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Longitudinal assessment of angiogenic markers in prediction of adverse outcome in women with confirmed pre-eclampsia

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Short title: Post-diagnosis surveillance of pre-eclampsia

Keywords: adverse maternal outcome, adverse perinatal outcome, longitudinal assessment, angiogenic markers, placental growth factor, pre-eclampsia, prediction, soluble fms-like tyrosine kinase-1, sFlt-1/PlGF ratio

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/uog.26276

CONTRIBUTION

What are the novel findings of this work?

Our findings underline a potential benefit of longitudinal maternal serum angiogenic marker assessment in the prediction of adverse maternal and perinatal outcomes compared to standard surveillance strategies in pregnancies with confirmed pre-eclampsia.

What are the clinical implications of this work?

Post-diagnosis longitudinal angiogenic marker assessment might be beneficial compared to conventional laboratory parameters alone for predicting both maternal and perinatal adverse outcomes in pregnancies with pre-eclampsia. Surveillance strategies in women with confirmed pre-eclampsia potentially benefit from the inclusion of longitudinal angiogenic marker assessment.

ABSTRACT

Objectives: Angiogenic marker assessment such as the ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF), is known to be a useful tool in the prediction of pre-eclampsia. However, evidence on surveillance strategies in pregnancies with pre-eclampsia diagnosis is lacking. Therefore, we aimed to assess the predictive performance of longitudinal maternal serum angiogenic marker assessment for adverse maternal and perinatal outcomes compared to standard laboratory parameters in pregnancies with confirmed pre-eclampsia.

Methods: This was a retrospective analysis of prospectively collected data from January 2013 to December 2020 at the Medical University of Vienna. The inclusion criteria were singleton pregnancies with confirmed pre-eclampsia and post-diagnosis maternal serum angiogenic marker assessment in at least two time points. The primary outcome was the predictive performance of longitudinal sFlt-1 and PlGF assessment for adverse maternal and perinatal outcomes compared to longitudinal conventional laboratory monitoring measured at the same time as the angiogenic markers in pregnancies with confirmed pre-eclampsia. Composite maternal adverse outcomes included intensive care unit (ICU) admission, pulmonary edema, eclampsia, and death. Composite adverse perinatal outcomes included stillbirth, neonatal death, placental abruption, neonatal intensive care unit (NICU) admission, intraventricular hemorrhage, necrotizing enterocolitis, respiratory distress syndrome, and mechanical ventilator support.

Results: In total 885 post-diagnosis sFlt-1/PlGF ratio measurements were taken from 323 pregnant women with confirmed pre-eclampsia. For composite maternal adverse outcome, the highest stand-alone predictive accuracy was obtained using maternal serum sFlt-1/PlGF ratio (AUROC 0.72, 95% CI 0.62–0.81), creatinine (AUROC 0.71, 95% CI 0.62–0.81), and lactate dehydrogenase (LDH) levels (AUROC 0.73, 95% CI 0.65–0.81). Maternal platelet levels (AUROC 0.65, 95% CI 0.55–0.74), serum alanine transaminase (ALT) (AUROC 0.59,

95% CI 0.49–0.69) and aspartate aminotransferase (AST) (AUROC 0.61, 95% CI 0.51–0.71) levels had poor stand-alone predictive accuracy. The best prediction model consisted of a combination of maternal serum LDH, creatinine levels and sFlt-1/PlGF ratio, which had an AUROC of 0.77 (95% CI 0.68–0.85), significantly higher than sFlt-1/PlGF ratio alone (P=0.037). For composite perinatal adverse outcome, the highest stand-alone predictive accuracy was obtained using maternal serum sFlt-1/PlGF ratio (AUROC 0.82, 95% CI 0.75–0.89) and creatinine (AUROC 0.74, 95% CI 0.67–0.80) levels, while sFlt-1/PlGF ratio was superior to creatinine alone (P<0.001). Maternal serum LDH levels (AUROC 0.65, 95% CI 0.53–0.74), platelet count (AUROC 0.57, 95% CI 0.44–0.67), ALT (AUROC 0.58, 95% CI 0.48–0.67) and AST (AUROC 0.58, 95% CI 0.48–0.67) levels had poor stand-alone predictive accuracy. No combination of biomarkers was superior to maternal serum sFlt-1/PlGF ratio alone for composite perinatal adverse outcome (P>0.05 for all).

Conclusions: Longitudinal maternal serum angiogenic marker assessment is a good predictor of adverse maternal and perinatal outcomes and superior to some of the conventional laboratory parameters in pregnancies with pre-eclampsia. More studies should focus on the optimal surveillance following the diagnosis of pre-eclampsia.

INTRODUCTION

Pre-eclampsia is a multi-system pregnancy-specific disorder, affecting 2-8% of pregnancies, associated with hypertension and end-organ dysfunction. It remains to be one of the leading causes of maternal and perinatal morbidity and mortality worldwide[1]. Recently, angiogenic marker assessment has revolutionized the prediction and diagnosis of hypertensive disorders of pregnancy (HDP)[2-18] and has been recommended as a useful tool in several international guidelines[19-21]. Compared with conventional diagnostic methods such as blood pressure measurement and assessment of proteinuria, angiogenic markers show superior predictive accuracy for adverse outcomes in pregnancies complicated by pre-eclampsia[15,22-31].

Published studies have demonstrated that a maternal serum soluble fms-like tyrosine kinase 1 to placental growth factor (sFlt-1/PlGF) ratio between 38 and 85, in a cohort of women with suspected pre-eclampsia, indicates a higher risk of developing pre-eclampsia later in pregnancy, so re-testing two to three weeks after the initial assessment is advised in this cohort[32].

However, the value of repeat assessment of the maternal serum sFlt-1/PlGF ratio in pregnant women with an established diagnosis of pre-eclampsia is yet to be determined and information on the timing and frequency of testing is currently lacking. Recommendations on surveillance strategies in women with diagnosed pre-eclampsia involve laboratory parameters suggestive of end-organ damage such as liver transaminases, creatinine and platelets[19,33,34]. Unfortunately, their predictive performance for adverse pregnancy outcomes in women affected by pre-eclampsia is either uncertain or poor[22].

According to current guidelines, angiogenic marker assessment is not part of surveillance protocols in women with an established diagnosis of pre-eclampsia to date[19,33,34], even though it can be hypothesized it might be beneficial according to current data on the predictive performance of the sFlt-1/PlGF ratio for adverse maternal and perinatal outcomes[35-38].

Therefore, the aim of this study was to evaluate the prognostic and additive value of repeat assessment of maternal serum sFlt-1/PlGF ratio in women with an established clinical diagnosis of pre-eclampsia compared to routine laboratory assessment.

METHODS

This was a retrospective analysis of prospectively routinely collected data recorded in an electronic database (Viewpoint 5.6.8.428, Wessling, Germany) between January 2013 and December 2020. The local research ethics committee of the Medical University of Vienna approved the study (approval number 1882/2018) and advised that written informed consent was not required from study participants.

Inclusion criteria were singleton pregnancies and confirmed diagnosis of pre-eclampsia. Those women, who had a history of cardiac disease, chronic kidney disease, pregnancies with aneuploidy, genetic syndromes or major structural fetal anomalies were excluded. Women, who did not deliver at the Department of Obstetrics and Fetomaternal Medicine at the Medical University of Vienna were also excluded due to missing perinatal and neonatal outcome parameters.

As part of the routine assessment in women with pre-eclampsia a blood sample was taken by venipuncture and stored in a collection tube without anticoagulant to analyze maternal serum levels of sFlt-1, PlGF and their ratio. The angiogenic marker concentrations were assessed in parallel by commercially available fully automated assays on Elecsys® (Roche Diagnostics, Penzberg, Germany) platform. The analysis was undertaken by biomedical technicians who were blinded to all clinical details, but the results were available to healthcare professionals. Testing was generally repeated 48 hours after first assessment and according to the patient's clinical presentation and clinicians' medical evaluation afterwards. The number of sFlt-1/PlGF assessments was not fixed ranging from 2-4 assessments with a median of 3 assessments.

Pre-eclampsia and superimposed pre-eclampsia were defined according to the revised criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP) in 2018[34]. This required high blood pressure (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) either predating pregnancy or recognized prior to 20 weeks of gestation (for superimposed pre-eclampsia) or after 20 weeks of gestation (for pre-eclampsia). The

diagnosis of pre-eclampsia and superimposed pre-eclampsia was made when one or more of the following features of pre-eclampsia were present: new-onset significant proteinuria, acute kidney injury (creatinine >1mg/dL), elevated liver enzymes (transaminase levels >40 IU/L), low platelet count (<150.000/μL), or neurological symptoms of pre-eclampsia (i.e. persistent visual scotomata, altered mental status, blindness, stroke, hyperreflexia accompanied by clonus, severe headaches accompanied by hyperreflexia or eclampsia). Significant proteinuria was diagnosed with either protein/creatinine ratio (PCR) of ≥30mg/mmol or ≥300mg protein excretion in 24 hours. Hemolysis, Elevated Liver enzymes, Low Platelets (HELLP) syndrome were defined as increased transaminases (aspartate aminotransferase and alanine aminotransferase concentrations more than twice the upper reference interval), reduced platelet count (<100,000/μL) plus at least one hemolysis criterion (increased lactate dehydrogenase concentration more than twice the upper reference interval or serum indirect bilirubin concentration > 1.2 mg/dL or reduced serum haptoglobin concentration < 0.3 g/L). Pre-eclampsia and superimposed pre-eclampsia were not diagnosed solely on the basis of worsening of hypertension or presence of fetal growth restriction (FGR).

The primary outcome was the utility of angiogenic markers for the prediction of adverse maternal and perinatal outcomes as well as the additive value of angiogenic marker assessment compared to conventional laboratory monitoring in women with pre-eclampsia after the diagnosis has been established. Composite maternal adverse outcomes included intensive care unit (ICU) admission, which was due to severe hypertension with the need of continuous blood pressure monitoring, liver dysfunction defined as elevated liver enzymes with transaminase levels twice the upper limit of normal[21] or acute kidney injury determined as creatinine of >1.0 mg/dL[21], pulmonary edema, eclampsia, and death. Composite adverse perinatal outcomes included stillbirth, neonatal death, intraventricular hemorrhage, retinopathy of prematurity and necrotizing enterocolitis. Pregnancies were delivered in case of severe hypertension (≥160/110mmHg) despite two types of antihypertensive drugs in appropriate doses, progressive thrombocytopenia, severe dyspnea, liver dysfunction, acute

kidney injury, HELLP syndrome, placental abruption or fetal compromise (abnormal fetal Doppler or abnormal cardiotocography). Pregnancies affected by growth restriction prior to 32 weeks of gestation were managed according to the criteria of the TRUFFLE study protocol[39]. After 32 weeks growth restricted fetuses were delivered for reverse flow in the umbilical artery and for zero flow in the umbilical artery after 34 weeks of gestation. For abnormalities in the cerebroplacental ratio (CPR), the delivery decision was based on the clinicians.

Statistical analysis

The longitudinal change in laboratory values was modeled using linear mixed-effects regression. Intercepts were allowed to vary between individuals and slopes were allowed to vary between different time points. The change in laboratory variables with time was modeled using piecewise polynomials (fourth order cubic B-splines) for gestational age to allow for non-linear change trajectories. Interaction term for the variance-covariance structure of random effects were assumed to be constant across the subjects. Regression diagnostics were performed for each model to test the normality of residuals, points of high leverage, normality of random effects, and constant variance of residuals. To assess the predictive capabilities of each laboratory variable, random effects from each model were extracted and their association with composite adverse outcomes was tested with logistic regression models. Predictive capabilities were assessed using the area under the receiver operating characteristics curve (AUROC). AUROC values were compared with De Long's test. *P*-values <0.05 were considered statistically significant. All analyses were conducted in R for Statistical Computing Software (v4.0.3) using `lme4` and `pROC` packages.

RESULTS

A total of 885 post-diagnosis sFlt1/PlGF ratio measurements were taken from 323 pregnant individuals with pre-eclampsia. The median gestational age at diagnosis was 34.0 weeks and at birth 35.3 weeks. The gestation at diagnosis was prior to 32 weeks' gestation in 37.8% of all cases, while 38.4% were diagnosed between 32- and 37-weeks' gestation, with 23.8% were diagnosed after 37 weeks' gestation. Histograms showing the distribution of gestational ages at sampling and delivery are available in Figure S1.

The median follow-up duration was 5 (interquartile range, 2-11) days, and the median number of repeat measurements was 3 (interquartile range, 2-4). The rate of composite maternal adverse outcomes in women with pre-eclampsia was 11.4% (37/323) and the rate of composite perinatal outcomes was 14.6% (47/323). Baseline and birth characteristics of pregnancies included in the study, stratified by composite adverse outcome type, are summarized in Table 1 and Table 2. Reasons for delivery (maternal, fetal and both) are displayed in Table S3. The longitudinal change in laboratory variables is depicted visually with spaghetti plots (Figure 1: adverse maternal; Figure 2: adverse perinatal).

Maternal adverse outcomes

Women with composite adverse maternal outcome were significantly younger (30.0 vs 33.0 years, P=0.006), had similar nulliparity rate (P=0.330) and body mass index (BMI) (P=0.946) compared to those without composite adverse maternal outcome. Presenting symptoms of pre-eclampsia were similar between the groups except for epigastric pain, which was more common in women who developed composite adverse outcome (16% vs. 8%) but the difference did not reach statistical significance threshold (P=0.082). Laboratory abnormalities at any time before birth were quite prevalent in both groups, but significantly more common in women with adverse outcomes (Table 1). However, none of the differences in these laboratory markers reached statistical significance apart from abnormal sFlt-1/PlGF ratio (91.9% vs 77.6%, P=0.043) (Table 1). The gestational age at diagnosis (28.4 vs 33.8 weeks, P=0.001)

and birth (29.6 vs 35.6 weeks, P<0.001) were significantly earlier in women with adverse maternal outcomes compared to those without.

Mixed-effects regression results showing the association of laboratory variables with maternal adverse outcomes are available in Table S1.

For composite maternal adverse outcome, the highest stand-alone predictive performance was obtained using maternal serum sFlt-1/PlGF ratio (AUROC 0.72, 95% CI 0.62–0.81), creatinine (AUROC 0.71, 95% CI 0.62–0.81), and LDH levels (AUROC 0.73, 95% CI 0.65–0.81) (Table 3). Maternal platelet levels (AUROC 0.65, 95% CI 0.55–0.74), serum ALT (AUROC 0.59, 95% CI 0.49–0.69) and AST (AUROC 0.61, 95% CI 0.51–0.71) levels had poor stand-alone predictive accuracy. The best prediction model consisted of a combination of maternal serum LDH, creatinine levels and sFlt-1/PlGF ratio, which had an AUROC of 0.77 (95% CI 0.68–0.85), significantly higher than sFlt-1/PlGF ratio alone (P=0.037) (Figure 1).

Perinatal adverse outcomes

Women with composite adverse perinatal outcome were of similar age (P=0.167), had similar nulliparity rate (P=0.322) and BMI (P=0.341) compared to those without composite perinatal adverse outcome. The presenting symptoms of pre-eclampsia were also similar between the groups (P>0.05 for all, Table 2). Laboratory abnormalities at any time before birth were prevalent in both groups, but significantly more common in pregnancies with composite adverse perinatal outcome (Table 1). However, none of the differences in these markers reached statistical significance except for abnormal maternal serum sFlt-1/PlGF ratio (95.7% vs 76.4%, P=0.002), LDH (40% vs. 21%, P=0.003) and thrombocytopenia (21% vs 8%, P=0.004). The gestational age at diagnosis (26.7 vs 34.1 weeks, P<0.001) and birth (28.0 vs 36.1 weeks, P<0.001) were significantly earlier in women with adverse perinatal outcomes compared to those without.

Mixed-effects regression results showing the association of laboratory variables with perinatal adverse outcomes is available in Table S2.

For composite perinatal adverse outcomes, the highest stand-alone predictive performance was obtained using maternal serum sFlt-1/PlGF ratio (AUROC 0.82, 95% CI 0.75–0.89) and creatinine (AUROC 0.74, 95% CI 0.67–0.80) levels, while sFlt-1/PlGF ratio was superior to creatinine alone (P<0.001) (Table 3). Maternal serum LDH levels (AUROC 0.65, 95% CI 0.53–0.74), platelet count (AUROC 0.57, 95% CI 0.44–0.67), ALT (AUROC 0.58, 95% CI 0.48–0.67) and AST (AUROC 0.58, 95% CI 0.48–0.67) levels had poor stand-alone predictive capabilities. No combination of biomarkers was superior to maternal serum sFlt-1/PlGF ratio alone (P>0.05 for all) (Figure 2).

There was no significant difference between maternal serum sFlt-1/PlGF ratio and PlGF alone in terms of predicting composite perinatal adverse outcomes (AUC 0.82 vs 0.81, respectively, P=0.412) and composite maternal adverse outcomes (AUC 0.72 vs 0.69, P=0.102).

DISCUSSION

Principal findings

Our study demonstrates moderate accuracy of repeat maternal serum angiogenic marker assessment in predicting composite adverse maternal and perinatal outcomes in singleton pregnancies with confirmed pre-eclampsia. Repeat measurements of maternal serum sFlt-1/PlGF ratio, in addition to some of the standard laboratory assessment, significantly improved the predictive accuracy for composite adverse maternal outcomes while angiogenic markers alone were best for predicting adverse perinatal outcomes. Maternal serum sFlt-1/PlGF, creatinine and LDH levels combined had the best predictive performance for composite adverse maternal adverse outcome compared to any other laboratory parameters combination. Predictive performance of platelets, AST and ALT was poor.

Comparison with the existing literature

Recently, maternal serum angiogenic marker imbalance has been acknowledged in several international guidelines as a criterion for diagnosis of pre-eclampsia. Moreover, the maternal serum sFlt-1/PlGF ratio has been shown to be an important tool not only for the diagnosis of pre-eclampsia in women with suspected pre-eclampsia, but also in the prognosis of the disease such as estimating the time until delivery and prediction of adverse maternal and perinatal adverse outcomes[2,3,6,7,36,40,41]. Repeated assessment of the ratio is advised in women with suspected pre-eclampsia with a ratio between 38 and 85 or 110 after 34 weeks' gestation, respectively, as well as for the surveillance of high-risk cohorts such as women with chronic hypertension[7,32]. Data on repeated angiogenic marker assessment in the surveillance of pregnancies with confirmed pre-eclampsia are lacking.

Lately, prognostic models such as Pre-eclampsia Integrated Estimate of Risk (PIERS), Prediction of Risks in early- onset Pre-eclampsia (PREP) or models using machine learning approaches, that include laboratory parameters used in pre-eclampsia surveillance, were

developed to guide clinical decision making and avoid adverse outcomes in women with early onset pre-eclampsia[25,42,43]. However, data comparing standard pre-eclampsia surveillance including the well-established models (fullPIERS and PREP) and angiogenic marker assessment are scarce.

Recently, Reddy et al[35] evaluated the predictive performance of the sFlt-1/PlGF ratio for adverse maternal and perinatal outcomes in combination with cardiovascular, fetal and placental indices in 123 women with suspected and confirmed pre-eclampsia. The ratio performed well for the prediction of adverse perinatal outcome, consistent with the findings of the present study, but failed to be predictive of adverse maternal outcome. This might be due to intervention bias and delivery before the onset of maternal adverse events, the lack of repeated angiogenic marker assessment or the smaller number of women evaluated in this cohort.

Peguero et al[44] conducted a prospective cohort study including 63 women with confirmed early-onset pre-eclampsia, evaluating the predictive performance of longitudinal angiogenic marker assessment for time to delivery and adverse outcome. In this study 26 women (41.3%) developed a complication that was predicted in 6.2% by the standard risk score and sFlt-1 at the time of hospital admission. Prediction was improved to 25.3% when longitudinal sFlt-1 assessment was added. Interestingly, maternal serum sFlt- 1 levels alone showed better performance than PlGF or the ratio in this cohort, which could be due to the fact that only women with severe early-onset pre-eclampsia were included. Maternal serum sFlt-1 has been shown to be the main cause of endothelial dysfunction in this cohort driving disease severity, this is in contrast to our findings, that demonstrate better predictive performance using the ratio compared to sFlt-1 and PlGF alone. This finding might be explained by the cohort of women with both early and late onset pre-eclampsia included in this study. The full-PIERS group[45] also evaluated their model regarding PlGF assessment and prediction of adverse outcome but could not demonstrate improved performance of their model when maternal serum PlGF was added. This was explained by intervention bias, as expectant management

was not the preferred strategy of care in this cohort. Furthermore, maternal serum PlGF levels were not assessed longitudinally as they were in our study.

Suresh et al[36] demonstrated that an elevated sFlt-1/PlGF ratio >85 best predicted adverse maternal outcomes at triage in women with suspected pre-eclampsia. In contrast to our findings sFlt-1/PlGF failed to improve diagnostic test value for fetal adverse events in women with confirmed PE, which might be due to the small number of fetal adverse events, to the smaller proportion of women presenting with an sFlt-1/PlGF ratio >85 (17%) or lack of longitudinal assessment in their cohort.

Recently, Schmidt et al[41] evaluated a machine learning based approach including standard laboratory parameters and sFlt-1/PlGF for their predictive performance of adverse outcomes in women with suspected pre-eclampsia. In line with our findings, high accuracy, sensitivity and PPV for the prediction of adverse outcome could be reached using this model, underlining the added value of angiogenic marker assessment in this population. Furthermore, the ratio also performed well in a machine learning model designed to predict delivery within 7 days after diagnosis of early onset pre-eclampsia as shown by Villalain et al[43].

Clinical and research implications

Current guidelines do not include angiogenic marker assessment for the surveillance of pregnancies with an established diagnosis of pre-eclampsia, even though the predictive performance of standard laboratory parameter assessment alone seems poor[22].

Recent studies together with our work, suggest good predictive performance of the maternal serum sFlt-1/PlGF ratio for adverse maternal and perinatal outcomes and challenge the current concept of pre-eclampsia surveillance[35,36,41,44]. Hence, our data add to the existing body of literature that longitudinal angiogenic marker assessment is useful for the prediction of both maternal and perinatal adverse pregnancy outcomes in the post diagnosis

surveillance. Data on longitudinal angiogenic marker assessment for pre-eclampsia surveillance should be further studied in a prospective, preferentially in a blinded setting.

Strengths and limitations

This is a large cohort comparing repeat angiogenic marker assessment in the surveillance of pregnancies with confirmed pre-eclampsia with routine laboratory assessment. Our study, in contrast to the existing literature, does not focus exclusively on women with early-onset severe pre-eclampsia but also includes women with late-onset pre-eclampsia, underlining the clinical relevance of this data.

This study has some limitations. Clinicians were not blinded to the results of angiogenic marker assessment and therefore intervention bias cannot be ruled out completely. However, there are no international guidelines or an institutional protocol in place recommending delivery due to an increase in maternal serum angiogenic markers only. Neither are there any international recommendations on longitudinal maternal serum angiogenic marker assessment. However, any bias in that aspect would have a negative impact on the predictive capabilities of angiogenic markers for maternal adverse outcomes. A delivery earlier than what would be indicated by standard laboratory assessment would constitute intervention bias and would appear as false positives because earlier delivery would have prevented the adverse maternal eventuality. However, this does not hold true for adverse perinatal outcomes as earlier delivery is often connected to prematurity and complications resulting from it. Moreover, this sort of bias applies to all markers assessed in this study and each one of them would be affected to a similar degree allowing for a direct comparison between them, which was our main aim.

Conclusion

Longitudinal maternal serum angiogenic marker assessment improves the predictive potential for adverse perinatal outcomes in singleton pregnancies with confirmed pre-eclampsia and might also be beneficial to predict adverse maternal outcomes, compared to established

laboratory assessment routines alone. Pre-eclampsia surveillance and management protocols may benefit from incorporating maternal serum angiogenic marker imbalance, in order to improve our ability to identify those destined to develop adverse outcomes.

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FIGURE LEGENDS

Figure 1 Spaghetti plots showing longitudinal changes of sFlt-1/PlGF ratio, serum LDH levels and serum creatinine levels with gestational age and its association with composite adverse maternal outcome (blue line: adverse maternal outcome).

Figure 2 Spaghetti plots showing longitudinal changes of sFlt-1/PlGF ratio, serum LDH levels and serum creatinine levels with gestational age and its association with composite adverse perinatal outcome (blue line: adverse perinatal outcome).

Table 1. Baseline and disease characteristics of women with and without composite maternal adverse outcome

Variables	**Pregnancies with composite maternal adverse outcome* (n=37)**	**Pregnancies without composite maternal adverse outcome (n=286)**	***P*-value**
Maternal age, years	30.0 (28.0 – 33.0)	33.0 (29.0 – 37.0)	0.006
Nulliparous, n (%)	21 (57)	138 (48)	0.330
Body mass index, kg/m^2	26.1 (21.1 – 29.7)	25.6 (21.8 – 28.5)	0.946
Conception method, n (%)			
• Spontaneous	36 (97)	246 (86)	0.052
• Assisted	1 (3)	40 (14)	
Symptoms of pre-eclampsia, n (%)			
• Epigastric pain	6 (16)	22 (8)	0.082
• New onset edema	17 (5)	114 (40)	0.614
• Dyspnea	1 (3)	7 (2)	0.925
• Neurological symptoms	8 (2)	55 (19)	0.729
Laboratory assessment, n (%)			
• sFlt-1/PlGF abnormal before birth	34 (91.9)	222 (77.6)	0.043
• Creatinine > 1mg/dL, any time before birth	4 (11)	17 (6)	0.258
• Transaminase >66 IU/L, any time before birth	5 (13.5)	53 (18.5)	0.454
• LDH > 280 IU/L, any time before birth	17 (46)	86 (30)	0.051
• Thrombocytopenia, any time before birth	7 (19)	25 (9)	0.051
Gestational age at diagnosis, weeks	28.4 (25.6 – 33.6)	33.8 (29.3 – 36.9)	0.001
Gestational age at birth, weeks	29.6 (27.0 – 34.9)	35.6 (31.5 – 38.1)	<0.001
Adverse maternal outcomes, n (%)			
ICU admission	32 (86.5)	0 (0.0)	–
Lung edema	5 (13.5)	0 (0.0)	–
Seizures	6 (16.2)	0 (0.0)	–
Maternal death	0 (0.0)	0 (0.0)	–

All values are presented either as median (interquartile range) or n (%). *Seizure, intensive care unit admission, lung edema or maternal death. sFlt-1/PlGF = soluble fms-like tyrosine kinase 1 to placental growth factor ratio; LDH = Lactate dehydrogenase.

Table 2. Baseline and disease characteristics of women with and without composite perinatal adverse outcome

Variables	**Pregnancies with composite perinatal adverse outcome* (n=47)**	**Pregnancies without composite perinatal adverse outcome (n=276)**	**P value**
Maternal age, years	32.0 (26.5 – 36.0)	33.0 (29.0 – 37.0)	0.167
Nulliparous, n (%)	20 (43)	139 (50)	0.322
Body-mass index, kg/m^2	24.4 (20.0 – 30.4)	25.7 (21.8 – 28.6)	0.341
Conception method, n (%)			
• Spontaneous	44 (94)	238 (86)	0.159
• Assisted	3 (6)	38 (14)	
Symptoms of pre-eclampsia, n (%)			
• Epigastric pain	5 (11)	23 (83)	0.603
• New onset edema	15 (32)	116 (42)	0.191
• Dyspnea	1 (2)	7 (3)	0.867
• Neurological symptoms	6 (13)	57 (21)	0.207
Laboratory assessment, n (%)			
• sFlt-1/PlGF abnormal before birth	45 (95.7)	211 (76.4)	0.002
• Creatinine> 1mg/dL, any time before delivery	2 (4)	19 (7)	0.499
• Transaminase >66 IU/L, any time before delivery	13 (27.6)	45 (16.3)	0.060
• LDH> 280 IU/L, any time before delivery	19 (40)	57 (21)	0.003
• Thrombocytopenia, any time before delivery	10 (21)	22 (8)	0.004
Gestational age at diagnosis, weeks	26.7 (24.8 – 30.3)	34.1 (30.2 – 37.1)	<0.001
Gestational age at birth, weeks	28.0 (25.4 – 30.8)	36.1 (32.4 – 38.2)	<0.001
Perinatal adverse outcomes, n (%)			
Intraventricular hemorrhage	12 (25.5)	0 (0.0)	–
Retinopathy of prematurity	24 (51.1)	0 (0.0)	–
Necrotizing enterocolitis	7 (14.9)	0 (0.0)	–
Perinatal death	12 (25.5)	0 (0.0)	–

All values are presented either as median (interquartile range) or n (%). *Intraventricular hemorrhage, necrotizing enterocolitis, retinopathy of prematurity, perinatal death.

Table 3. Area under the receiver operating characteristics curve (AUROC) values of models predicting composite maternal and perinatal adverse outcomes.

Variables	Composite maternal adverse outcome, AUROC	Sensitivity (10% FPR)	Composite perinatal adverse outcome, AUROC	Sensitivity (10% FPR)
Creatinine levels, all cases	0.71, 95% CI: 0.62 – 0.81	32.4%	0.74, 95% CI: 0.67 – 0.80	21.3%
- Early onset	0.72, 95% CI: 0.62 – 0.83	32.1%	0.64, 95% CI: 0.56 – 0.74	18.6%
- Late onset	0.54, 95% CI: 0.33 – 0.75	22.2%	0.52, 95% CI: 0.20 – 0.84	0.0%
LDH, all cases	0.73, 95% CI: 0.65 – 0.81	32.4%	0.65, 95% CI: 0.53 – 0.74	27.7%
- Early onset	0.68, 95% CI: 0.58 – 0.79	28.6%	0.65, 95% CI: 0.55 – 0.74	27.9%
- Late onset	0.78, 95% CI: 0.64 – 0.93	33.3%	0.55, 95% CI: 0.25 – 0.84	0.0%
ALT, all cases	0.59, 95% CI: 0.49 – 0.69	18.9%	0.58, 95% CI: 0.48 – 0.67	21.3%
- Early onset	0.58, 95% CI: 0.46 – 0.70	17.8%	0.59, 95% CI: 0.48 – 0.69	23.3%
- Late onset	0.63, 95% CI: 0.45 – 0.80	22.2%	0.46, 95% CI: 0.19 – 0.73	0.0%
AST, all cases	0.61, 95% CI: 0.51 – 0.71	18.9%	0.58, 95% CI: 0.48 – 0.67	21.2%
- Early onset	0.58, 95% CI: 0.46 – 0.70	17.8%	0.58, 95% CI: 0.48 – 0.69	23.2%
- Late onset	0.62, 95% CI: 0.475 – 0.80	22.2%	0.46, 95% CI: 0.19 – 0.73	0.0%
Platelet count, all cases	0.65, 95% CI: 0.55 – 0.74	24.3%	0.57, 95% CI: 0.44 – 0.67	29.7%
- Early onset	0.64, 95% CI: 0.53 – 0.75	25.0%	0.55, 95% CI: 0.44 – 0.66	25.6%
- Late onset	0.57, 95% CI: 0.35 – 0.80	11.0%	0.58, 95% CI: 0.44 – 0.73	0.0%
sFlt-1/PlGF ratio, all cases	0.72, 95% CI: 0.62 – 0.81	35.1%	0.82, 95% CI: 0.75 – 0.89	53.1%
- Early onset	0.69, 95% CI: 0.58 – 0.80	28.6%	0.77, 95% CI: 0.69 – 0.86	34.8%
- Late onset	0.64, 95% CI: 0.38 – 0.89	33.3%	0.55, 95% CI: 0.11 – 0.98	25.0%

sFlt-1/PlGF = soluble fms-like tyrosine kinase 1 to placental growth factor ratio.