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Prognostic value of angiogenic markers in pregnancies with fetal growth restriction

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CONTRIBUTION

What are the novel findings of this work?

This study documents the prognostic potential of angiogenic markers sFlt-1, PlGF and their ratio for predicting the development of preeclampsia in pregnancies with fetal growth restriction and suspected preeclampsia.

What are the clinical implications of this work?

Establishing improved strategies to identify pregnant women at increased risk for preeclampsia is of great significance in the management of fetal growth restriction. This work highlights the superiority of sFlt-1/PlGF ratio to PlGF alone in pregnancies with fetal growth restriction and the preserved rule-out potential of sFLT-1/PlGF ratio for the development of preeclampsia using the established cut-offs.

ABSTRACT

Objective: Pregnancies with fetal growth restriction are at increased risk of preeclampsia. Angiogenic markers including soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are altered in pregnancies complicated by fetal growth restriction (FGR). The utility of these markers as a predictor of preeclampsia in women with growth-restricted fetuses is still uncertain. This study aims to evaluate the prognostic value of angiogenic markers for predicting the development of preeclampsia in pregnancies with FGR and suspected preeclampsia.

Methods: This study included 93 women with FGR, defined according to Delphi consensus criteria, who were assessed for angiogenic markers sFlt-1 and PlGF for suspicion of preeclampsia at the Department of Obstetrics and feto-maternal Medicine at the Medical University of Vienna between 2013 and 2020. Women with established diagnosis of preeclampsia at sampling were excluded. Cox regression analysis and logistic regression were performed to demonstrate the association of angiogenic markers with the outcome.

Results: Within this cohort, 14 women (15.1%) developed preeclampsia within one week from sampling, 21 (22.6%) within two weeks, 38 (40.9%) at any time. The sFLT-1/PLGF ratio consistently showed a stronger association with development of preeclampsia compared to sFlt-1 or PlGF alone in pregnancies with fetal growth restriction (PE within a week, AUC 0.85 vs 0.82 and 0.72, respectively). Models including sFlt-1/PlGF were more strongly associated with preeclampsia hazard compared to sFlt-1 and PlGF alone models (C-index: 0.79±0.046 vs 0.76±0.048 and 0.75±0.047, respectively). Risk classification capabilities of sFlt-1/PlGF decreased after the two-week time point. The established cut-off value for ruling out preeclampsia (sFlt-1/PlGF ratio <38) was effective with a negative predictive value of 93.3% and sensitivity of 95.2%.

Conclusion: Combined use of sFlt-1/PlGF can be preferred to PlGF alone in pregnancies with fetal growth restriction. Moreover, established cut-offs for ruling-out development of preeclampsia seem to be effective in these patients.

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 due to an underlying condition, uteroplacental insufficiency being one of the most common causes [2,5,6]. FGR, as an expression of impaired placental function, shows similarities to clinical and pathophysiological features of preeclampsia [7] and pregnancies with growth-restricted fetuses are at increased risk for developing preeclampsia, a potentially life-threatening condition for both the mother and the fetus [7,8]. The diagnosis and monitoring of FGR are currently based on ultrasound assessment comprising fetal weight estimation, assessment of the amniotic fluid volume and doppler indices [9-13], while various recent studies were able to show a higher sensitivity in discovering and monitoring placental dysfunction with a combination of biophysical and biochemical parameters [14,15]. Imbalance of the angiogenic biomarkers soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), characterized by an increased sFlt-1/PlGF ratio, has been identified to play an important role in the development of placental dysfunction and related diseases such as preeclampsia [16-19] and FGR [18,20]. Increased maternal serum sFlt-1/PlGF values have also been demonstrated to be predictive of adverse perinatal outcomes and shorter time to delivery in early-onset SGA and FGR [7,20,21] and were suggested as potential additive tools for fetal surveillance in these patients [20,22]. However, the utility of sFlt-1/PlGF as a predictor of preeclampsia in pregnancies with growth-restricted fetuses is still uncertain. Angiogenic marker levels have been found to be altered in FGR in presence as well as in absence of preeclampsia [23], which complicates the prediction of development of preeclampsia and raises the question whether predefined cut-offs of the sFlt-1/PlGF ratio or PlGF for preeclampsia prediction and diagnosis can be applied equally in pregnancies with or without FGR. As preeclampsia is often a limiting factor in pregnancies complicated by FGR, evaluating the risk of maternal complications, in particular the development of preeclampsia, is a key factor and presents one of the main clinical challenges in uteroplacental insufficiency, manifested as FGR. Recommended methods of maternal surveillance in women with FGR are currently limited to conventional strategies, such as blood

pressure monitoring. Improved surveillance strategies would be helpful to identify the mother at risk for delivery due to maternal complications such as preeclampsia and would aid counselling of pregnant women. Therefore, this study aims to demonstrate the prognostic value of maternal serum angiogenic markers (sFlt-1 and PlGF) for predicting the development of preeclampsia in pregnancies with a growth restricted fetus.

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. No written informed consent for study participation was required.

The study population consisted of women with singleton pregnancies and established diagnosis of FGR, defined according to the Delphi consensus definition using estimated fetal weight at time of assessment [2].

that underwent sampling of angiogenic markers sFlt-1, PlGF and their ratio, for suspicion of preeclampsia. Suspicion of preeclampsia was based on presenting with any of the following symptoms: high blood pressure, de novo proteinuria, de novo edema, elevated liver enzymes, epigastric pain, low platelets, dyspnea or neurological symptoms including visual disturbances and severe headaches. Women with established diagnosis of preeclampsia at time of sampling were excluded, as well as women suffering from chronic kidney disease, pregnancies with aneuploidy, genetic syndromes or major structural fetal anomalies. Women who did not deliver at the Department of Obstetrics and feto-maternal Medicine at the Medical University of Vienna were also excluded due to missing outcome data.

Preeclampsia was defined as new-onset systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or worsening of preexisting hypertension and additional organ manifestation including proteinuria (protein/creatinine ratio ≥30 mg/mmol or ≥300 mg protein/24 hours) and/or elevated liver enzymes (twice the upper reference values), thrombocytopenia (platelet count <100 000/μL), kidney dysfunction (serum creatinine > 1.1mg/dL), visual disturbances and neurological symptoms (e.g. altered mental state, severe headaches, persistent visual scotoma, eclampsia) and pulmonary edema [24].

As part of the routine assessment in women with suspected preeclampsia, 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 Elecsys (Roche Diagnostics, Penzberg, Germany) platform. The analysis was undertaken by biomedical technicians.

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

Statistical analysis

Explanatory variables were presented as median and interquartile ranges (IQR) for continuous data and as n (%) for categorical data. Categorical variables were compared by X^2-test or Fisher's exact test, while continuous data were compared using either independent samples t-test or Mann-Whitney U test. Association of angiogenic markers for development of preeclampsia was assessed with Cox-regression and logistic regression models. In time-to-event analyses, hazard of preeclampsia development was assessed by taking assessment-to-diagnosis interval into account. Those who delivered without a diagnosis of preeclampsia were considered right censored. Cox models were compared according to their C-index. Predicted risk estimates from Cox-models were obtained and development of preeclampsia at certain time periods were compared among different risk categories. The same endpoints (preeclampsia development at certain time frames) were also assessed with logistic regression analyses. Logistic models were compared with area under the receiver operating characteristics curves using De Long's test. Sensitivity, specificity, negative and positive predictive values were calculated using pre-established cut-offs and the Youden index of

logistics models. All analyses were conducted using R for statistical computing software (v.4.2.2).

RESULTS

The study included 93 singleton pregnancies complicated by FGR that underwent sampling of maternal serum sFlt-1, PlGF and their ratio for suspicion of preeclampsia. The median gestational age (GA) at diagnosis of FGR was 26.4 (IQR: 24.3 to 31.0) weeks. 38 (40.9%) women among the cohort developed preeclampsia. The median GA at diagnosis of preeclampsia was 29.6 (IQR: 25.9 – 33.9) weeks. There were no significant differences in maternal demographics (Table 1) between pregnancies with isolated FGR and women who developed preeclampsia later in pregnancy. GA at sampling of angiogenic markers was significantly lower in the group that developed preeclampsia compared to those who did not (median 28.4 weeks in the preeclampsia group vs 30.6 weeks in the no preeclampsia group, p=0.044), while symptoms for suspicion of preeclampsia were similar between the two groups. The most common reason for angiogenic marker assessment due to suspicion of preeclampsia was maternal raised blood pressure. Women who developed preeclampsia later in pregnancy presented with high blood pressure significantly more often than women who were non-preeclamptic (92.1% vs 70.9%, p=0.026). The indication for delivery was FGR-related in 56 patients (60.2%), preeclampsia combined with FGR in 22 patients (23.7%), purely preeclampsia in 12 (12.9%), preterm labor in two cases (2.1%) and due to placental abruption (1.1%) in one case. The birthweight percentile did not differ significantly between the two groups (p=0.317).

Stillbirth occurred in 3 cases (7.9%), all among the preeclampsia cohort. We found no significant differences in neonatal adverse outcome measures including low 5-minute APGAR (p=0.132), admission to neonatal intensive care unit (NICU) (p=0.727), need for ventilation support (p=0.708), necrotizing enterocolitis (NEC) (p=0.128) and intraventricular hemorrhage (IVH) (p=0.999) between the two study groups (Table 1).

Association of baseline characteristics and angiogenic markers with the risk of preeclampsia

Cox regression analysis was performed to investigate the association of baseline characteristics and angiogenic markers with the hazard of developing preeclampsia (Table 2). We calculated three models, separately testing for the association of sFlt-1, PlGF and the sFlt-1/PlGF ratio. All angiogenic markers were entered the models in log-transformed and scaled fashion, meaning hazard ratios correspond to one standard unit change in the log—unit of respective parameter. All models included baseline characteristics that present established risk factors for preeclampsia (age, nulliparity, BMI, smoking and chronic hypertension) [25] as well as GA at FGR diagnosis and GA at sampling. The analysis demonstrated the strongest association of sFlt-1/PlGF ratio alone (HR 3.94, $p<0.001$), despite significant associations for smoking and GA at sampling (HR 3.23, $p=0.04$ and HR 0.71, $p=0.03$). The sFlt-1/PlGF ratio model was superior to sFlt-1 and PlGF models (C-index:0.79 vs. 0.759 and 0.755, respectively).

Figure 1 shows the risk of preeclampsia development compared between predicted relative risk groups from Cox-model, categorized as follows: very low risk (predicted relative risk ≤0.5), low risk (>0.5 to ≤0.75), baseline (>0.75 to ≤1.33), moderate (>1.33 to ≤2) and high risk (>2). Risk stratification was performed according to results calculated in the cox regression model (Table 2) using sFlt-1, PlGF and GA at sampling. Development of preeclampsia was stratified for time of disease onset, time points were grouped as follows: development of preeclampsia within one week (Figure 1a), within two weeks (Figure 1b) or at any time after assessment (Figure 1c). In the study cohort, 14 women (15.1%) developed preeclampsia within one week from sampling, 21 (22.6%) within two weeks, 38 (40.9%) at any time. According to risk stratification categories from sFlt-1, PlGF and GA at sampling combined model, very low, low, baseline moderate risk categories showed good differentiation of absolute risk up to two weeks from sampling. Risk classification capability decreased after the two-week time point.

Logistic regression analysis was performed additionally to evaluate the predictive value of sFlt-1, PlGF and their ratio in combination with baseline characteristics for the risk of

preeclampsia in patients with FGR within one week, two weeks and at any time after sampling (Table 3). The sFlt-1/PlGF ratio performed well as a predictive marker for development of preeclampsia within one week and two weeks (AUC 0.87 and 0.80, respectively) and there was significant drop in the AUC values if model considered preeclampsia developed later than 2 weeks (AUC: 0.69) (Table 3). sFlt-1 alone showed good performance within one week, however, was inferior to the sFlt-1/PlGF model (AUC 0.82 vs 0.87, *p*=0.002). Performance of sFlt-1 alone decreased significantly after one week (AUC 0.82 vs 0.73). In comparison, the PlGF model was inferior to both models (Table 3). The sFlt-1/PlGF ratio was superior to both PlGF and sFlt-1 alone for all time points.

Predictive accuracy characteristics was calculated for sFlt-1, PlGF and sFlt-1/PlGF ratio separately using Youden index cut-offs. We observed high predictive performance of the sFlt-1/PlGF ratio for prediction of preeclampsia within one week, (PPV 0.968, sensitivity 0.833, specificity 0.857) and two weeks (PPV 0.911, sensitivity 0.761, specificity 0.737), while predictive accuracy decreased after two weeks (PPV 0.842, sensitivity 0.320, specificity 0.917). The predictive performance of sFlt-1 and PlGF alone similarly decreased over time, both showing overall inferior performance compared to the sFlt-1/PlGF ratio (Table 4).

Performance of established cut-offs

We tested predefined cut-off values [17] for the sFlt-1/PlGF ratio (sFlt-1/PlGF ratio < 38 for ruling out preeclampsia within 2 weeks, sFlt-1/PlGF ratio >85 before 34 weeks and >110 after 34 weeks of gestation for ruling in preeclampsia within one week), for their predictive accuracy within our cohort of women with FGR and suspected preeclampsia (Table 5). The rule-out cut-off of 38 showed a high negative predictive value and high sensitivity for the development of preeclampsia within two weeks from assessment, although specificity was poor (NPV 93.3, PPV 25.6, sensitivity 95.2, specificity 19.4). Cut-off values > 85 and > 110 (before and after 34 weeks of gestation, respectively) were evaluated for their capability to predict development of preeclampsia within one week from assessment. Ruling-in cut-offs showed suboptimal predictive capability in this cohort (PPV 19.4, NPV 96.1, Sensitivity 92.8, Specificity 31.6). The

predictive performance of the sFlt-1/PlGF ratio combined with GA at assessment performed equally before and after 34 weeks of gestation (Supplementary Figure 1).

DISCUSSION

Summary of the key findings

Maternal serum angiogenic markers are strongly associated with the development of preeclampsia in pregnancies with FGR. The sFlt-1/PlGF ratio was superior to sFlt-1 and PlGF alone in all investigated models while association weakened drastically two weeks after the initial assessment. The established cut-off value of the sFlt-1/PlGF ratio below 38 was still effective in ruling out preeclampsia within 2 weeks in pregnancies with FGR. Cut-off values for ruling in preeclampsia within one week of assessment, defined as sFlt-1/PlGF ratio > 85 before 34 weeks of gestation and > 110 after 34 weeks of gestation, showed suboptimal predictive capability in this cohort. There was no conclusive evidence regarding the superior performance of angiogenic markers prior to 34 weeks of gestation, compared to after 34 weeks of gestation.

Interpretation of the study findings and comparison with existing literature

In this study, we were able to demonstrate that angiogenic markers are capable of predicting the development of preeclampsia in the short-term in pregnancies with FGR that were assessed for symptoms of preeclampsia but did not fulfill criteria of preeclampsia diagnosis. The sFlt-1/PlGF ratio has been widely established as a predictive marker for the development of preeclampsia and particularly for ruling out preeclampsia in patients presenting with classic signs of preeclampsia 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 preeclampsia as well as FGR alone, while values were higher in patients with co-existing preeclampsia [18,23,28,29]. Increased sFlt-1/PlGF values have been demonstrated to be predictive of adverse outcomes and shorter time to delivery in early-onset SGA and FGR [7,20,21,30] and were suggested as potential additive tools for fetal surveillance in these patients [20,22]. Various studies have demonstrated a 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 outcomes [7,21,31,32]. Bonacina et al. confirmed the high predictive performance of angiogenic markers for

detecting higher risks of fetal distress, proposing the implementation of sFlt-1/PlGF ratio assessment in surveillance protocols of pregnancies with FGR [22]. The utility of angiogenic markers as a predictor of preeclampsia in pregnancies with growth-restricted fetuses and suspicion of preeclampsia, however, has not been evaluated to date. Previous literature on the utility of angiogenic markers in FGR has mainly focused on fetal surveillance, while this study, consisting of a cohort of patients with confirmed FGR, according to Delphi consensus criteria [2], evaluated the usefulness of angiogenic marker assessment to predict preeclampsia within this cohort. Our data support the importance of angiogenic marker assessment in pregnancies complicated by FGR for risk assessment of maternal complications. The established cut-off to rule out preeclampsia in the short term (sFlt-1/PlGF < 38) [17] similarly showed high rule-out potential within our cohort of pregnancies with growth restricted fetuses, supporting its utility. The performance of pre-defined cut-offs to rule in preeclampsia [17], however, did not perform well within our cohort, raising the question whether these cut-off values of the sFlt-1/PlGF can be used for preeclampsia diagnosis in patients with FGR and suspected preeclampsia.

Strengths and weaknesses

The novel aspect of this study is the assessment of the prognostic value of angiogenic markers for predicting the development of preeclampsia in pregnancies with FGR diagnosed with a stringent criterion. This study provides valuable information on the comparative performance of the angiogenic markers sFlt-1, PlGF and their ratio in women with FGR and suspected preeclampsia. Recent recommendations by the International Society for the Study of Hypertension in Pregnancy [33], suggest including uteroplacental dysfunction such as FGR as a diagnostic criterion of preeclampsia. In this retrospective study, however, we did not consider the diagnosis of FGR as a diagnostic criterion for preeclampsia, as these criteria are not universally accepted and, to date, have not been implemented internationally. The diagnosis of preeclampsia was based on universally established diagnostic criteria as mentioned above [24], which were applied for clinical guidance at our department in the

observed time period. Our study has some limitations. Clinicians were not blinded to sFlt-1/PlGF values in women with suspected preeclampsia. Therefore, we cannot rule out the possibility of intervention bias. There is, however, no local protocol or any 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, due to the uniformity of the cohort resulting from strict inclusion criteria, we are confident of the relevance of the data. Furthermore, we acknowledge some limitation in the sensitivity analysis of angiogenic markers in late onset cases compared to cases with onset prior to 34 weeks of gestation, due to the high number of early onset cases within our cohort.

Clinical and Research implications

In this study, the sFlt-1/PlGF ratio showed strong association the development of preeclampsia, in pregnancies with FGR presenting with a suspicion of the diagnosis, chiefly due to elevated blood pressure. Pregnancies with growth-restricted fetuses are at elevated risk for developing preeclampsia, a potentially life-threatening condition for both the mother and the baby. Identifying the woman at increased risk for preeclampsia presents one of the main challenges in the management of FGR, preeclampsia often being the limiting factor in prolonging these pregnancies. These patients should be closely monitored for maternal complications and improved strategies including angiogenic marker assessment in addition to conventional surveillance strategies, such as blood pressure monitoring, to identify preeclampsia, as well as to predict the time to delivery, are highly necessary. Our data support the use of the established sFlt-1/PlGF ratio cut-off of <38 to rule out preeclampsia within 2 weeks in pregnancies with fetal growth restriction, emphasizing usefulness of angiogenic markers in short-term risk assessment. Predefined rule-in cut-offs for the prediction of preeclampsia need to be reevaluated within a larger cohort of pregnancies complicated by FGR.

Conclusions

In conclusion, we demonstrated that maternal serum angiogenic markers when used in combination (sFlt-1 & PlGF) provide stronger prognostic potential compared to solitary use (i.e. PlGF alone). They offer sound short-term risk stratification capabilities up to two-weeks in terms of preeclampsia development. The predefined cut-off of <38 proved to be effective in ruling-out preeclampsia in pregnancies complicated by FGR with suspected preeclampsia. There was no conclusive change in their performance between early and late-onset cases in this small cohort.

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FIGURE LEGENDS

Figure 1a-c. Risk stratification according to the results of cox model using sFlt-1, PlGF and gestational age at sampling. Preeclampsia development is stratified according to time of disease onset (Figure 1a = within one week, Figure 1b = within two weeks, Figure 1c = any time). In predicting development of preeclampsia after assessment in dependence of the different time points, the model including sFlt-1/PlGF ratio and gestational age at sampling performed well for prediction of development within 1 and 2 weeks, showing the highest predictive value for the high and moderate risk groups.

Table 1. Baseline characteristics and outcomes and symptoms at presentation suspicious for preeclampsia. Cohort divided into women who developed preeclampsia or no preeclampsia.

Data variables	levels	Preeclampsia (n=38)	No preeclampsia (n=55)	P value
Maternal age in years	Median (IQR)	32.5 (30.0 to 36.0)	33.0 (27.5 to 36.0)	0.338
Nulliparous, n (%)	Yes	21 (55.3)	37 (67.3)	0.229
	No	17 (44.7)	16 (29.1)	
	(Missing)	0 (0.0)	2 (3.6)	
Conception, n (%)	spontaneous	31 (81.6)	45 (81.8)	0.711
	IVF	2 (5.3)	3 (5.5)	
	egg cell donation	0 (0.0)	1 (1.8)	
	(Missing)	5 (13.2)	6 (10.9)	
Smoking, n (%)	Yes	8 (21.1)	7 (12.7)	0.482
	No	28 (73.7)	43 (78.2)	
	(Missing)	2 (5.3)	5 (9.1)	
Maternal body mass index (kg/m2)	Median (IQR)	25.7 (21.6 to 29.0)	24.0 (21.9 to 27.5)	0.663
Epigastric pain, n (%)	Yes	2 (5.3)	2 (3.6)	1.000
	No	36 (94.7)	53 (96.4)	
Edema, n (%)	Yes	6 (15.8)	5 (9.1)	0.511

Data variables	levels	Preeclampsia (n=38)	No preeclampsia (n=55)	P value
	No	32 (84.2)	50 (90.9)	
Proteinuria, n (%)	Yes	3 (7.9)	8 (14.5)	0.516
	No	35 (92.1)	47 (85.5)	
Elevated liver enzymes, n (%)	Yes	0 (0.0)	3 (5.5)	0.386
	No	38 (100.0)	52 (94.5)	
High blood pressure, n (%)	Yes	35 (92.1)	39 (70.9)	0.026
	No	3 (7.9)	16 (29.1)	
Dyspnea, n (%)	Yes	1 (2.6)	0 (0.0)	0.852
	No	37 (97.4)	55 (100.0)	
Neurological symptoms, n (%)	Yes	3 (7.9)	2 (3.6)	0.669
	No	35 (92.1)	53 (96.4)	
Gestational age at sampling (weeks)	Median (IQR)	28.4 (24.9 to 31.5)	30.6 (26.6 to 34.5)	0.044
Gestational age at diagnosis (weeks)	Median (IQR)	26.4 (24.3 to 31.0)	28.9 (24.3 to 33.1)	0.284
Maternal ICU admission, n (%)	Yes	4 (10.5)	0 (0.0)	0.052
	No	34 (89.5)	55 (100%)	

Data variables	levels	Preeclampsia (n=38)	No preeclampsia (n=55)	P value
Birthweight percentile	Median (IQR)	4.8 (2.3 to 8.7)	6.7 (2.8 to 9.8)	0.317
Outcome n (%)	Livebirth	33 (86.8)	49 (89.1)	0.082
	Stillbirth	3 (7.9)	0 (0.0)	
	Early NND	2 (5.3)	6 (10.9)	
5-minute APGAR <7, n(%)	Yes	3 (7.9)	0 (0.0)	0.132
	No	35 (91.1)	54 (98.2)	
	(Missing)	0 (0.0)	1(1.8)	
Preterm birth <37 weeks, n (%)	Yes	34 (89.5)	42 (73.4)	0.182
	No	4 (10.5)	13 (23.6)	
Preterm birth <32 weeks, n (%)	Yes	21 (55.3)	23 (41.3)	0.287
	No	17 (44.7)	32 (58.2)	
NICU admission, n (%)	Yes	25 (65.8)	33 (60)	0.727
	No	13 (34.2	22 (40.0)	
Ventilation support, n (%)	Yes	21 (55.3)	27 (49.4)	0.708
	No	17 (44.7)	28 (50.9	
NEC, n (%)	Yes	3 (7.9)	0 (0.0	0.128

Data variables	levels	Preeclampsia (n=38)	No preeclampsia (n=55)	P value
	No	35 (92.1	55 (100.0)	
IVH, n (%)	Yes	1 (2.6)	2 (3.6)	0.999
	No	37 (97.4	53 (96.4)	

Data are presented as median (IQR) for continuous variables, categorical variables are presented as absolute frequencies and percentages, *n* (%). IQR: interquartile range, IVH: intraventricular hemorrhage, NEC: necrotizing enterocolitis, NICU: neonatal intensive care unit, NND: neonatal death

Table 2. Cox-regression model showing the association of baseline characteristics and angiogenic markers with the hazard of preeclampsia diagnosis.

Variable	Beta (SE)	HR (95% CI)	P value	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 (Kh/m2)	-0.00 (0.04)	1.00 (0.93, 1.07)	0.96	-
Smoking	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	-0.35 (0.17)	0.70 (0.51, 0.98)	**0.04**	-
GA at FGR diagnosis	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 (kg/m2)	-0.04 (0.04)	0.96 (0.89, 1.03)	0.26	-
Smoking	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	-
Gestational age at sampling	-0.17 (0.14)	0.85 (0.64, 1.12)	0.24	-
GA at FGR diagnosis	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 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 (kg/m2)	-0.03 (0.04)	0.97 (0.90, 1.05)	0.43	-
Smoking	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	-0.35 (0.16)	0.71 (0.51, 0.97)	**0.03**	-
GA at FGR diagnosis	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)

BMI: body-mass index, C-index: concordance index, GA: gestational age, HR: hazard ratio, PlGF: placental growth factor, SE: standard error, sFlt-1: soluble fms-like tyrosine kinase-1; *Variables other than angiogenic markers are included in all the models. Concordance index is reported for three models that include each angiogenic marker separately.

Table 3. Logistic regression model showing the association of baseline characteristics and angiogenic markers with the odds of preeclampsia diagnosis at specific time points.

	PE within a week (n=14)		**PE within two 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 model (AUC, 95% CI)	AUC: 0.87 (0.76 – 0.97)		AUC: 0.80 (0.69 – 0.92)		AUC: 0.69 (0.57 – 0.80)	
Smoking	3.56 (0.67, 19.27)	0.13	3.50 (0.77, 16.70)	0.1	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	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	AUC: 0.82 (0.72 – 0.92)		AUC: 0.73 (0.62 – 0.84)		AUC: 0.69 (0.58 – 0.80)	
Smoking	2.29 (0.46, 10.69)	0.29	2.43 (0.60, 9.57)	0.2	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	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 (AUC, 95% CI)	AUC: 0.72 (0.58 – 0.86)		AUC: 0.72 (0.59 – 0.84)		AUC: 0.65 (0.54 – 0.77)	

Smoking	3.31 (0.79, 14.45)	0.1	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
Gestational age at sampling	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

GA: gestational age, OR: odds ratio, PE: preeclampsia, PlGF: placental growth factor, sFlt-1: soluble fms-like tyrosine kinase-1

All presented analyses are multivariable.

Table 4. Predictive accuracy characteristics of different angiogenic markers and gestational age at assessment models at their Youden index cut-off.

	sFlt-1 & PlGF	**sFlt-1**	**PlGF**
PE within a 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 two 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

NPV: negative predictive value sFlt-1: soluble fms-like tyrosine kinase-1, PE: preeclampsia, PlGF: placental growth factor, PPV: positive predictive value

Table 5. Predictive accuracy of established sFlt-1/PlGF ratio cut-offs for preeclampsia prediction in patients with FGR (sFlt-1/PlGF ratio < 38 to rule out preeclampsia within the next two weeks, sFlt-1/PlGF ratio > 85 before 34 weeks of gestation, > 110 above 34 weeks of gestation to rule in preeclampsia within the next week)

	sFlt-1/PlGF < 38 at any GA (ruling-out < 2 weeks)	**Abnormal sFlt-1/PlGF* (rule-in < 1 week)**
Sensitivity	95.2	92.8
Specificity	19.4	31.6
PPV	25.6	19.4
NPV	93.3	96.1

GA: gestational age, NPV: negative predictive value, PE: preeclampsia, PlGF: placental growth factor, PPV: positive predictive value, sFlt-1: soluble fms-like tyrosine kinase-1

* abnormal sFlt-1/PlGF ratio was defined as >85 below 34 weeks of gestation and > 110 above 34 weeks of gestation

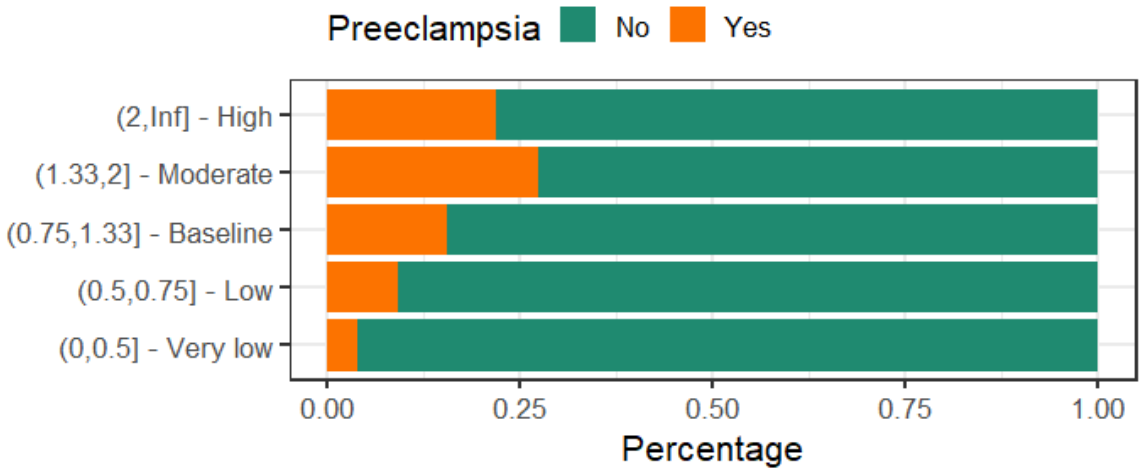

Figure 1a

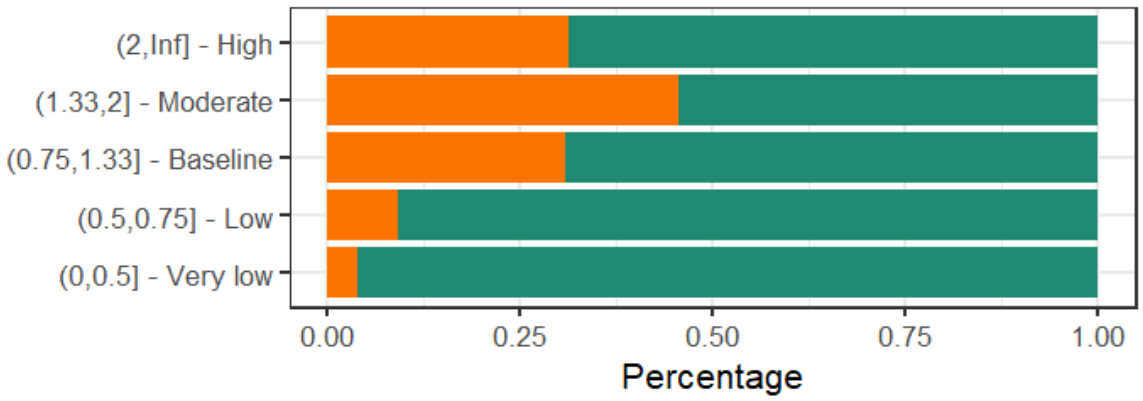

Figure 1b

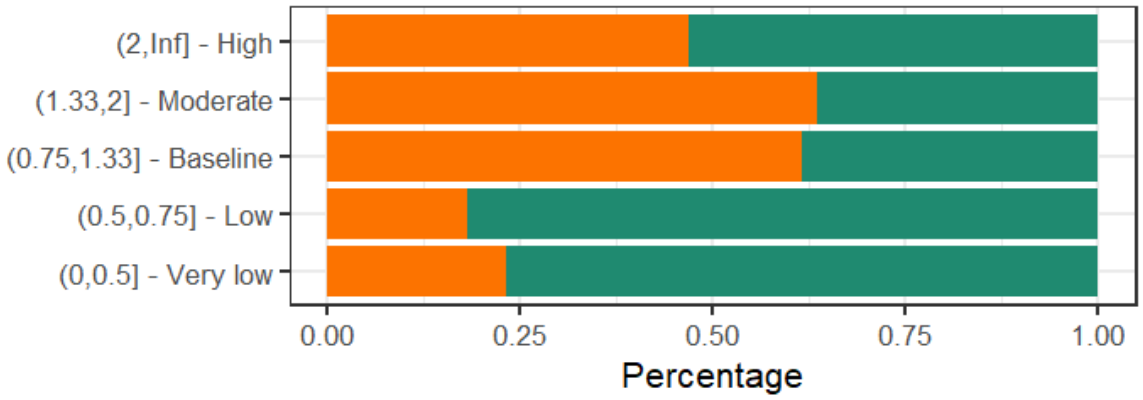

Figure 1c