspicion of pre-eclampsia at the Medical University of Vienna, Vienna, Austria, between 2013 and 2020. Women with an established diagnosis of pre-eclampsia at sampling were excluded. Cox regression analysis and logistic regression analysis were performed to evaluate the association of angiogenic markers with the development of pre-eclampsia at various timepoints.*

Results *In this cohort of 93 women, pre-eclampsia was diagnosed in 14 (15.1%) women within 1 week after sampling, 21 (22.6%) within 2 weeks after sampling and 38 (40.9%) at any time after assessment. The sFlt-1/PlGF ratio consistently showed a stronger association with the development of pre-eclampsia compared to sFlt-1 or PlGF alone (pre-eclampsia within 1 week: area under the receiver-operating-characteristics curve, 0.87 vs 0.82 vs 0.72). Models including the sFlt-1/PlGF ratio were associated more strongly with pre-eclampsia hazard compared to models including sFlt-1 or PlGF alone (concordance index, 0.790 vs 0.759 vs 0.755). The risk classification capability of the sFlt-1/PlGF ratio decreased after the 2-week timepoint. The established cut-off value for the sFlt-1/PlGF ratio of < 38 was effective for ruling out pre-eclampsia within 2 weeks, with a negative predictive value of 0.933 and sensitivity of 0.952.*

Conclusions *Use of the sFlt-1/PlGF ratio is preferrable to the use of PlGF alone for the prediction of pre-eclampsia in pregnancies with FGR. Established cut-offs for ruling out the development of pre-eclampsia in the short term seem to be effective in these patients.* © *2023 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

Correspondence to: Dr J. Binder, Department of Obstetrics and Feto-Maternal Medicine, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria (e-mail: julia.binder@meduniwien.ac.at)

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INTRODUCTION

Fetal growth restriction (FGR) is associated with maternal and perinatal morbidity and mortality[1–4]. The growth-restricted fetus does not reach its biological growth potential owing to an underlying condition, the most common being uteroplacental insufficiency[2,5,6]. FGR, as an expression of impaired placental function, shares clinical and pathophysiological features with pre-eclampsia[7], and FGR pregnancies are at increased risk for developing pre-eclampsia, a potentially life-threatening condition for both the mother and fetus[7,8]. The diagnosis and monitoring of FGR are currently based on ultrasound assessment, comprising fetal weight estimation, assessment of amniotic fluid volume and Doppler examination[9–13], although recent studies have shown a higher sensitivity in detecting and monitoring placental dysfunction with the use of a combination of biophysical and biochemical parameters[14,15]. Imbalance between the angiogenic biomarkers soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), characterized by an increased sFlt-1/PlGF ratio, is thought to play an important role in the development of placental dysfunction and related diseases, such as pre-eclampsia[16–19] and FGR[18,20]. Increased maternal serum sFlt-1/PlGF ratio has also been shown to be predictive of adverse perinatal outcome and shorter time to delivery in early-onset small-for-gestational-age (SGA) and FGR fetuses[7,20,21], and has been suggested as a potential additive tool for fetal surveillance in these patients[20,22].

However, the utility of the sFlt-1/PlGF ratio as a predictor of pre-eclampsia in pregnancies with FGR is still uncertain. Angiogenic marker levels have been found to be altered in FGR in the presence as well as in the absence of pre-eclampsia[23]. This raises the question as to whether predefined cut-offs in the sFlt-1/PlGF ratio or PlGF level for the prediction and diagnosis of pre-eclampsia can be applied equally to pregnancies with and those without FGR. As pre-eclampsia is often a limiting factor in prolonging pregnancies complicated by FGR, evaluating the risk of maternal complications, particularly pre-eclampsia, represents one of the key clinical challenges in managing uteroplacental insufficiency. Recommended methods of surveillance in women with FGR are limited to conventional strategies, such as blood-pressure monitoring. Improved surveillance strategies would facilitate the identification of women at risk for delivery owing to maternal complications, such as pre-eclampsia, and aid counseling of pregnant women. Therefore, this study aimed to assess the prognostic value of maternal serum angiogenic markers (sFlt-1 and PlGF) for predicting the development of pre-eclampsia in pregnancies with FGR.

METHODS

This was a retrospective analysis of prospectively collected data recorded in an electronic database (Viewpoint 5.6.8.428, Wessling, Germany) between January 2013 and December 2020. The study was approved by the local research ethics committee (approval number 1882/2018) of the Medical University of Vienna, Vienna, Austria. No written informed consent for study participation was required.

The study population consisted of women with a singleton pregnancy and an established diagnosis of FGR, defined according to the Delphi consensus[2] and using estimated fetal weight at the time of assessment, who underwent sampling of sFlt-1 and PlGF for suspicion of pre-eclampsia. Suspicion of pre-eclampsia was based on presentation with any of the following symptoms: high blood pressure, *de-novo* proteinuria, *de-novo* edema, elevated liver enzymes, epigastric pain, low platelet count, dyspnea or neurological symptoms, including visual disturbance, and severe headache. Women with an established diagnosis of pre-eclampsia at the time of sampling were excluded, as were those suffering from chronic kidney disease and those with aneuploidy, genetic syndrome and/or major fetal structural anomaly. Women who did not deliver at the Department of Obstetrics and Feto-Maternal Medicine at the Medical University of Vienna were also excluded owing to missing outcome data.

Pre-eclampsia was defined as new-onset systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg or worsening of pre-existing hypertension and additional organ manifestation, including proteinuria (protein/creatinine ratio $\geq$ 30 mg/mmol or $\geq$ 300 mg protein/24 h), elevated liver enzymes (twice the upper reference value), thrombocytopenia (platelet count $<$ 100 000/μL), kidney dysfunction (serum creatinine $>$ 1.1 mg/dL), neurological symptoms (e.g. altered mental state, severe headache, persistent visual scotoma, eclampsia) and pulmonary edema[24].

As part of routine assessment in women with suspected pre-eclampsia, a blood sample was taken by venipuncture and stored in a collection tube without anticoagulants to analyze maternal serum levels of sFlt-1, PlGF and their ratio. The angiogenic marker concentrations were assessed by commercially available, fully automated assays on the Elecsys platform (Roche Diagnostics, Penzberg, Germany). The analysis was undertaken by biomedical technicians.

The primary outcome of the study was the usefulness of sFlt-1, PlGF and their ratio for predicting the development of pre-eclampsia in pregnancies complicated by FGR and suspected pre-eclampsia. The outcome groups were classified as follows: diagnosis of pre-eclampsia within 1 week, diagnosis of pre-eclampsia within 2 weeks or diagnosis of pre-eclampsia at any time after assessment for suspicion of pre-eclampsia. The physicians were not blinded to the laboratory results. However, to date, there are no published guidelines, nor was there a local protocol, recommending the inclusion of the sFlt-1/PlGF ratio in the management of FGR.

Statistical analysis

Explanatory variables are presented as median (interquartile range (IQR)) for continuous data and as *n* (%) for

categorical data. Categorical variables were compared using the χ^2-test or Fisher's exact test, while continuous data were compared using the independent samples *t*-test or Mann–Whitney *U*-test. Association of angiogenic markers with the development of pre-eclampsia was assessed using Cox regression and logistic regression models. In time-to-event analyses, the hazard of pre-eclampsia development was assessed by considering the assessment-to-diagnosis interval. Those who delivered without a diagnosis of pre-eclampsia were considered right-censored. Cox models were compared according to their concordance index (C-index). Predicted risk estimates from Cox models were obtained and the rates of diagnosis of pre-eclampsia at certain timepoints were compared among risk categories. The same endpoints (development of pre-eclampsia at certain timepoints) were also assessed using logistic regression analysis. Logistic models were compared according to the area under the receiver-operating-characteristics curve (AUC), using DeLong's test. Sensitivity, specificity and negative predictive values (NPV) and positive predictive values (PPV) were calculated using pre-established cut-offs and the Youden index for logistics models. Statistical analysis was carried out using R software (v.4.2.2; R Foundation for Statistical Computing Platform, Vienna, Austria). $P < 0.05$ was considered to indicate statistical significance.

RESULTS

This study included 93 singleton pregnancies complicated by FGR that underwent sampling of maternal serum sFlt-1 and PlGF for suspicion of pre-eclampsia. The median gestational age (GA) at the diagnosis of FGR was 26.4 (IQR, 24.3–31.0) weeks. Overall, 38 (40.9%) women in the cohort developed pre-eclampsia. The median GA at the diagnosis of pre-eclampsia was 29.6 (IQR, 25.9–33.9) weeks. There were no significant differences in maternal demographic characteristics between women with isolated FGR and those who developed pre-eclampsia later in gestation (Table 1). GA at sampling of angiogenic markers was significantly lower in women who developed pre-eclampsia compared with those who did not (median, 28.4 *vs* 30.6 weeks; $P = 0.044$), while symptoms suspicious for pre-eclampsia were largely similar between the two groups. The most common reason why women underwent angiogenic-marker assessment for suspicion of pre-eclampsia was raised maternal blood pressure. Women who developed pre-eclampsia later in pregnancy

Table 1 Baseline characteristics, symptoms suspicious for pre-eclampsia (PE) at presentation and pregnancy outcome in 93 women in study cohort, according to whether or not they developed PE

Variable	*PE (n = 38)*	*No PE (n = 55)*	P
Maternal age (years)	32.5 (30.0–36.0)	33.0 (27.5–36.0)	0.338
Nulliparous	21 (55.3)	37/53 (69.8)	0.229
Mode of conception			0.711
Spontaneous	31/33 (93.9)	45/49 (91.8)	
IVF	2/33 (6.1)	3/49 (6.1)	
Egg cell donation	0/33 (0)	1/49 (2.0)	
Smoker	8/36 (22.2)	7/50 (14.0)	0.482
Maternal BMI (kg/m^2)	25.7 (21.6–29.0)	24.0 (21.9–27.5)	0.663
Epigastric pain	2 (5.3)	2 (3.6)	1.000
Edema	6 (15.8)	5 (9.1)	0.511
Proteinuria	3 (7.9)	8 (14.5)	0.516
Elevated liver enzymes	0 (0)	3 (5.5)	0.386
High blood pressure	35 (92.1)	39 (70.9)	0.026
Dyspnea	1 (2.6)	0 (0)	0.852
Neurological symptoms	3 (7.9)	2 (3.6)	0.669
GA at sampling (weeks)	28.4 (24.9–31.5)	30.6 (26.6–34.5)	0.044
GA at FGR diagnosis (weeks)	26.4 (24.3–31.0)	28.9 (24.3–33.1)	0.284
Maternal ICU admission	4 (10.5)	0 (0)	0.052
Birth-weight percentile	4.8 (2.3–8.7)	6.7 (2.8–9.8)	0.317
Outcome			0.082
Live birth	33 (86.8)	49 (89.1)	
Stillbirth	3 (7.9)	0 (0)	
Early NND	2 (5.3)	6 (10.9)	
5-min Apgar score < 7	3 (7.9)	0/54 (0)	0.132
Preterm birth < 37 weeks	34 (89.5)	42 (76.4)	0.182
Preterm birth < 32 weeks	21 (55.3)	23 (41.8)	0.287
NICU admission	25 (65.8)	33 (60.0)	0.727
Ventilation support	21 (55.3)	27 (49.1)	0.708
Necrotizing enterocolitis	3 (7.9)	0 (0)	0.128
Intraventricular hemorrhage	1 (2.6)	2 (3.6)	0.999

Data are given as median (interquartile range), *n* (%) or *n*/*N* (%). BMI, body mass index; FGR, fetal growth restriction; GA, gestational age; ICU, intensive care unit; IVF, *in-vitro* fertilization; NICU, neonatal intensive care unit; NND, neonatal death.

presented with high blood pressure significantly more often than did women who were non-pre-eclamptic (92.1% *vs* 70.9%; $P = 0.026$). The indication for delivery was FGR-related in 56 (60.2%) patients, pre-eclampsia combined with FGR in 22 (23.7%) patients, purely pre-eclampsia in 12 (12.9%) patients, preterm labor in two (2.2%) patients and placental abruption in one (1.1%) patient. The birth-weight percentile did not differ significantly between the two groups ($P = 0.317$).

Stillbirth occurred in three cases, of which all were in the pre-eclampsia group. We found no significant differences in the rate of neonatal adverse outcomes, including low 5-min Apgar score ($P = 0.132$), admission to the neonatal intensive care unit ($P = 0.727$), need for ventilation support ($P = 0.708$), necrotizing enterocolitis ($P = 0.128$) and intraventricular hemorrhage ($P = 0.999$), between the two study groups (Table 1).

Association of characteristics and biomarkers with pre-eclampsia risk

Cox regression analysis was performed to investigate the association of baseline characteristics and angiogenic markers with the hazard of developing pre-eclampsia (Table 2). We assessed three models, testing separately for the association of sFlt-1, PlGF and the sFlt-1/PlGF ratio. All angiogenic markers were entered into the models in a log-transformed and scaled fashion, meaning hazard ratios (HR) correspond to one standard unit change in the log-unit of the respective parameter. All models included baseline characteristics that constitute established risk factors for pre-eclampsia (age, nulliparity, body mass index, smoking and chronic hypertension)[25], as well as GA at diagnosis of FGR and GA at sampling. The strongest association was for sFlt-1/PlGF ratio alone (HR, 3.94; $P < 0.001$), despite significant associations for smoking (HR, 3.23; $P = 0.04$) and GA at sampling (HR, 0.71; $P = 0.03$). The sFlt-1/PlGF ratio model (C-index, 0.790) was superior to the sFlt-1 model (C-index, 0.759) and PlGF model (C-index, 0.755).

Figure 1 shows the risk of development of pre-eclampsia according to the predicted relative risk from Cox regression, categorized as follows: very low risk, ≤ 0.5; low risk, > 0.5 to ≤ 0.75; baseline risk, > 0.75 to ≤ 1.33; moderate risk, > 1.33 to ≤ 2; and high risk, > 2. Risk stratification was based on the results of the Cox regression model using sFlt-1, PlGF and GA at sampling (Table 2). Development of pre-eclampsia was stratified according to the time of onset of disease: within 1 week (Figure 1a), within 2 weeks (Figure 1b) or at any time (Figure 1c) after assessment. In the study cohort, 14 (15.1%) women developed pre-eclampsia within 1 week, 21 (22.6%) developed pre-eclampsia within 2 weeks and 38 (40.9%) developed pre-eclampsia at any time after assessment. According to

Table 2 Cox regression model showing association of baseline characteristics and angiogenic markers with hazard of pre-eclampsia diagnosis

Variable	*Beta (SE)*	*HR (95% CI)*	P	*C-index (SE)*
sFlt-1 model				
Maternal age (in years)	0.05 (0.04)	1.05 (0.97–1.13)	0.25	—
Nulliparous	−0.30 (0.39)	0.74 (0.34–1.60)	0.45	—
Maternal BMI (in kg/m^2)	−0.00 (0.04)	1.00 (0.93–1.07)	0.96	—
Smoker	1.14 (0.53)	3.11 (1.10–8.79)	0.03	—
Chronic hypertension	−0.55 (0.59)	0.58 (0.18–1.84)	0.35	—
GA at sampling (in weeks)	−0.35 (0.17)	0.70 (0.51–0.98)	0.04	—
GA at FGR diagnosis (in weeks)	0.16 (0.11)	1.17 (0.95–1.44)	0.14	—
sFlt-1	0.92 (0.23)	2.52 (1.59–3.99)	< 0.001	0.759 (0.047)
PlGF model				
Maternal age (in years)	0.03 (0.04)	1.03 (0.96–1.11)	0.43	—
Nulliparous	−0.42 (0.43)	0.66 (0.28–1.51)	0.32	—
Maternal BMI (in kg/m^2)	−0.04 (0.04)	0.96 (0.89–1.03)	0.26	—
Smoker	1.14 (0.55)	3.12 (1.07–9.14)	0.04	—
Chronic hypertension	−0.19 (0.60)	0.83 (0.26–2.69)	0.76	—
GA at sampling (in weeks)	−0.17 (0.14)	0.85 (0.64–1.12)	0.24	—
GA at FGR diagnosis (in weeks)	0.18 (0.10)	1.20 (0.99–1.45)	0.07	—
PlGF	−0.92 (0.27)	0.40 (0.23–0.68)	< 0.001	0.755 (0.048)
sFlt-1/PlGF ratio model				
Maternal age (in years)	0.03 (0.04)	1.03 (0.95–1.12)	0.42	—
Nulliparous	−0.53 (0.43)	0.59 (0.25–1.37)	0.22	—
Maternal BMI (in kg/m^2)	−0.03 (0.04)	0.97 (0.90–1.05)	0.43	—
Smoker	1.17 (0.56)	3.23 (1.07–9.73)	0.04	—
Chronic hypertension	−0.13 (0.64)	0.88 (0.25–3.07)	0.84	—
GA at sampling (in weeks)	−0.35 (0.16)	0.71 (0.51–0.97)	0.03	—
GA at FGR diagnosis (in weeks)	0.20 (0.11)	1.23 (1.00–1.51)	0.05	—
sFlt-1/PlGF ratio	1.37 (0.33)	3.94 (2.05–7.59)	< 0.001	0.790 (0.046)

Variables other than angiogenic markers are included in all models. Concordance index (C-index) is reported for three models that include each angiogenic marker separately. Angiogenic marker values are log-transformed. BMI, body mass index; FGR, fetal growth restriction; GA, gestational age; HR, hazard ratio; PlGF, placental growth factor; SE, standard error; sFlt-1, soluble fms-like tyrosine kinase-1.

risk stratification by the model comprising sFlt-1, PlGF and GA at sampling, the very low, low, baseline and moderate risk categories showed good differentiation of absolute risk up to 2 weeks after sampling, but risk classification capability decreased after the 2-week timepoint.

Logistic regression analysis was performed to evaluate the predictive value of sFlt-1, PlGF and their ratio in combination with baseline characteristics for the risk of developing pre-eclampsia within 1 week, 2 weeks and at any time after sampling in patients with FGR (Tables 3 and 4). The sFlt-1/PlGF ratio performed well as a predictive marker for the development of pre-eclampsia within 1 week (AUC, 0.87) and 2 weeks (AUC, 0.80), but there was a significant drop in performance if pre-eclampsia development later than 2 weeks was considered (AUC, 0.69). sFlt-1 alone showed a good performance for the development of pre-eclampsia within 1 week, although it was inferior to the sFlt-1/PlGF model (AUC, 0.82 *vs* 0.87; $P = 0.002$). The performance of sFlt-1 alone decreased significantly when pre-eclampsia developed after 1 week (AUC, 0.73). The sFlt-1/PlGF ratio was superior to both PlGF and sFlt-1 alone, while the PlGF model had the poorest performance at all timepoints.

Predictive accuracy characteristics were calculated for the sFlt-1, PlGF and sFlt-1/PlGF ratio models separately using Youden index cut-offs. The sFlt-1/PlGF ratio had high performance for the prediction of pre-eclampsia within 1 week (PPV, 0.968; sensitivity, 0.833; specificity, 0.857) and within 2 weeks (PPV, 0.911; sensitivity, 0.761; specificity, 0.737) from assessment, while predictive accuracy decreased after 2 weeks (PPV, 0.842; sensitivity, 0.320; specificity, 0.917). Similarly, the predictive performance of sFlt-1 and PlGF in isolation decreased over time, with both showing overall inferior performance compared with that of the sFlt-1/PlGF ratio (Table 5).

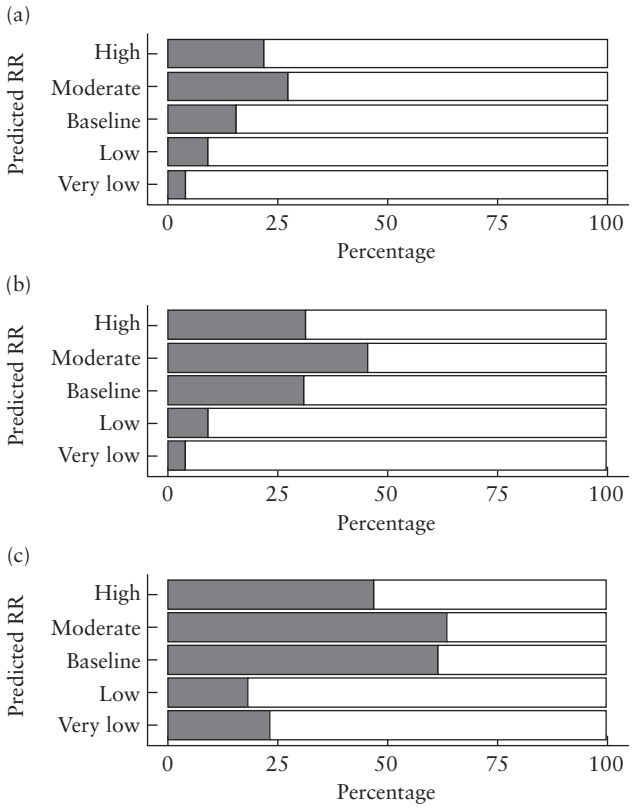

Figure 1 Risk stratification for development of pre-eclampsia (■) within 1 week (a), within 2 weeks (b) and at any time (c) after assessment, according to results of Cox regression model using soluble fms-like tyrosine kinase-1, placental growth factor and gestational age at sampling. Very low relative risk (RR), ≤ 0.5; low RR, > 0.5 to ≤ 0.75; baseline RR, > 0.75 to ≤ 1.33; moderate RR, > 1.33 to ≤ 2; high RR, > 2. □, no pre-eclampsia.

Table 3 Areas under receiver-operating-characteristics curves for models predicting development of pre-eclampsia (PE) at specific timepoints after assessment

Model	*PE within 1 week (n = 14)*	*PE within 2 weeks (n = 21)*	*PE at any time (n = 38)*
sFlt-1/PlGF ratio	0.87 (0.76–0.97)	0.80 (0.69–0.92)	0.69 (0.57–0.80)
sFlt-1	0.82 (0.72–0.92)	0.73 (0.62–0.84)	0.69 (0.58–0.80)
PlGF	0.72 (0.58–0.86)	0.72 (0.59–0.84)	0.65 (0.54–0.77)

Variables other than angiogenic markers are included in all models. Data in parentheses are 95% CI. PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

Performance of established cut-offs

We tested the following predefined cut-off values for the sFlt-1/PlGF ratio to predict pre-eclampsia in our cohort[17]: sFlt-1/PlGF ratio < 38 for ruling out pre-eclampsia within 2 weeks, and sFlt-1/PlGF ratio > 85 before 34 weeks' gestation and > 110 at or after 34 weeks for ruling in pre-eclampsia within 1 week (Table 6). The rule-out cut-off of < 38 showed a high NPV (0.933) and high sensitivity (0.952) for the development of pre-eclampsia within 2 weeks from assessment, although specificity was poor (0.194). The rule-in cut-off values of > 85 and > 110 (before and at/after 34 weeks' gestation, respectively) showed suboptimal predictive capability in this cohort (PPV, 0.194; NPV, 0.961; sensitivity, 0.928; specificity, 0.316). The predictive performance of the sFlt-1/PlGF ratio combined with GA at sampling performed equally before and at/after 34 weeks (AUC, 0.83 *vs* 0.93; $P = 0.333$) (Figure S1).

DISCUSSION

Summary of key findings

Maternal serum angiogenic markers are associated strongly with the development of pre-eclampsia in pregnancies with FGR. The sFlt-1/PlGF ratio was superior to sFlt-1 or PlGF alone in all investigated models, although the association weakened drastically beyond 2 weeks after the initial assessment. The established cut-off value of the sFlt-1/PlGF ratio < 38 was effective in ruling out pre-eclampsia within 2 weeks in pregnancies with FGR. Cut-off values for ruling in pre-eclampsia within 1 week

of assessment, defined as sFlt-1/PlGF ratio > 85 before 34 weeks' gestation and > 110 at or after 34 weeks, showed suboptimal predictive capability in this cohort. There was no conclusive evidence to suggest superior performance of angiogenic markers prior to 34 weeks compared with that at or after 34 weeks.

Interpretation of study findings and comparison with literature

In this study, we demonstrated that angiogenic markers are capable of predicting the development of pre-eclampsia in the short term in pregnancies with FGR that were assessed for symptoms of pre-eclampsia but did not fulfill the criteria for a pre-eclampsia diagnosis. The sFlt-1/PlGF ratio has been widely established as a predictive marker for the development of pre-eclampsia and particularly for ruling out pre-eclampsia in patients presenting with classic signs of the disease, such as hypertension and proteinuria[17,26,27]. In recent years, it has been shown that angiogenic imbalance is present in pregnancies with FGR combined with pre-eclampsia, as well as in those with FGR alone, while the imbalance is greater in patients with co-existing pre-eclampsia[18,23,28,29]. Increased sFlt-1/PlGF ratio has been shown to be predictive of adverse outcome and shorter time to delivery in early-onset SGA and FGR[7,20,21,30] and has been suggested as a potential additive tool for fetal surveillance in these patients[20,22]. Various studies have demonstrated the value of the sFlt-1/PlGF ratio in the management of pregnancies complicated by FGR, showing an association between increased maternal serum sFlt-1/PlGF values and shorter time to delivery as well as higher rates of adverse perinatal outcome[7,21,31,32]. Bonacina *et al.*[22] confirmed the high predictive performance of angiogenic markers for fetal distress, and proposed incorporating assessment of the sFlt-1/PlGF ratio into surveillance protocols for

Table 5 Predictive accuracy characteristics of angiogenic marker models for pre-eclampsia (PE) at their Youden index cut-off

Characteristic	*sFlt-1/PlGF ratio model*	*sFlt-1 model*	*PlGF model*
PE within 1 week			
Sensitivity	0.833	0.778	0.736
Specificity	0.857	0.857	0.643
PPV	0.968	0.966	0.914
NPV	0.500	0.429	0.321
PE within 2 weeks			
Sensitivity	0.761	0.687	0.403
Specificity	0.737	0.789	0.947
PPV	0.911	0.920	0.964
NPV	0.467	0.417	0.310
PE at any time			
Sensitivity	0.320	0.540	0.480
Specificity	0.917	0.778	0.778
PPV	0.842	0.771	0.750
NPV	0.493	0.549	0.519

All models included gestational age at sampling of placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1). NPV, negative predictive value; PPV, positive predictive value.

Table 6 Predictive accuracy of established cut-offs for soluble fms-like tyrosine kinase-1 to placental growth factor (sFlt-1/PlGF) ratio for pre-eclampsia development in pregnancies with fetal growth restriction

Characteristic	*sFlt-1/PlGF ratio < 38 at any GA (rule out < 2 weeks)*	*Abnormal sFlt-1/PlGF ratio (rule in < 1 week)**
Sensitivity	0.952	0.928
Specificity	0.194	0.316
PPV	0.256	0.194
NPV	0.933	0.961

*Defined as > 85 before 34 weeks' gestation and > 110 at or after 34 weeks. GA, gestational age; NPV, negative predictive value; PPV, positive predictive value.

Table 4 Logistic regression model showing association of baseline characteristics and angiogenic markers with odds of developing pre-eclampsia (PE) at specific timepoints after assessment

	PE within 1 week (n = 14)		*PE within 2 weeks* (n = 21)		*PE at any time* (n = 38)	
Variable	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P
sFlt-1/PlGF ratio model						
Smoker	3.56 (0.67–19.27)	0.13	3.50 (0.77–16.70)	0.10	2.62 (0.76–9.67)	0.13
Chronic hypertension	1.98 (0.24–12.96)	0.48	1.36 (0.23–6.89)	0.71	1.59 (0.43–5.91)	0.48
GA at sampling (in weeks)	1.21 (1.03–1.45)	0.02	1.09 (0.94–1.26)	0.26	0.93 (0.82–1.04)	0.21
sFlt-1/PlGF ratio	10.49 (3.08–53.39)	0.001	6.61 (2.46–23.12)	< 0.001	1.56 (0.94–2.77)	0.10
sFlt-1 model						
Smoker	2.29 (0.46–10.69)	0.29	2.43 (0.60–9.57)	0.20	2.44 (0.71–8.99)	0.16
Chronic hypertension	1.83 (0.22–11.55)	0.53	0.96 (0.17–4.44)	0.96	1.59 (0.44–5.83)	0.48
GA at sampling (in weeks)	1.01 (0.87–1.18)	0.87	0.93 (0.81–1.06)	0.28	0.89 (0.79–0.99)	0.03
sFlt-1	4.91 (2.05–15.46)	0.002	2.54 (1.35–5.44)	0.008	1.64 (1.02–2.77)	0.05
PlGF model						
Smoker	3.31 (0.79–14.45)	0.10	2.82 (0.62–12.14)	0.16	2.62 (0.77–9.51)	0.13
Chronic hypertension	0.88 (0.15–4.13)	0.88	1.02 (0.13–5.38)	0.98	1.37 (0.38–4.92)	0.63
GA at sampling (in weeks)	1.10 (0.94–1.29)	0.23	1.16 (0.98–1.38)	0.08	0.93 (0.81–1.04)	0.22
PlGF	0.25 (0.09–0.59)	0.004	0.30 (0.10–0.74)	0.02	0.77 (0.43–1.34)	0.37

All presented analyses are multivariable. Angiogenic markers are log-transformed. GA, gestational age; OR, odds ratio; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

pregnancies with FGR. However, the utility of angiogenic markers as predictors of pre-eclampsia in pregnancies with FGR and suspicion of pre-eclampsia has not been evaluated to date.

Previous literature on the utility of angiogenic markers in FGR has focused mainly on fetal surveillance, while this study, comprising a cohort of patients with confirmed FGR according to Delphi consensus criteria[2], evaluated the usefulness of angiogenic-marker assessment to predict pre-eclampsia. Our data attest to the importance of angiogenic-marker assessment in pregnancies complicated by FGR for assessing the risk of maternal complications. The established cut-off to rule out pre-eclampsia in the short term (sFlt-1/PlGF < 38)[17] showed high rule-out potential in our cohort. The performance of pre-defined cut-offs to rule in pre-eclampsia[17], however, did not perform well in our cohort, questioning their applicability to patients with FGR and suspected pre-eclampsia.

Strengths and limitations

A novel aspect of this study is its assessment of the prognostic value of angiogenic markers for predicting the development of pre-eclampsia in pregnancies diagnosed with FGR according to stringent criteria. This study provides valuable information on the comparative performance of sFlt-1, PlGF and their ratio in women with FGR and suspected pre-eclampsia. Recent recommendations of the International Society for the Study of Hypertension in Pregnancy[33] suggest including uteroplacental dysfunction, such as FGR, as a diagnostic criterion for pre-eclampsia. This retrospective study did not follow this recommendation, because it is not universally accepted and, to date, has not been implemented internationally. The diagnosis of pre-eclampsia was based on universally established diagnostic criteria[24], which were used for clinical guidance at our department during the observed time period.

A limitation of our study is that clinicians were not blinded to values of the sFlt-1/PlGF ratio in women with suspected pre-eclampsia. Therefore, we cannot rule out the possibility of intervention bias. However, there is no local protocol or national/international guideline recommending delivery solely based on angiogenic markers in women with FGR, minimizing the likelihood of intervention bias. We acknowledge additional limitations resulting from the small sample size. However, owing to the uniformity of the cohort resulting from strict inclusion criteria, we are confident in the relevance of the data. Furthermore, we acknowledge that our sensitivity analysis of angiogenic markers in late- *vs* early-onset cases was limited, because of the high proportion of early-onset (< 34 weeks) cases within our cohort.

Clinical and research implications

In this study, the sFlt-1/PlGF ratio was associated strongly with the development of pre-eclampsia in pregnancies with FGR presenting with symptoms suspicious of pre-eclampsia, chiefly elevated blood pressure. Pregnancies with FGR are at elevated risk for developing pre-eclampsia, a potentially life-threatening condition for both the mother and the fetus. Identifying women at increased risk for pre-eclampsia presents one of the main challenges in the management of FGR, pre-eclampsia often being the limiting factor in prolonging these pregnancies. These patients should be monitored closely for maternal complications, and improved surveillance strategies, including angiogenic marker assessment, are needed to complement conventional methods, such as blood-pressure monitoring, in order to identify pre-eclampsia and predict the time to delivery. Our data support the use of the established sFlt-1/PlGF ratio cut-off of < 38 to rule out pre-eclampsia within 2 weeks in pregnancies with FGR. Predefined rule-in cut-offs for the prediction of pre-eclampsia should be re-evaluated within a larger cohort of pregnancies complicated by FGR.

Conclusions

We have shown that the maternal serum angiogenic markers sFlt-1 and PlGF have greater prognostic potential when used in combination compared with their solitary use. They offer sound short-term risk stratification capability for pre-eclampsia for up to 2 weeks after assessment. The predefined cut-off of < 38 proved to be effective in ruling out pre-eclampsia in pregnancies complicated by FGR with suspected pre-eclampsia. There was no conclusive difference in the performance of biomarkers for early- *vs* late-onset pre-eclampsia in this small cohort.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Performance of soluble fms-like tyrosine kinase-1 to placental growth factor ratio and gestational age at sampling for predicting development of pre-eclampsia within 1 week after assessment, stratified by gestational age at sampling.