Ultrasound Obstet Gynecol 2023; **62**: 843–851
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.26276.

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

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

CONTRIBUTION

What are the novel findings of this work?
Our findings underline the potential benefit of longitudinal maternal serum angiogenic marker assessment in the prediction of adverse maternal and perinatal outcomes when compared to standard surveillance strategies in pregnancies with confirmed pre-eclampsia (PE).

What are the clinical implications of this work?
Post-diagnosis longitudinal angiogenic marker assessment might be beneficial when compared to conventional laboratory parameters alone in predicting both maternal and perinatal adverse outcomes in pregnancies with PE. Surveillance strategies in women with confirmed PE may 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 (PE). However, evidence from surveillance strategies in pregnancies with a PE diagnosis is lacking. Therefore, we aimed to assess the predictive performance of longitudinal maternal serum angiogenic marker assessment for both maternal and perinatal adverse outcomes when compared to standard laboratory parameters in pregnancies with confirmed PE.*

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 pregnancy with confirmed PE and post-diagnosis maternal serum angiogenic marker assessment at a minimum of two timepoints. The primary outcome was the predictive performance of longitudinal sFlt-1 and PlGF assessment for adverse maternal and perinatal outcomes compared to conventional laboratory monitoring at the same time in pregnancies with confirmed PE. Composite adverse maternal outcome included intensive care unit admission, pulmonary edema, eclampsia and/or death. Composite adverse perinatal outcome included stillbirth, neonatal death, placental abruption, neonatal intensive care unit admission, intraventricular hemorrhage, necrotizing enterocolitis, respiratory distress syndrome and/or mechanical ventilator support.*

Results *In total, 885 post-diagnosis sFlt-1/PlGF ratio measurements were obtained from 323 pregnant women with confirmed PE. For composite adverse maternal outcome, the highest standalone predictive accuracy was obtained using maternal serum sFlt-1/PlGF ratio (area under the receiver-operating-characteristics curve (AUC), 0.72 (95% CI, 0.62–0.81)), creatinine (AUC, 0.71 (95% CI, 0.62–0.81)) and lactate dehydrogenase (LDH) levels (AUC, 0.73 (95% CI, 0.65–0.81)). Maternal platelet levels (AUC, 0.65 (95% CI, 0.55–0.74)), serum alanine aminotransferase (ALT) (AUC, 0.59 (95% CI, 0.49–0.69)) and aspartate aminotransferase (AST) (AUC, 0.61 (95% CI, 0.51–0.71) levels had poor standalone predictive accuracy. The best prediction model consisted of a combination of maternal serum LDH, creatinine levels and sFlt-1/PlGF ratio, which had an AUC of 0.77 (95% CI, 0.68–0.85), significantly higher*

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Accepted: 12 May 2023

than sFlt-1/PlGF ratio alone (P = 0.037). For composite adverse perinatal outcome, the highest standalone predictive accuracy was obtained using maternal serum sFlt-1/PlGF ratio (AUC, 0.82 (95% CI, 0.75–0.89)) and creatinine (AUC, 0.74 (95% CI, 0.67–0.80)) levels, sFlt-1/PlGF ratio being superior to creatinine alone (P < 0.001). Maternal serum LDH levels (AUC, 0.65 (95% CI, 0.53–0.74)), platelet count (AUC, 0.57 (95% CI, 0.44–0.67)), ALT (AUC, 0.58 (95% CI, 0.48–0.67)) and AST (AUC, 0.58 (95% CI, 0.48–0.67)) levels had poor standalone predictive accuracy. No combination of biomarkers was superior to maternal serum sFlt-1/PlGF ratio alone for prediction of composite adverse perinatal outcome (P > 0.05 for all).

Conclusions *In pregnancies with confirmed PE, longitudinal maternal serum angiogenic marker assessment is a good predictor of adverse maternal and perinatal outcomes and superior to some conventional laboratory parameters. Further studies should focus on optimal surveillance following diagnosis of PE.* © *2023 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Pre-eclampsia (PE) is a multisystem pregnancy-specific disorder, affecting 2–8% of all pregnancies, and is associated with hypertension and end-organ dysfunction. It remains 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[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 outcome in pregnancy complicated by PE[15,22–31].

It has been demonstrated that a maternal serum soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio between 38 and 85, in a cohort of women with suspected PE, indicates a higher risk of developing PE later in pregnancy, so retesting 2–3 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 PE 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 PE 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 outcome in women affected by PE 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 PE[19,33,34]; even though it can be hypothesized that it might be beneficial according to current data on the predictive performance of the sFlt-1/PlGF ratio for adverse maternal and perinatal outcome[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 compared to routine laboratory assessment in women with an established clinical diagnosis of PE.

METHODS

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

Inclusion criteria were singleton pregnancy, confirmed diagnosis of PE and post-diagnosis maternal serum angiogenic marker assessment at a minimum of two timepoints. Women with a history of cardiac disease, chronic kidney disease, pregnancy with fetal aneuploidy, genetic syndrome or major structural anomaly 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 PE, a blood sample was taken by venipuncture and stored in a collection tube without anticoagulant to analyze maternal serum levels of sFlt-1 and PlGF, and to calculate their ratio. The angiogenic marker concentrations were assessed in parallel by commercially available fully automated assays on the 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 h after the first assessment, according to patient clinical presentation and following clinician medical evaluation. The number of sFlt-1/PlGF assessments was not fixed, ranging from two to four with a median of three.

PE and superimposed PE 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 PE) or after 20 weeks of gestation (for PE). The diagnosis of PE and superimposed PE was made when one or more of the following features of PE were present: new-onset significant proteinuria, acute kidney injury (creatinine ≥ 1 mg/dL), elevated liver enzymes (transaminase levels

> 40 IU/L), low platelet count (< 150 000/μL) or neurological symptoms of PE (i.e. persistent visual scotomata, altered mental status, blindness, stroke, hyper-reflexia accompanied by clonus, severe headaches accompanied by hyper-reflexia or eclampsia). Significant proteinuria was diagnosed with either protein/creatinine ratio of ≥ 30 mg/mmol or ≥ 300 mg protein excretion in 24 h. HELLP syndrome was defined as increased transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations more than twice the upper reference interval), reduced platelet count (< 100 000/μL), plus at least one hemolysis criterion (increased lactate dehydrogenase (LDH) 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). PE and superimposed PE were not diagnosed solely on the basis of worsening of hypertension or presence of fetal growth restriction.

The primary outcome was the utility of angiogenic markers for the prediction of adverse maternal and perinatal outcome, as well as the additive value of angiogenic marker assessment compared to conventional laboratory monitoring in women with PE after the diagnosis has been established. Composite adverse maternal outcome included intensive care unit admission (due to severe hypertension with the need for 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 concentration > 1.0 mg/dL[21]), pulmonary edema, eclampsia and/or death. Composite adverse perinatal outcome included stillbirth, neonatal death, intraventricular hemorrhage, retinopathy of prematurity and/or necrotizing enterocolitis. Pregnancies were delivered in case of severe hypertension (≥ 160/110 mmHg) 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 reversed flow in the umbilical artery, and for zero flow in the umbilical artery after 34 weeks of gestation. For abnormalities in the cerebroplacental ratio, the delivery decision was based on the clinician's assessment.

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 timepoints. 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 was 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 capability was assessed using the area under the receiver-operating-characteristics curve (AUC). AUC values were compared using DeLong's test. *P*-values < 0.05 were considered statistically significant. All analyses were conducted in R for Statistical Computing Software (v4.0.3; https://www.r-project.org/) using lme4 and pROC packages.

RESULTS

A total of 885 post-diagnosis sFlt1/PlGF ratio measurements were taken from 323 pregnant individuals with PE. The median gestational age at diagnosis was 34.0 weeks and at birth was 35.3 weeks. The gestational age at diagnosis was prior to 32 weeks in 37.8% of all cases, while 38.4% were diagnosed between 32 and 37 weeks' gestation, with 23.8% after 37 weeks. 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 (IQR), 2–11) days, and the median number of repeat measurements was 3 (IQR, 2–4). The rate of composite adverse maternal outcome in women with PE was 11.5% (37/323) and the rate of composite adverse perinatal outcome was 14.6% (47/323). Baseline and disease characteristics of PE pregnancies included in the study, stratified by composite adverse outcome type, are summarized in Tables 1 and 2. Reasons for delivery (maternal, fetal and both) are displayed in Table S1. The longitudinal change in laboratory variables for composite adverse maternal and perinatal outcomes is depicted visually with spaghetti plots in Figures 1 and 2, respectively.

Adverse maternal outcome

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 PE 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 the statistical significance threshold ($P = 0.082$). Abnormal laboratory results at any time before birth were quite prevalent in both groups, but generally more common in women with adverse outcomes (Table 1). However, none of the differences in these laboratory markers reached statistical significance

Table 1 Baseline and disease characteristics of 323 pre-eclamptic (PE) pregnancies, with or without composite adverse maternal outcome*

Parameter	*Composite adverse maternal outcome* (n = *37*)	*No composite adverse maternal outcome* (n = *286*)	P
Maternal age (years)	30.0 (28.0–33.0)	33.0 (29.0–37.0)	0.006
Nulliparous	21 (56.8)	138 (48.2)	0.330
Body mass index (kg/m^2)	26.1 (21.1–29.7)	25.6 (21.8–28.5)	0.946
Conception method			
Spontaneous	36 (97.3)	246 (86.0)	0.052
Assisted	1 (2.7)	40 (14.0)	
PE symptom			
Epigastric pain	6 (16.2)	22 (7.7)	0.082
New-onset edema	17 (45.9)	114 (39.9)	0.614
Dyspnea	1 (2.7)	7 (2.4)	0.925
Neurological symptoms	8 (21.6)	55 (19.2)	0.729
Laboratory assessment†			
sFlt-1/PlGF abnormal	34 (91.9)	222 (77.6)	0.043
Creatinine > 1 mg/dL	4 (10.8)	17 (5.9)	0.258
Transaminase > 66 IU/L	5 (13.5)	53 (18.5)	0.454
LDH > 280 IU/L	17 (45.9)	86 (30.1)	0.051
Thrombocytopenia	7 (18.9)	25 (8.7)	0.051
GA at PE diagnosis (weeks)	28.4 (25.6–33.6)	33.8 (29.3–36.9)	0.001
GA at birth (weeks)	29.6 (27.0–34.9)	35.6 (31.5–38.1)	< 0.001
Adverse maternal outcome			
ICU admission	32 (86.5)	0 (0)	—
Lung edema	5 (13.5)	0 (0)	—
Seizure	6 (16.2)	0 (0)	—
Maternal death	0 (0)	0 (0)	—

Data are given as median (interquartile range) or *n* (%). *Seizure, intensive care unit (ICU) admission, lung edema and/or maternal death. †Any time before birth. GA, gestational age; LDH, lactate dehydrogenase; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

Table 2 Baseline and disease characteristics of 323 pre-eclamptic (PE) pregnancies, with or without composite adverse perinatal outcome*

Parameter	*Composite adverse perinatal outcome* (n = *47*)	*No composite adverse perinatal outcome* (n = *276*)	P
Maternal age (years)	32.0 (26.5–36.0)	33.0 (29.0–37.0)	0.167
Nulliparous	20 (42.6)	139 (50.4)	0.322
Body mass index (kg/m^2)	24.4 (20.0–30.4)	25.7 (21.8–28.6)	0.341
Conception method			
Spontaneous	44 (93.6)	238 (86.2)	0.159
Assisted	3 (6.4)	38 (13.8)	
PE symptom			
Epigastric pain	5 (10.6)	23 (8.3)	0.603
New-onset edema	15 (31.9)	116 (42.0)	0.191
Dyspnea	1 (2.1)	7 (2.5)	0.867
Neurological symptoms	6 (12.8)	57 (20.7)	0.207
Laboratory assessment†			
sFlt-1/PlGF abnormal	45 (95.7)	211 (76.4)	0.002
Creatinine > 1 mg/dL	2 (4.3)	19 (6.9)	0.499
Transaminase > 66 IU/L	13 (27.7)	45 (16.3)	0.060
LDH > 280 IU/L	19 (40.4)	57 (20.7)	0.003
Thrombocytopenia	10 (21.3)	22 (8.0)	0.004
GA at PE diagnosis (weeks)	26.7 (24.8–30.3)	34.1 (30.2–37.1)	< 0.001
GA at birth (weeks)	28.0 (25.4–30.8)	36.1 (32.4–38.2)	< 0.001
Adverse perinatal outcome			
Intraventricular hemorrhage	12 (25.5)	0 (0)	—
Retinopathy of prematurity	24 (51.1)	0 (0)	—
Necrotizing enterocolitis	7 (14.9)	0 (0)	—
Perinatal death	12 (25.5)	0 (0)	—

Data are given as median (interquartile range) or *n* (%). *Intraventricular hemorrhage, necrotizing enterocolitis, retinopathy of prematurity and/or perinatal death. †Any time before birth. GA, gestational age; LDH, lactate dehydrogenase; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

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 outcome compared to those without.

Mixed-effects regression results showing the association of laboratory variables with adverse maternal outcome are available in Table S2.

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

Adverse perinatal outcome

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 adverse perinatal outcome. The presenting symptoms of PE 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 generally more common in pregnancies with composite adverse perinatal outcome (Table 2). However, only differences in 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%; $P = 0.004$) reached statistical significance. The gestational age at diagnosis (26.7 *vs* 34.1 weeks; $P < 0.001$) and at birth (28.0 *vs* 36.1 weeks; $P < 0.001$) were significantly earlier in women with adverse perinatal outcome compared to those without.

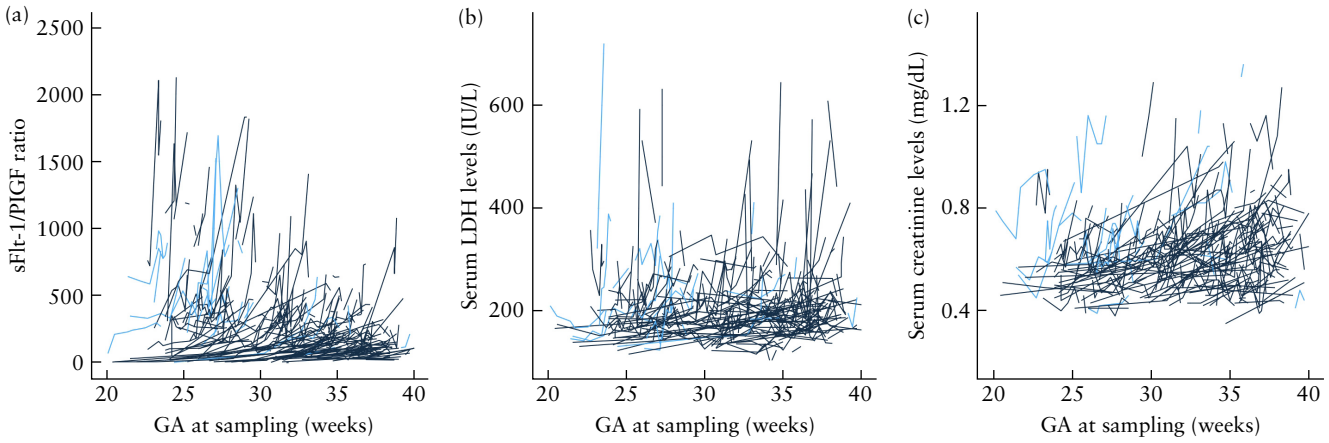

Figure 1 Spaghetti plots showing longitudinal changes of: (a) soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio, (b) serum lactate dehydrogenase (LDH) level and (c) serum creatinine level, according to gestational age (GA), and association with composite adverse maternal outcome. Blue lines indicate adverse maternal outcome.

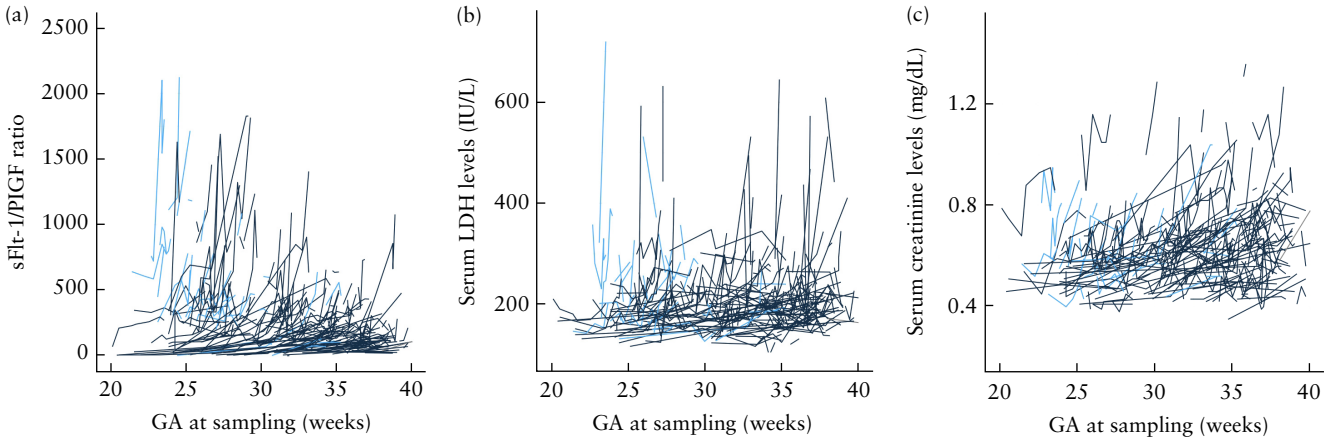

Figure 2 Spaghetti plots showing longitudinal changes of: (a) soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio, (b) serum lactate dehydrogenase (LDH) level and (c) serum creatinine level, according to gestational age (GA), and association with composite adverse perinatal outcome. Blue lines indicate adverse perinatal outcome.

Table 3 Areas under receiver-operating-characteristics curve (AUC) for models predicting composite adverse maternal or perinatal outcome in all, early- and late-onset pre-eclamptic pregnancies

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

Sensitivities are given as %. FPR, false-positive rate; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1.

Mixed-effects regression results showing the association of laboratory variables with adverse perinatal outcome are available in Table S3.

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

There was no significant difference between maternal serum sFlt-1/PlGF ratio and PlGF alone in terms of predicting composite adverse perinatal outcome (AUC, 0.82 (95% CI, 0.75–0.89) *vs* 0.81 (95% CI, 0.74–0.88); $P = 0.412$) and composite adverse maternal outcome (AUC, 0.72 (95% CI, 0.62–0.81) *vs* 0.69 (95% CI, 0.59–0.79); $P = 0.102$).

DISCUSSION

Principal findings

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

Comparison with existing literature

Recently, maternal serum angiogenic marker imbalance has been acknowledged in several international guidelines as a criterion for diagnosis of PE. Moreover, the maternal serum sFlt-1/PlGF ratio has been shown to be an important tool, not only for the diagnosis of PE in women with suspected PE, but also in the prognosis of the disease; for example, estimating the time until delivery and prediction of adverse maternal and perinatal outcome[2,3,6,7,18,36,40]. Repeat assessment of the ratio is advised in women with suspected PE, with a ratio between 38 and 85 or 110 after 34 weeks' gestation, as well as for the surveillance of high-risk cohorts, such as women with chronic hypertension[7,32]. Data on repeat angiogenic marker assessment in the surveillance of pregnancies with confirmed PE 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 PE surveillance, were developed to guide clinical decision-making and avoid adverse outcome in women with early-onset PE[25,41,42]. However, data comparing standard PE 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 outcome in combination with cardiovascular, fetal and placental indices in 123 women with suspected and confirmed PE. 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 the cohort.

Peguero *et al.*[43] conducted a prospective cohort study including 63 women with confirmed early-onset PE, evaluating the predictive performance of longitudinal angiogenic marker assessment for time to delivery and adverse outcome. They found that 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 had better performance than PlGF or the ratio in their cohort, which could be due to the fact that only women with severe early-onset PE were included. Maternal serum sFlt-1 was shown to be the main cause of endothelial dysfunction driving disease severity in their cohort; 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 PE included in this study. The fullPIERS group[44] 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 their cohort. Furthermore, maternal serum PlGF levels were not assessed longitudinally, as they were in this study.

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

Recently, Schmidt *et al.*[40] evaluated a machine-learning-based approach, which included standard laboratory parameters and sFlt-1/PlGF for their predictive performance of adverse outcome in women with suspected PE. In line with our findings, high accuracy, sensitivity and positive predictive value 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 PE, as shown by Villalain *et al.*[42].

Clinical and research implications

Current guidelines do not include angiogenic marker assessment for the surveillance of pregnancies with an established diagnosis of PE, 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 outcome and challenge the current concept of PE surveillance[35,36,40,43]. Hence, our data add to the existing body of literature that shows longitudinal angiogenic marker assessment is useful for the prediction of both maternal and perinatal adverse pregnancy outcomes in post-diagnosis surveillance. Data on longitudinal angiogenic marker assessment for PE surveillance should be studied further in a prospective, preferentially blinded, setting.

Strengths and limitations

This is a large cohort comparing repeat angiogenic marker assessment with routine laboratory testing in the surveillance of pregnancies with confirmed PE. This study, in contrast with the existing literature, does not focus exclusively on women with early-onset severe PE, but also includes women with late-onset PE, underlining the clinical relevance of these 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 capability of angiogenic markers for adverse maternal outcome. Delivery earlier than that 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 outcome as earlier delivery is often connected to prematurity and complications resulting

from it. Moreover, this type 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.

Conclusions

Longitudinal maternal serum angiogenic marker assessment improves the predictive potential for adverse perinatal outcome in singleton pregnancy with confirmed PE and might also be beneficial to predict adverse maternal outcome, when compared to established laboratory assessment alone. PE 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 outcome.

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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 Histograms showing distribution of gestational age at assessment and delivery.

Table S1 Reasons for delivery in study population ($n = 323$)

Table S2 Mixed-effects regression models for association of gestational age at sampling with laboratory variables. Interaction term for composite adverse outcome is maternal. Gestational age at sampling divided into five categories: 1st degree represents earliest gestational ages and 5th degree represents most advanced gestational ages

Table S3 Mixed-effects regression models for association of gestational age at sampling with laboratory variables. Interaction term for composite adverse outcome is perinatal. Gestational age at sampling divided into five categories: 1st degree represents earliest gestational ages and 5th degree represents most advanced gestational ages