The diagnostic value of angiogenic and antiangiogenic factors in differential diagnosis of preeclampsia

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The definition of preeclampsia is changing. However, with the addition of organ symptoms to the presence of hypertension in pregnancy instead of relying only on proteinuria, a more precise detection of women at risk of preeclampsia-associated adverse events has not been achieved. Instead, under the new definitions of the American College of Obstetricians and Gynecologists and of the International Society for the Study of Hypertension in Pregnancy, more women are classified as preeclamptic, with a tendency to milder disease. Furthermore, angiogenic and antiangiogenic factors have emerged as essential tools for predicting and diagnosing preeclampsia at high accuracies. Next to being rooted in the pathophysiology of the disease, they have been proven to be reliable tools for predicting and diagnosing the disease. In addition, 2 cutoffs have been evaluated for the clinical setting. As shown in the Prediction of Short-Term Outcome in Pregnant Women With Suspected Preeclampsia Study, at the soluble fms-like tyrosine kinase-1—to—placental growth factor ratio cutoff of 38, a preeclampsia can be ruled out for 1 week with a negative predictive value of 99.3% (95% confidence interval, 97.9—99.9) and ruled in with a positive predictive value of 36.7% (95% confidence interval, 28.4—45.7). The diagnostic cutoff of 85 has been shown to accurately identify women with preeclampsia, with a sensitivity of up to 88% and a specificity of 99.5%. In this review, we highlight the central role of angiogenic and antiangiogenic factors in the differential diagnosis of women presenting at high risk of the disease, such as patients with chronic hypertension or chronic kidney disease. We will focus on their ability to predict preeclampsia-associated adverse fetal and maternal outcomes. This is only possible when critically reviewing the evolution of the definition of "preeclampsia." We show how changes in this definition shape our clinical picture of the condition and how angiogenic and antiangiogenic biomarkers might be included to better identify women destined to develop preeclampsia-related adverse outcomes.

Key words: chronic hypertension, chronic kidney disease, differential diagnosis of preeclampsia, preeclampsia, pregnancy, sFlt-1/PlGF

The Evolution of the Definition of Preeclampsia

Preeclampsia (PE) is a multisystem disorder in pregnancy. The definitions have evolved, depending on advances in means to detect distinct features of the syndrome. Proteinuria, which was first described in association with eclampsia in 1843, is no longer mandatory for the diagnosis of the disease in the 2018 definition of the International Society for the Study of Hypertension in Pregnancy (ISSHP).[1,2] Although the triad of hypertension, proteinuria, and edema served as the definition of the disease for most of the 20th century, Leon Chesley[2] suggested in 1976 to exclude the symptom "edema" because of a lack of specificity for the condition. However, the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy published revised PE classification criteria only in 2000. In their report, PE was classified as a pregnancy-specific syndrome characterized by new-onset hypertension in a previously normotensive woman after 20 weeks' gestation with proteinuria.[3] Blood pressure (BP) criteria included a systolic BP of >140 mm Hg or a diastolic BP of >90 mm Hg. Proteinuria was defined as urinary excretion of ≥0.3 g of protein in a 24-hour specimen, which correlates with a random ≥1+ urine dipstick in the absence of a urinary tract infection. The American College of Obstetricians and Gynecologists (ACOG) followed that definition, as published in their 2002 Practice Bulletin.[4] From a perspective of the research definition, "the new-onset of hypertension (>140/90 mm Hg) and proteinuria (>300 mg/24 h or a protein-to-creatinine ratio of ≥30 mg/mmol) after 20 weeks' gestation" had the most impact on scientific literature over the last 20 years. Thus, until recently, the term "preeclampsia" is implying "hypertension and proteinuria." This is critical to understand when elucidating the impact of the angiogenic and antiangiogenic factors for the differential diagnosis of PE.

The Ability of the Gold Standard Definition to Detect Preeclampsia-Associated Adverse Outcomes

Hypertension and proteinuria are 2 clinical features of PE and the results of a complex pathophysiological cascade. The aim of every practicing obstetrician and obstetrical physician is the early detection of women who are at risk of potentially lethal PE-associated maternal

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Received July 13, 2020; revised Sept. 22, 2020; accepted Sept. 25, 2020.

Stefan Verlohren received speaker fees and participated in advisory boards from Roche Diagnostics, ThermoFisher and Alexion. Lisa-Antonia Dröge received speaker fees from Roche Diagnostics.

This paper is part of a supplement.

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0002-9378/$36.00 • https://doi.org/10.1016/j.ajog.2020.09.046

or fetal adverse outcomes. For a long time, the best practice to predict these adverse outcomes was to use the proxy "hypertension and proteinuria," partially because they are "convenient to measure" as James Roberts put it in his seminal work.[5] Meanwhile, it is well accepted that "hypertension and proteinuria" have a low positive predictive value (PPV) for detecting associated complications. Roberts has shown that PPV of hypertension and proteinuria to detect PE-associated complications is approximately 20%.[5] In their publication, Zhang et al[5] state that BP and proteinuria are not specific enough to define this disorder. Furthermore, they conclude that an overdiagnosis will increase sensitivity at a cost of including more false-positive subjects (reducing specificity) and subsequently overtreating patients in whom the maternal and perinatal outcomes will be normal. Therefore, they see the identification of a good biomarker as the "ultimate solution" for this problem: "The marker should be sensitive and specific to the pathophysiology of this disorder and might be used as a sole criterion or along with BP and proteinuria."

The Impact of the New Definition of Preeclampsia on Pregnancy Outcome

In the recent revision of the definition of preeclampsia by the ISSHP, PE is defined as the new onset of hypertension plus organ symptoms, such as liver dysfunction, hemolysis, or thrombocytopenia, or fetal growth restriction.[6] It is questionable if this new definition increases the specificity and PPV to better identify women at risk of adverse outcomes. The group of Nicolaides has recently shown that the new definition by the ISSHP increased the number of women being diagnosed with PE by about 21% and by 7% when the new ACOG definition is applied. This is however accompanied by a decreased severity in outcomes, such as gestational age at delivery, birthweight, birth of a small-for-gesational-age (SGA) neonate, and perinatal death.[7] In their retrospective analysis, they only focused on these pregnancy outcomes and did not record further maternal and fetal adverse events. Their findings, however, agree with other published works that made the new definitions of PE and an impact on the outcome. Homer et al[8] found that women with proteinuric PE compared with those with nonproteinuric PE delivered earlier (36.7 [2.8] vs 37.3 [2.2] gestational weeks), more often had severe hypertension (38.7 vs 29.7%), and had a higher incidence of perinatal mortality (25.2/1000 vs 6.67/1000). The authors concluded that nonproteinuric PE is a more benign condition than proteinuric PE. Tochio et al[9] investigated a cohort of 308 women in Japan. Applying the new ISSHP definition increased the number of women labeled as preeclamptic by 155. This was not paralleled, however, by an increase in adverse fetal or maternal outcomes; they remained approximately unchanged (maternal adverse outcomes [15.15% vs 20%] and fetal adverse outcomes [17% vs 13.3%]). Kallela et al[10] investigated the impact of the new definition in a large pregnancy cohort in Finland where they could also show an increase in additional diagnoses of PE, as 27.9% of women who were previously classified as having gestational hypertension were now labeled as preeclamptic. A change in pregnancy outcome was not recorded in that study. This host of publications uniformly showed that the proportion of women classified as preeclamptic is increased when the new definitions of the ISSHP and ACOG are used. This, however, is not paralleled by an increase in severe outcomes in these women.

General Concepts to Improve the Prediction of Adverse Outcomes of Preeclampsia with Angiogenic and Antiangiogenic Factors

From 2003, the group of Karumanchi set the most essential milestone in the understanding of the pathophysiology of PE. They first showed that women with PE have increased placental expression of soluble fms-like tyrosinekinase-1 (sFlt-1) and decreased expression of placental growth factor (PlGF) and vascular endothelial growth factor (VEGF).[11,12] They furthermore showed that concentrations of sFlt-1 were elevated, whereas that of PlGF were decreased in the peripheral blood of women with PE. The degree of alteration correlated in a dose-response—like relationship to the severity of the disease: the more dysregulated the placental expression and circulating concentrations in peripheral blood, the more severe the disease. In the same study, the results of an animal experiment were presented. Adenoviral administration of sFlt-1 in pregnant rats resulted in hypertension, proteinuria, and glomerular endotheliosis in these animals. Induction of preeclamptic features by high sFlt-1 concentrations confirmed its etiologic role.[13]

VEGF, PlGF, and sFlt-1 are angiogenic and antiangiogenic factors that play a major role in physiological and pathologic angiogenesis, the formation and maintenance of blood vessel structures. The interplay between VEGF and the structurally highly homologous PlGF and its receptors, VEGF receptor 1 (VEGR1 synonymous fms-like tyrosinkinase-1), Flt-1, and Flt-2, guides transmembrane signaling of angiogenic signals. In pregnancy however, an alternative, soluble splice variant of VEGFR1 (sVEGFR-1 or sFlt-1) binds to circulating VEGF and PlGF and inhibits signaling on the membrane-bound receptors, thereby exerting an antiangiogenic effect. When concentrations of sFlt-1 are massively elevated, a decreased angiogenic signaling results.[14]

Diagnostic Cutoffs for Preeclampsia

The ability of angiogenic and antiangiogenic factors, most notably the sFlt-1—to—PlGF ratio, to aid in prediction and diagnosis of PE has henceforth been shown in multiple clinical studies following Karumanchi's pioneering work.[11,15–20] Among others, our group was able to show that the ratio of sFlt-1 and PlGF is useful to predict and diagnose PE.[21–25] Over the past decade, different automated assays for sFlt-1 and PlGF were evaluated and clinical cutoffs determined.[26–29] We have shown that the sFlt-1—to—PlGF ratio at the cutoff of 85 is able to detect PE at <34 0/7 weeks' gestation with a sensitivity of 89% and a specificity of 97%.[21] A two-phase cutoff for diagnosing PE was evaluated, with a lower cutoff of 33 for the whole gestational phase after 20 weeks and an upper cutoff of 85 for <34 weeks and 110 for ≥34 weeks. This two-phase cutoff results

in a sensitivity of up to 88% and a specificity of up to 99.5%.[22] In the last few years, the sFlt-1—to—PlGF ratio has been established in the obstetrical routine in Germany and Europe. In hospital and in outpatient clinics, the sFlt-1—to—PlGF ratio is a daily routine parameter, and results are available within 24 hours. The use of the angiogenic and antiangiogenic factors is encouraged in the guideline of the German-speaking societies of obstetrics and gynecology (Austrian, German, and Swiss) for predicting and diagnosing the disease in women at high risk.

Predictive Cutoffs for Preeclampsia

The Prediction of Short-Term Outcome in Pregnant Women With Suspected Preeclampsia Study (PROGNOSIS) evaluated whether the sFlt-1—to—PlGF ratio is able to rule in or rule out PE in women with suspicion of PE between 24 0/7 and 36 6/7 weeks' gestation.[30] In this large prospective multicenter study, a total of 1273 patients were enrolled at 30 international study sites. They were eligible for enrollment if they had either a new onset of hypertension or a new onset of proteinuria or 1 or more signs and symptoms indicative of PE, such as headache, epigastric pain, excessive edema, severe swelling, visual disturbances, sudden weight gain, or a pathologic uterine artery Doppler (resistance index of the uterine arteries <95th percentile or bilateral uterine notching). The primary endpoint was to demonstrate that low ratios of sFlt-1 and PlGF predict the absence of PE, eclampsia, or hemolysis, elevated liver enzymes, and a low platelet count (HELLP) syndrome within 1 week of baseline visit and that high ratios of sFlt-1 and PlGF predict the diagnosis of PE, eclampsia, or HELLP syndrome within 4 weeks of baseline visit. Secondary objectives included the use of the use of the sFlt-1—to—PlGF ratio to predict PE-related maternal and fetal adverse outcomes; the correlation of ratio dynamics with diagnosis and severity of PE, eclampsia, or HELLP syndrome; and the correlation of the sFlt-1—to—PlGF ratio with preterm delivery and time to delivery. PROGNOSIS was designed to derive and validate a cutoff-based prediction model for each prediction claim (1-week rule out or 4-week rule in) in a 2-step approach. In the first development study, 500 subjects were enrolled, and a cutoff was derived, which was tested in a subsequent validation study of 550 patients. The cutoff that was derived was 38. An sFlt-1—to—PlGF ratio of ≤38 had a negative predictive value (NPV) of 99.3% (95% confidence interval [CI], 97.9—99.9) to rule out PE in women presenting with signs and symptoms of the disease. Up to 4 weeks after testing, the high NPV of this cutoff prevailed, with 97.9%, 95.7%, and 94.3% at 2, 3, and 4 weeks, respectively.[31] The PPV of a sFlt-1—to—PlGF ratio of >38 to rule in PE within the next 4 weeks was 36.7% (95% CI, 28.4—45.7), corresponding to a sensitivity of 66.2% (95% CI, 54.0—77.0) and a specificity of 83.1% (95% CI, 79.4—86.3). Thus, PROGNOSIS was able to show that the sFlt-1—to—PlGF ratio is able to rule out the disease for 1 week in women presenting at high risk with a high NPV. An sFlt-1—to—PlGF ratio of 38 and more is indicative of the development of PE within the next 4 weeks, with a PPV of 36.7%.

Sovio et al[32] evaluated the sFlt-1—to—PlGF ratio in unselected nulliparous women in different gestational ages and a priori risk groups: In the low-risk group, the sFlt-1—to—PlGF ratio cutoff of 38 at 28 weeks yielded a PPV of 33.3% and an NPV of 99.5%. Thus, the ratio cutoff of 38 is also feasible to rule out the condition in women at low risk. The evidence on the performance of angiogenic and antiangiogenic factors has been limited for twin pregnancies.[33] Recently, Binder et al[33] showed the applicability of the cutoff of 38 to rule out a delivery because of PE in twin pregnancies for 1 and 2 weeks at an NPV of 98.8% and 96.4%, respectively.[34]

Following single measurements, repeated measurements are important: patients with a delta between 2 measurements 2 and 3 weeks apart have a higher risk of PE. In the group of women that eventually developed PE, the mean delta of the sFlt-1—to—PlGF ratio within 2 weeks was 31.2 (interquartile range [IQR], 6.48—62.36), whereas in the group of patients that did not develop PE in the end, it was 1.45 (IQR −0.12 to 9.41).[31]

A host of studies has evaluated the cutoffs of 38 and 85.[35—40] The Table summarizes the studies that have evaluated these cutoffs in different clinical settings and with different assay systems.

Multimarker Modeling Including Angiogenic Factors in Outcome Prediction

The integration of angiogenic and antiangiogenic factors into multimarker modeling approaches led to improved prediction of the disease. Perry et al[41] combined maternal factors and sFlt-1 and PlGF to predict a PE-related delivery within 1 and 2 weeks. They showed the added value of including the sFlt-1—to—PlGF ratio as a continuous marker rather than using a fixed cutoff. Saleh et al[42] compared the predictive value of the sFlt-1—to—PlGF ratio with cutoff values and as a continuous marker with suspected PE and confirmed the better performance for adverse maternal and fetal outcomes when measuring the sFlt-1—to—PlGF ratio continuously. The findings of Ciobanou et al[43] confirmed an increased predictive performance of the combination of history, BP, and the sFlt-1—to—PlGF ratio over using the biomarkers alone to predict adverse outcomes in the third trimester of pregnancy.[44]

This host of convincing clinical studies has underpinned the clinical importance of the angiogenic and antiangiogenic factors and has led to the reimbursement and guideline implementation in many parts of the world.[45—47] Following the advances in first-trimester screening, where many studies have shown the excellent performance of the predictive algorithms, the sFlt-1—to—PlGF ratio is an important means for follow-up of patients at high risk as identified by early screening.[48] The Combined Multimarker Screening and Randomized Patient Treatment with Aspirin for Evidence-Based Preeclampsia Prevention (ASPRE) study has clearly shown the necessity of early screening and prophylaxis. The early initiation of a 150 mg per day intake of aspirin with therapy adherence of ≥90%, starting from 11 to 14 weeks' gestation to 36 weeks' gestation, decreased the presence of early-onset PE with an odds ratio

TABLE

Summary of relevant studies evaluating the predictive and diagnostic utility for a PE or a PE-related outcome of the sFlt-1—to—PIGF ratio, sorted for the cutoffs 38 and 85

Cutoff	Study, year	Study type	Number and type of patients	Endpoint	Results (%)
sFlt-1—to—PIGF cutoff of 38	McCarthy et al,[28] 2019	COMPARE Prospespective randomized clinical trial	198 women Women with suspected preterm PE before 35 wk (gestational age) with serum samples	Prediction of delivery within 14 d with different test (PIGF alone and sFlt-1—to—PIGF)	AUC of 0.875 (75.00 sensitivity, 90.02 specificity) NPV to exclude delivery within 2 wk 95.30 (95% CI 91.10—97.60) PPV to predict delivery within 2 wk 57.50 (95% CI, 44.90—69.20)
	Zeisler et al,[30] 2016	PROGNOSIS Prospective multicenter observational study	500 in the development cohort and 550 in the validation cohort (101 and 98 patients with PE or HELLP) Patients with suspicion of PE in 24 0/7 to 36 6/7 wk (gestational age)	Derivation and validation of a sFlt-1—to—PIGF ratio to rule out the development of a PE within 1 wk and to rule in the development of a PE within 4 wk if PE is suspected	NPV to rule out PE within 1 wk 99.30 (95% CI, 97.90—99.90), (80.00 sensitivity, 78.30 specificity) PPV to rule in PE in 4 wk 36.70 (95% CI, 28.40—45.70), (66.20 sensitivity, 83.10 specificity)
	Sovio et al,[32] 2017	POP study Prospective cohort study	4099 in total 3751 controls, 26 preterm PE, 111 severe PE Patients for routine visits at 20, 28, and 36 wk (gestational age)	Evaluation of effectiveness of the sFlt-1—to—PIGF ratio as a screening test for PE in unselected nulliparous women	Sampling at 28 wk NPV for PE plus preterm birth 99.50(95% CI, 99.30—99.70) (23.10 sensitivity, 99.70 specificity) 54.70% PPV for PE plus preterm birth 31.60 (95% CI, 10.70—52.50) Sampling at 36 wk NPV for severe PE 98.50 (95% CI, 98.10—98.90) (54.70 sensitivity 86.20 specificity) PPV for severe PE 10.20 (95% CI, 7.70—12.72)
	Bian et al,[35] 2019	PROGNOSIS Asia Prospective multicenter observational study	700 in total 599 without PE 101 with PE or HELLP Patients with suspicion of PE in 18 0/7 to 36 6/7 wk (gestational age)	Primary endpoint: value of the sFlt-1—to—PIGF ratio for ruling out PE within 1 wk and ruling in PE within 4 wk Secondary endpoint: value of the ratio for predicting fetal adverse outcomes	NPV to rule out PE within 1 wk 98.60 (95% CI, 97.20—99.40) (76.50 sensitivity, 82.10 specificity) PPV to rule in PE in 4 wk 30.30 (95% CI, 23.00—38.50) (62.00 sensitivity, 83.90 specificity) NPV to rule out fetal adverse outcome within 1 wk 98.90 (95% CI, 97.60—99.60) (80.00 sensitivity, 81.80 specificity) PPV go rule in fetal adverse outcome within 4 wk 53.50 (95% CI, 45.00—61.80) (61.60 sensitivity, 88.10 specificity)

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(continued)

TABLE

Summary of relevant studies evaluating the predictive and diagnostic utility for a PE or a PE-related outcome of the sFlt-1—to—PIGF ratio, sorted for the cutoffs 38 and 85 *(continued)*

Cutoff	Study, year	Study type	Number and type of patients	Endpoint	Results (%)
	Dragan et al,[36] 2017	Prospective multicenter observational study	12,305 women 12,001 without PE 14 with PE at <1 wk 77 with PE at <4 wk n=22 PE at ≥4 wk Patients for routine visits at 30 4/7 to 34 6/7 and 35 0/7 to 36 6/7 wk	Evaluation of the sFlt-1—to—PIGF ratio to predict delivery of 38 patients with PE at 30—37 wk (gestational age)	NPV to rule out PE within 1 wk 899.97 (95% CI 99.94—100.00), (78.60 sensitivity, 95.50 specificity) PPV to rule in PE in 1 wk 1.90 (95% CI, 0.80—3.00) NPV to rule out PE within 4 wk 99.85 (95% CI, 99.78—99.92), (76.60 sensitivity, 95.90 specificity) PPV to rule in PE within ≥4 wk 8.30 (95% CI 6.00—10.60)
	Cerdeira et al 2019[37]	INSPIRE Prospespective randomized clinical trial	370 women in total (186 reveal vs 184 nonreveal) 85 with PE Patients with suspicion of PE between 24 0/7 and 37 0/7 wk (gestational age)	Evaluation of the sFlt-1—to—PIGF ratio of 38 patients to rule out PE within 7 d Primary endpoint: hospitalization within 24 h for testing Secondary endpoint: development of PE within 7 d	NPV to rule out admission within 24 h PPV to rule in admission within 24 h NPV to rule out PE within 7 d 100.00 (95% CI 97.10-100.00), (100.00 sensitivity, 77.80 specificity) PPV to rule in PE within 7 d 40.00 (95% CI 27.60—53.50)
sFlt-1—to—PIGF cutoff of 85	Verlohren et al,[21] 2010	2 arms, longitudinal study of controls, case-control study	Case-control: n=339 71 with PE (37 with early-onset PE, 34 with late onset PE), 268 gestational age-matched controls	Separation between PE and controls at diagnosis of PE with sFlt-1—to—PIGF ratio (1) Gesational age—independent PE (2) Late- and early-onset PE	PE vs controls AUC of 0.95 (82.00 sensitivity, 95.00 specificity) Early-onset vs controls AUC of 0.97 (89.00 sensitivity, 97.00 specificity) Late onset vs controls AUC of 0.89 (74.00 sensitivity, 89.00 specificity)
	Verlohren et al,[22] 2014	2 arms, longitudinal study of controls, case-control study	1149 patients in total 234 with PE 468 controls	Separation between PE or HELLP and controls at diagnosis of PE with sFlt-1—to—PIGF ratio. Late- and early-onset PE, establishment of new cutoffs, focusing on sensitivity and specificity with equivocal zone	AUC of 0.94 for late- and early-onset PE (75.60 sensitivity, 95.50 specificity) Early onset vs controls (88.00 sensitivity, 99.50 specificity
	Dröge et al,[26] 2017	2 arms, longitudinal study of controls, case-control study	32 patients with PE vs 132 controls 46 patients with PE-related outcome (IUGR and or PE) vs 167 controls	Separation between PE and controls and PE-related outcome (IUGR and or PE) at diagnosis with sFlt-1—to—PIGF ratio with the Kryptor assay	PE vs controls (87.88 sensitivity, 88.64 specificity) PE-related outcome vs controls (84.78 sensitivity, 91.02 specificity)

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TABLE

Summary of relevant studies evaluating the predictive and diagnostic utility for a PE or a PE-related outcome of the sFlt-1—to—PlGF ratio, sorted for the cutoffs 38 and 85 *(continued)*

Cutoff	Study, year	Study type	Number and type of patients	Endpoint	Results (%)
	Stepan et al,[27] 2019	Case-control study	383 patients in total 113 with PE or HELLP (39 early onset, 74 late onset)	Separation between PE and controls in early and late onset PE with different test kits (Elecsys Roche Diagnostics, Mannheim, Germany) with different sFlt-1—to—PlGF ratio cutoffs	PE vs Controls: AUC of 0.95 (72.90 sensitivity, 96.80 specificity) Early-onset PE vs controls: AUC of 100.00 (88.10 sensitivity, 100.00 specificity) Late onset vs controls: AUC of 0.91 (64.50 sensitivity, 94.80 specificity)
	Huhn et al,[38] 2018	Case-control study	34 with preterm PE 64 controls for preterm PE	Separation between PE and controls in early- and late-onset PE with different cutoff values	Early-onset PE vs controls AUC of 0.92 (94.00 sensitivity, 86.00 specificity)
	Salahuddin et al,[39] 2016	Case-control study	846 patients in total 412 patients with suspected PE 434 controls	Prediction of PE-associated adverse maternal and perinatal outcomes within 2 wk in women with suspected PE with presence of hypertension, proteinuria and sFlt-1—to—PlGF ratio of >85	Adverse outcome vs no adverse outcome sFlt-1—to—PlGF of >85 plus hypertension plus proteinuria AUC of 0.89 at <34 wk (BRAHMS Kryptor Assay [Thermo Fisher Scientific, Henningsdorf, Germany] and Elecsys Assay [Roche Diagnostics, Mannheim, Germany]), 0.80 (Kryptor Assay), −0.81 (Elecsys Assay) at ≥34 wk
	Rana et al,[40] 2012	Case-control study 348 without adverse outcome 268 with adverse outcome	616 women with suspected PE	Prediction of subsequent adverse maternal and perinatal outcome within 2 wk (adverse outcome, hypertension + at least 1 criteria: elevated liver enzymes, thrombocytopenia, DIC, placental abruption, pulmonary edema, cerebral hemorrhage, seizure, acute renal failure, maternal death, iatrogenic delivery because of PE, SGA, abnormal umbilical Doppler, fetal death)	All patients: AUC of 0.76 87.00% sensitivity Presentation at <34 wk: AUC 0.89 (72.90 sensitivity, 94.00 specificity, NPV 87.30)

AUC, area under the curve; *CI*, confidence interval; *DIC*, disseminated intravascular coagulation; *HELLP*, hemolysis, elevated liver enzymes, and a low platelet count; *IUGR*, intrauterine growth restriction; *NPV*, negative predictive value; *PE*, preeclampsia; *PlGF*, placental growth factor; *PPV*, positive predictive value; *sFlt-1*, soluble fms-like tyrosine kinase-1; *SGA*, small for gestational age.

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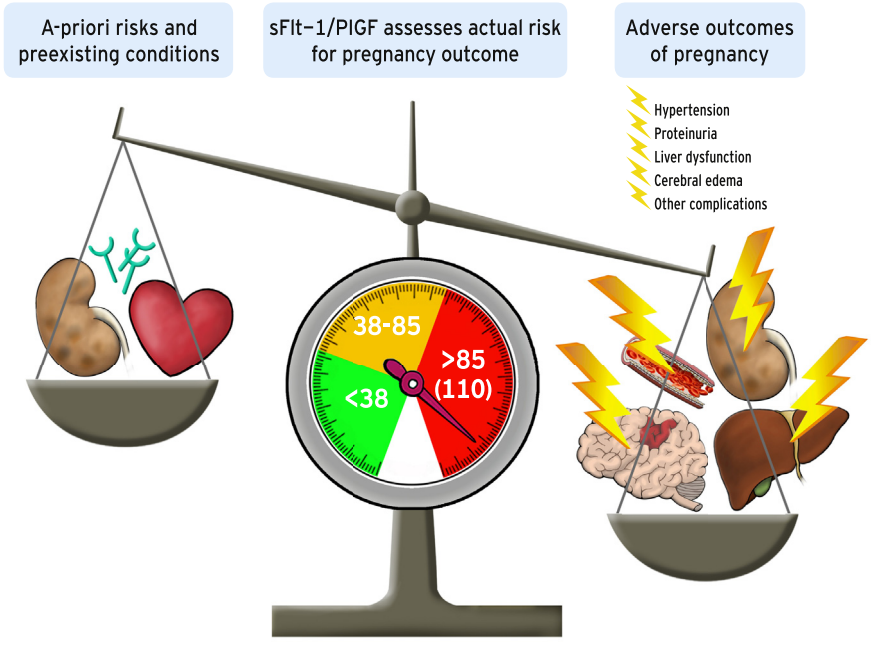
FIGURE
Evaluation of the preexisting conditions and new-onset symptoms with sFlt-1/PlGF scale



An illustration of the clinical evaluation of the patient's preexisting conditions and new-onset symptoms that can be weighted-out with an sFlt-1/PlGF scale, indicating not only the placental-caused proportion of the disease but also the pending maternal and fetal pregnancy outcomes.

PlGF, placental growth factor; *sFlt-1*, soluble fms-like tyrosine kinase-1.

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of 0.38 (95% CI, 0.20−0.74; *P*=.004).[49−53] However, a comprehensive approach of following up these patients during pregnancy includes repeated screening and surveillance with the help of angiogenic and antiangiogenic factors as women stay at high risk despite receiving aspirin.[48]

Predicting Preeclampsia-Related Adverse Outcomes with Angiogenic and Antiangiogenic Factors

Most clinical studies that evaluated the diagnostic and predictive use of biomarkers chose the endpoint "PE," as defined by the old ISSHP or ACOG definition. In 2012, Rana et al[41] was the first to evaluate the accuracy of the sFlt-1−to−PlGF ratio when focusing on PE-related complications: in a prospective clinical study on 616 women with suspected PE, they evaluated the use of the sFlt-1−to−PlGF ratio to predict adverse outcomes, defined as the presence of hypertension plus severe outcome features, such as delivery before 34 weeks' gestation, lung edema, and placental abruption. The women enrolled in this study presented with a clinical suspicion of the disease. However, the sFlt-1−to−PlGF ratio in women that developed adverse outcomes in the next 14 days was 47.0 (25th−75th percentile, 15.5−112.2) vs 10.8 (4.1−28.6; *P*<.0001) in women that did not experience adverse events. In the subgroup of women presenting at <34 weeks' gestation, the median sFlt-1−to−PlGF ratio in the adverse outcome vs no adverse outcome group was 226.6 (50.4−547.3) vs 4.5 (2.0−13.5; *P*<.0001). In a further analysis of the study cohort, they showed that in the 46 women with "hypertension and proteinuria" and a sFlt-1−to−PlGF ratio of <85, no adverse outcomes were recorded. In contrast, of the 51 women with "PE" and an sFlt-1−to−PlGF ratio of ≥85, 52.9% subjects had an adverse outcome. This provokes the question if a nonangiogenic PE is the benign variant of the disease spectrum.[54] The posthoc analysis of PROGNOSIS painted a similar picture. The sFlt-1−to−PlGF ratio at the cut-off of 38 had an NPV of 98.5% (95% CI, 96.9−99.5) for ruling out PE and PE-related adverse outcomes within 1 week and a PPV of 65.5% (95% CI, 56.3−74) to rule in the combined endpoint. Thus, when the meaningful clinical endpoint "PE-related adverse outcomes" is chosen, angiogenic and antiangiogenic factors are performing well, predicting these in women presenting with clinical suspicion of the disease.

The Figure depicts the importance of the sFlt-1−to−PlGF ratio as a relative "gauge" between preexisting risk conditions and PE-associated adverse outcomes. The sFlt-1−to−PlGF ratio weighs the actual risk considering preexisting conditions and can give a precise prediction of adverse outcomes.

Predicting Time to Delivery with Angiogenic and Antiangiogenic Factors

An important aspect of differential diagnosis in women presenting at high risk of the disease is the prediction of time to delivery. As a causative therapy of the disease is nonexistent, prophylactic treatment, such as the initiation of steroid therapy, is paramount in preventing adverse outcomes. The angiogenic factors can be used to predict the remaining pregnancy duration. A "dose-response" relationship between the concentration of the sFlt-1−to−PlGF ratio and the remaining pregnancy duration has been shown repeatedly: the higher the dysbalance of the markers, the shorter the remaining pregnancy duration. Below 34 weeks, an sFlt-1−to−PlGF ratio of >655 corresponds to significantly reduced time to delivery, with only 29.4% patients being pregnant after 48 hours and 5.9% of cases >7 days. After 34 weeks with an sFlt-1−to−PlGF ratio of >201, only 16.7% and 0% of patients remain pregnant after 2 and 7 days, respectively.[55] In a posthoc analysis of the PROGNOSIS cohort, women that had a sFlt-1−to−PlGF ratio of >38 had a median remaining pregnancy duration

of 17 days as compared with 51 days in women with a sFlt-1—to—PlGF ratio of ≤38, irrespective of the diagnosis of PE.[56] Women with an sFlt-1—to—PlGF ratio of >38 have a significantly reduced remaining time to delivery compared with those with an sFlt-1—to—PlGF ratio of ≤38, regardless of whether they developed PE or not (regression analysis factor, 0.61; 95% CI, 0.57—0.65).

Duhig et al[57] evaluated the impact of the physician's knowledge of the angiogenic marker concentration on the outcome. In their large real-world study, they found that consideration of the patients PlGF concentration resulted in a shorter time not only to diagnosis of PE but also to a significantly lower number of adverse maternal outcomes. In their study, they relied on PlGF alone. However, Stepan et al[58] indicated that the combined measurement of the sFlt-1—to—PlGF ratio provides improved diagnostic accuracy over the determination of the PlGF serum concentration alone.

Differential Diagnosis of Adverse Outcomes in Patients with Chronic Kidney Disease

As reviewed recently by Wiles et al,[59] patients with chronic kidney disease (CKD) have a 10-fold higher risk to develop PE, but the overlapping clinical presentation of both diseases with (preexisting) hypertension (mostly treated) and proteinuria (mostly in the nephrotic range) makes a causal differentiation of both underlying conditions difficult. Moreover, kidney-affecting diseases, such as systemic lupus erythematosus (SLE), thrombocytopenic purpura, or atypical hemolytic uremic syndrome, can first occur or aggravate in pregnancy, which makes the discrimination against placental-related complications as challenging as the prediction of the pregnancy outcome.[60,61] Biomarkers that have been investigated to distinguish between CKD and superimposed preeclampsia were linked to the renin-angiotensin system activation, the endothelial pathology, complement-activation dysfunction, and tubular injury. In a cohort of 60 patients with CKD (15 with superimposed PE and 45 without PE), the endothelial factors, hyaluronan and vascular cell adhesion molecule, separated women with CKD and CKD with PE with an AUC of 0.80 and 0.86, respectively; complement factors (C3a, C59, C5b-9); kidney injury markers, such as kidney injury molecule (KIM-1); lipocalin-2; and endothelial intercellular adhesion molecule (ICAM) and E-selectin and P-selectin and renin and angiotensin showed no discriminatory power.[62] With an overall PE-incidence of 20% and an SGA-incidence of 23% reported by Wiles et al[63] and an odds ratio (OR) of 2.38 (95% CI, 1.64—3.44) for SGA and an OR of 1.52 (95% CI, 1.16—1.99) for preterm delivery described by Kendrick et al,[64] additional diagnosis of PE and short-term pregnancy outcome were assessed.

As reviewed by Boulanger et al,[65] primarily, placental-caused deteriorations in hypertension and proteinuria have a less favorable outcome in pregnancy with an early indication to preterm delivery, whereas an underlying kidney disease might still allow prolongation of the pregnancy. In 2016, Bramham et al[66] evaluated the discriminatory value of cardiovascular B-type natriuretic peptide, neutrophil gelatinase-associated lipocalin, placental relaxin, and PlGF for an indicated delivery within 14 days because of PE when CKD was preexisting. Only a decreased PlGF level with an AUC of 0.85 in the receiver operating characteristic (ROC) analysis was a discriminatory marker for the outcome.[67] This finding agrees with the results of the study by Rolfo et al,[68] who aimed to distinguish patients with CKD (n=23; mean of 29.8 weeks' gestation) from those with PE (n=34; mean of 30.6 weeks' gestation) with the sFlt-1—to—PlGF ratio. Patients with CKD vs those with PE had significantly lower sFlt-1—to—PlGF values (4.00 [interquartile range (IQR), 0.52—136.59] vs 435.79 [IQR, 260.90—1153.53]; $P<.001$. In a follow-up study after 20 weeks' gestation with 67 patients, Rolfo et al[69] reevaluated these results and analyzed the added value of uterine Doppler measurements. An ROC analysis showed a sensitivity of 83% and a specificity of 91% when using an sFlt-1—to—PlGF ratio cutoff value of 32.8; the more frequent finding of normal uterine flow parameters in women with CKD was unfortunately not calculated in a multimarker-model. The findings of Rolfo et al[69] indicate that PE-related outcomes can be predicted with measurement of the sFlt-1—to—PlGF ratio and additional placental markers, such as the uterine artery flow might help in the distinction from otherwise caused diseases. Furthermore, PE-mimicking symptoms might also be uncovered if the sFlt-1—to—PlGF ratio is negative. Case reports, such as the one of Hirashima et al,[60] who reported the clinical presentation of a patient with unsuspicious sFlt-1—to—PlGF ratio but deteriorating kidney parameters and hypertension in pregnancy, revealing the initial diagnosis of an SLE and successful immunosuppressive therapy encourage the use of the sFlt-1—to—PlGF ratio. The clinical availability of angiogenic and antiangiogenic factors is thus helpful in the differential and challenging diagnoses of patients with CKD in pregnancy.

Differential Diagnosis of Adverse Outcomes in Patients with Pregnancy-Induced Hypertension and Chronic Hypertension

Another hallmark in the use of angiogenic and antiangiogenic factors is outcome prediction in patients with chronic hypertension. Affecting an increasing number of 0.5% to 1.5% of all pregnancies in the industrialized countries, these patients are at increased risk of superimposed PE.[62] A total of 26% of patients developed superimposed PE, 20% of patients experienced birth at <37 weeks' gestation, and 17% of patients had a delivery of an SGA infant, as shown in a large metaanalysis of 55 cohort studies.[70] In a large cohort study of 109,932 patients, Panaitescu et al[71] found similar numbers: a total of 23% of patients with chronic hypertension developed superimposed PE. The incidence of preterm PE was 8.5% and term PE 14.3%. They have nicely delineated after adjustment for confounding factors, chronic hypertension was associated with a 3.7-fold increase in the risk of iatrogenic preterm birth and a 1.8-fold increase in the risk of elective cesarean delivery as a consequence of iatrogenic intervention rather than a direct effect of

the preexisting disease. In the competing risk model for PE screening, chronic hypertension is one of the strongest contributors to a priori risk.[72] In a subanalysis of the ASPRE cohort, women with chronic hypertension did not benefit from an early aspirin intake as incidence of PE was not reduced in these women.

Based on these data, it is important to understand the role of chronic hypertension as a strong risk factor for adverse outcomes. The angiogenic and antiangiogenic factors help identify patients with chronic hypertension at risk of superimposed PE.[73] Verlohren et al[55] reported a significantly lower sFlt-1—to—PlGF ratio in patients with chronic and gestational hypertension compared with those with PE (both $P<.01$), implying that gestational hypertension per se is not associated with an increased sFlt-1—to—PlGF ratio. The sFlt-1—to—PlGF ratio cutoff of 85 was not exceeded in patients with a pregnancy outcome of chronic hypertension or gestational hypertension. Therefore, also with this PE-specific risk condition, the testing of the angiogenic biomarkers helps to identify those who develop an early adverse fetomaternal outcome and those in whom the outcome is uneventful.

Conclusion

The aim of appropriate antenatal care is the early identification and, as a result, prevention of adverse maternal and fetal pregnancy outcomes. As highlighted above, all iterations of the definition of PE had the goal to best predict PE-related adverse outcomes. The clinical features used as proxies for PE changed with the availability of respective diagnostic tools. Although "hypertension and proteinuria" are of limited predictive accuracy for adverse outcome, they have been the best available diagnostic means for a long time.[5] The advent of angiogenic and antiangiogenic markers is a paradigm shift in PE prediction. The placental biomarkers not only are "closer at the source" of the pathophysiological origin of the placental disorder PE but also have been proven to be a reliable tool in outcome prediction in numerous clinical studies over the past 17 years.

The recent change in the definition of PE of the ISSHP has unfortunately not embraced the clear evidence on the angiogenic biomarkers. Moreover, the 2018 revision did not result in an increased specificity but rather a decreased specificity, leading to further overdiagnosis and potentially more adverse outcomes because of iatrogenic intervention.[7] In the same year, the German, Swiss, and Austrian Societies of Obstetrics and Gynecology updated their guidelines on the management of hypertensive disorders in pregnancy. The definition of PE has been revisited. PE is now defined as the presence of any (also preexisting) hypertension in pregnancy of 140/90 mm Hg accompanied by a new onset of organ manifestations that cannot be attributed to another cause. In concordance with the ISSHP definition, these organ manifestations comprise renal, hematologic, hepatic, neurologic, pulmonary, and placental changes. In addition, in case of an absence of other organ manifestations, a change in "PE-specific systems," such as angiogenic and antiangiogenic factors, can indicate PE. This is the first international guideline that has included the evidence on the pathophysiological role and diagnostic capability of angiogenic and antiangiogenic factors in the definition of the disease "PE." Now, prospective clinical studies have to show if this iteration leads to a more specific diagnosis of PE and most of all to a more accurate prediction of adverse outcomes. However, considering the published evidence reviewed here, the integration of angiogenic marker results into the list of "organ manifestations" of PE is the next logical step with the potential to enhance the differential diagnosis and adverse outcome prediction.[74] ■

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