Predictive value of the sFlt-1/PlGF ratio in women with suspected preeclampsia: An update (Review)

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Abstract. Preeclampsia (PE) is a major complication of pregnancy with an incidence rate of 2-8% and is a leading cause of maternal mortality and morbidity. The various consequences of severe preeclampsia for the fetus, neonate and child include intrauterine growth retardation (IUGR), fetal hypoxia, oligohydramnios, intrauterine fetal demise, increased perinatal mortality and morbidity, neurodevelopmental disorders and even irreversible brain damage (cerebral palsy). A number of studies have demonstrated that differences in maternal serum concentrations of angiogenic factors between preeclampsia and normotensive pregnancies can be used as biomarkers, either alone or in combination with other markers, to predict the development of PE. The presence in the maternal circulation of two proteins of placental origin, placental growth factor (PlGF) and soluble fms-like tyrosine kinase 1 (sFlt-1), has been shown to be of clinical value, as the sFlt-1/PlGF ratio appears to be the optimal predictive tool for the development of PE. The measurement of their concentration in maternal serum in screening models, serves as predictive marker for the development of PE or IUGR later in gestation. However, further research is required to improve its clinical applicability and provide guidelines for its use worldwide to achieve more consistent clinical management of women with PE.

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Abbreviations: PE, preeclampsia; IUGR, intrauterine growth retardation; EOPE, early-onset PE; LOPE, late-onset preeclampsia; HELLP, hemolysis, elevated liver enzymes, and low platelets; STB, secondary syncytiotrophoblast; HIF-1α, hypoxia-inducible factor 1α; ET-1, endothelin-1; STBM, syncytiotrophoblast microparticles; AT1-AA, angiotensin II 1 receptor autoantibodies; NO, nitric oxide; OS, oxidative stress; ER, endoplasmic reticulum; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; sEng, soluble endoglin; VEGFRs, vascular endothelial growth factor (angiogenic) receptors; KDR, kinase domain region; EGFR, epidermal growth factor receptor; CPEP, calcium for preeclampsia prevention; uFP ratio, urinary sFlt-1-to-PlGF ratio; CI, confidence interval; ROC, receiver operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value; UtA-PI, mean uterine artery pulsatility index, MAP, mean arterial pressure; PROGNOSIS, PRediction of short-term Outcome in preGNant wOmen with Suspected preeclampsIa Study; PreOS, Preeclampsia Open Study; STEPS, study of early preeclampsia in Spain; NT-proBNP, N-terminal pro-B natriuretic peptide; POP, pregnancy outcome prediction, INSPIRE, interventional study evaluating the short-term prediction of preeclampsia/eclampsia in pregnant women with suspected preeclampsia; FPR, false-positive rate; FMF, Fetal Medicine Foundation; PAPP-A, serum pregnancy-associated plasma protein A; UAD, uterine artery Doppler

Key words: preeclampsia, IUGR, pregnancy, angiogenic growth factors, PlGF, sFlt-1

Contents

1. Introduction

Normal pregnancy is associated with significant maternal cardiovascular hemodynamic alterations, necessary for the optimal development of the growing fetus and the protection of the mother (1,2). Maternal blood volume progressively increases from the first weeks of gestation and reaches a peak of 40-50% above non-pregnant volumes at ~34-36 weeks of gestation, where it remains at these levels until term (1,3,4). The increase in maternal blood volume is required to provide increased blood flow throughout the placenta, a highly vascular organ, which is the primary site for maternal-fetal exchange of nutrients, gases and waste. Placental vascular network development requires vasculogenesis, and branching and non-branching angiogenesis, which are regulated by the

coordination between different vascular endothelial growth factors and cell types (5). The dysregulation of placental vascular development leads to placental dysfunction associated with various serious obstetric complications, such as preeclampsia (PE), intrauterine growth restriction (IUGR), pre-term birth and stillbirth (5,6).

PE is a pregnancy-specific multisystem disorder, affecting 2-7% of all pregnancies (7,8). It is a major cause of maternal and fetal/perinatal morbidity and mortality worldwide (15-20% in developed countries), leading to ~70,000 direct maternal deaths and ~500,000 perinatal deaths annually (7,9). The main criteria for the clinical diagnosis of PE are the new onset of hypertension and proteinuria or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following: Thrombocytopenia, elevated levels of liver transaminases, pulmonary edema, new-onset renal insufficiency, or cerebral or visual disturbances (8). Moreover, women who develop PE are at an increased risk of also developing cardiovascular complications later on in life (7). PE is classified as early-onset PE (EOPE), which accounts for 5-20% of all PE cases and develops prior to 34 weeks of gestation and late-onset (LOPE), which accounts for 80-95% of cases worldwide and develops after 34 weeks of gestation (10,11). EOPE is usually more severe and is associated with a high rate of IUGR, while LOPE is associated with eclampsia and hemolysis, elevated liver enzymes, and low platelets (HELLP) (10,11).

Although it is commonly acknowledged that PE is caused by placental dysfunction, its underlying pathophysiology remains incompletely understood (7). In 1991, Redman (12) introduced the two-stage model of PE pathophysiology: Stage 1 (preclinical, placental stage), which occurs during the 1st half of pregnancy, is caused by placental dysfunction with unremodeling of spiral arteries and uteroplacental malperfusion, leading to placental hypoxia (ischemia); and stage 2 (clinical, maternal stage), which occurs during the 2nd half of the pregnancy and is hypothesized to be a consequence of stage 1, as hypoxic placenta causes the increased release of numerous biological factors into the maternal circulation leading to endothelial dysfunction (Fig. 1A). An updated two-stage model was suggested in 2019 (11), in which at least two or more different pathways lead to stage 1 (Fig. 1B). The extrinsic placental pathway, is the classical dysfunctional placentation pathway leading to secondary syncytiotrophoblast (STB) stress and the release of pro-inflammatory factors into the maternal circulation, which develops early in pregnancy and leads to EOPE accompanied very often by IUGR. The intrinsic placental pathway arises due to the placental outgrowing uterine capacity with restricted intervillous perfusion, also causing STB stress, which develops late in pregnancy and leads to LOPE with normal fetal growth (11). Another potential pathway leading to STB stress is the excessive trophoblast senescence of ageing placenta. STB stress has been shown to stimulate the release of multiple factors in the maternal circulation including hypoxia-inducible factor 1α, endothelin-1, syncytiotrophoblast microparticles, angiotensin II 1 receptor autoantibodies (AT1-AA), nitric oxide (NO), oxidative stress, endoplasmic reticulum stress and angiogenic factors and their receptors (13,14). The updated model also incorporates maternal factors, including genetic, genetic, behavioral, immunological and environmental factors, which may affect both stages of PE.

2. Angiogenic growth factors and their receptors in PE

In normal placental vasculogenesis, extravillous cytotrophoblasts invade the myometrium spiral arteries and replace the endothelial layer of the uterine vessels, transforming them into elastic, soft, wide, low-resistance blood vessels, thus allowing increased uterine blood flow and adequate oxygen and nutrients supplies to the fetus (Fig. 2) (5,6,15). In PE, there is trophoblast dysfunction and the incomplete remodeling of spiral uterine arteries, causing placental hypoxia, oxidative stress and endothelial dysfunction responsible for the clinical symptoms (Fig. 2) (6,15-22). Interactions between angiogenic factors and their receptors contribute to placental angiogenic balance and are responsible for the maintenance and development of the placental vasculature (Fig. 2) (5,15,23-26).

VEGF and PlGF belong to the VEGF family and have angiogenic properties, while sFlt-1 and sEng exert anti-angiogenic effects (6). Reference values for pro- and anti-angiogenic factors and their protein tyrosine kinase receptors (VEGFRs) in the serum of normal pregnant women have been established (27) and different serum concentrations of these factors have been found in women with PE compared to those in women with normotensive pregnancies, indicating their inolvement in the pathogenesis of PE (Fig. 2) (5,6,13-15,18-22). The complex etiology and pathophysiology of PE emphasizes the need for a clinically useful biochemical marker for the diagnosis and subsequent prediction and management of PE.

VEGF (or VEGF-A) is a member of the human VEGF family, which also includes VEGF-B, VEGF-C, VEGF-D and PlGF, and their signals are mediated by their receptors, VEGFR-1/sFlt-1, VEGFR-2/KDR and VEGFR-3/Flt-4 (6,20,21,26,28). VEGF is produced by several cell types, including macrophages, keratinocytes, T-cells, tumor cells and cytotrophoblasts, and plays a key role in the regulation and differentiation of the vascular system (6,20,21,26,28). VEGF-A in the placenta induces vascular permeability and endothelial cell proliferation, maintains the integrity of newly formed capillaries and regulates trophoblast proliferation, differentiation and invasion (6,20). VEGF is a 45-kDa glycoprotein encoded by the VEGF gene, which is located on chromosome 6p21.1 (28,29). Through alternative mRNA splicing, several VEGF-A subtypes are generated, with VEGF-A165 being the predominant one (26,28).

PlGF was first identified in a placenta cDNA library in 1991 (30). It belongs to the cysteine-knot growth factor family and it has both angiogenic and pro-inflammatory functions (6,31). PlGF is expressed in trophoblasts, endothelial and epithelial cells, the skin, certain tumors, and the heart, lungs, thyroid and skeletal muscle (31). PIGF is a 45-50-kDa dimeric glycoprotein produced by the PIGF gene, which is located on chromosome 14q24 (6,31). Due to alternative mRNA splicing, four isoforms are encoded composed of 131, 152, 203 and 224 amino acids (31). PlGF shares a 53% homology with VEGF (6,30,31).

VEGF binds with high affinity to both sFlt-1 and KDR receptors and promotes branching angiogenesis (first trimester of pregnancy), while PlGF binds with high affinity exclusively to sFlt-1 and leads to non-branching angiogenesis (second

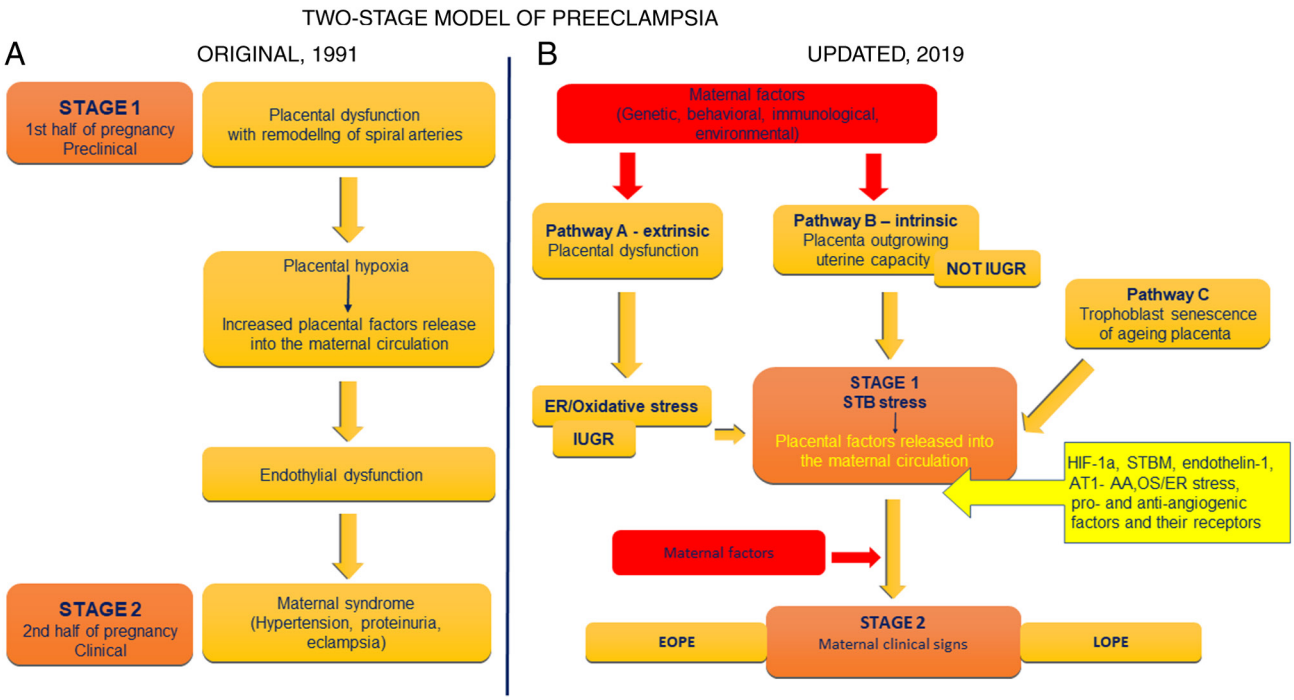

Figure 1. Two-stage model of PE pathophysiology. (A) Original model proposed in 1991 and (B) updated model suggested in 2019. PE, preeclampsia; ER, endoplasmic reticulum; IUGR, intrauterine growth retardation; EOPE, early-onset PE; STB, secondary syncytiotrophoblast; HIF-1α, hypoxia-induced factor 1α; STBM, syncytiotrophoblast microparticles; OS, oxidative stress; AT1-AA, angiotensin II 1 receptor autoantibodies; LOPE, late-onset preeclampsia.

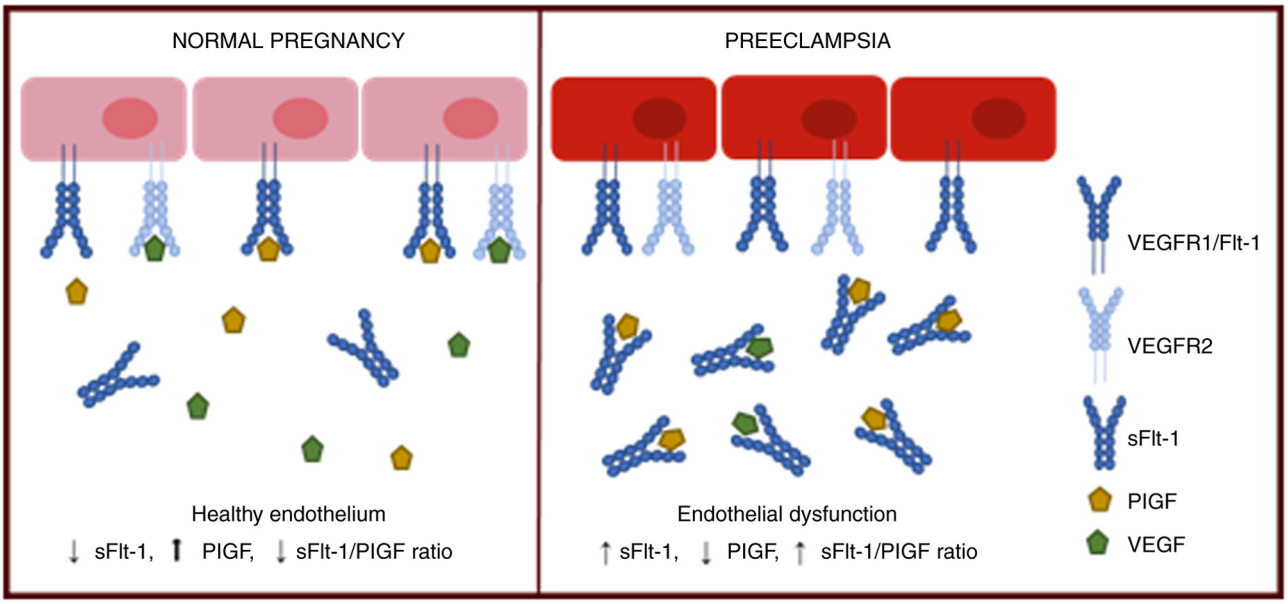

Figure 2. Angiogenic factors in normal pregnancy and preeclampsia. sFlt-1, soluble fms-like tyrosine kinase 1; PlGF, placental growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor (angiogenic) receptor.

trimester) (5,6,26,31-33). sFlt-1 and KDR receptors both have an extra cellular ligand binding domain, a transmembrane domain and an intracellular tyrosine kinase domain (6,15).

The Flt-1 gene is located on chromosome 13q12 and encodes a 186-kDa glycoprotein (6,15,34). The sFlt-1 protein is a splice variant of VEGFR-1, a 100-kDa variably glycosylated protein, which includes the extracellular ligand-binding domain and lacks the transmembrane and intracellular domains, thus it is secreted (soluble), and acts as a VEGF and PlGF antagonist, thus preventing their activity (15,26,33,34). sFlt1 is found in endothelial cells, monocytes, trophoblasts, vascular smooth muscle cells, dendritic cells, renal mesangial cells and various human tumor cell types (6,15,33). Multiple isoforms of sFlt1 have been reported, which are differentially expressed and distributed in human tissues, and may be associated with a variety of physiological and pathological roles (34,35). In humans, sFLT-1 i13 is the main sFLT-1 variant and is widely expressed in the majority of tissues, whereas the sFLT-1 e15a

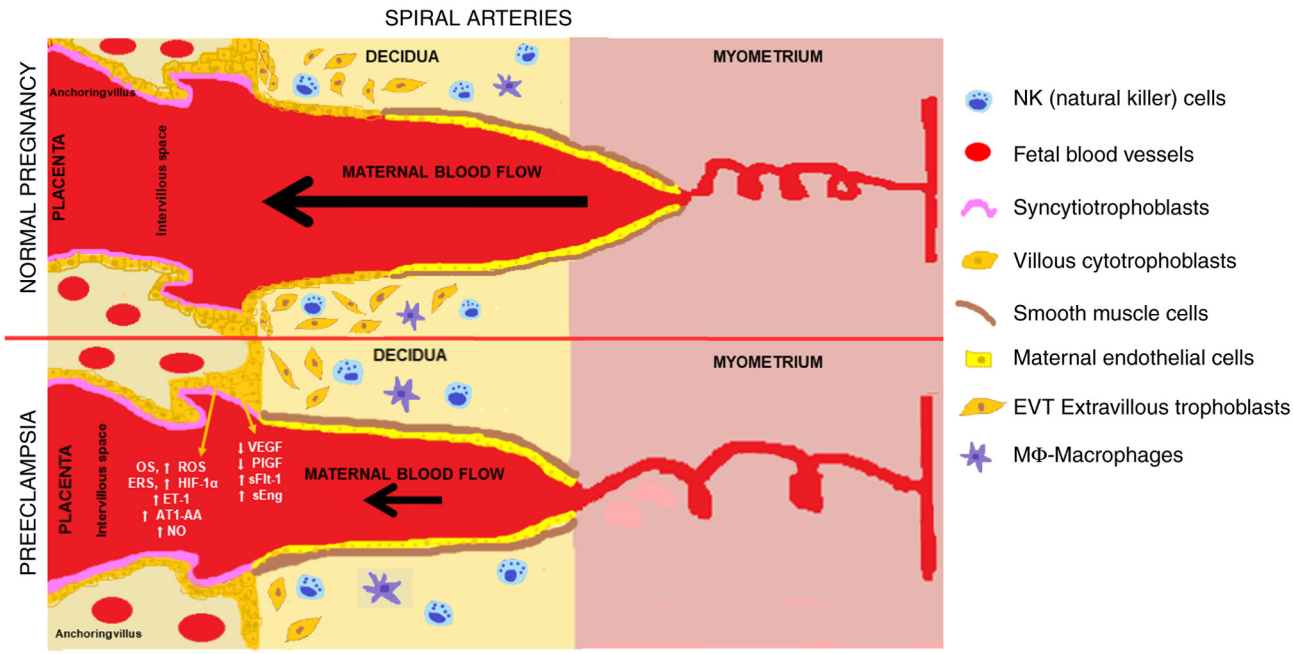

Figure 3. Spiral arteries remodeling in normal pregnancy and in preeclampsia. OS, oxidative stress; ROS, reactive oxygen species; ERS, endoplasmic reticulum stress; HIF-1α, hypoxia-induced factor 1α; NO, nitric oxide; AT1-AA, angiotensin II 1 receptor autoantibodies; PlGF, placental growth factor; VEGF, vascular endothelial growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; sEng, soluble endoglin.

variant appears to be the main protein in the circulation of women with PE (34,35). Although sFLt-1 placental derivation is well known, the upstream mechanisms regulating its release are poorly characterized. A recent study identified that epidermal growth factor receptor and mitochondrial signaling pathways positively regulated the placental release of sFlt-1 and may play central roles in the pathogenesis of PE (36).

Other factors have also been observed in the plasma of women with PE, such as increased sEng, NO, AT1-AA, cellular fibronectin and decreased heme oxygenase-1 and prostacyclin levels, suggesting a possible involvement in the pathogenesis of PE (Fig. 3) (19,37).

3. PlGF and sFlt-1 in PE

Extensive research has demonstrated the role of VEGF angiogenic factors and their receptors in the pathophysiology of PE and numerous scientists have been focused on their evaluation as candidate biomarkers in order to develop an efficient screening test with diagnostic and predictive potential for PE (6,15-19). Free VEGF plasma concentrations during pregnancy are low and often below the detection limit of most commercially available diagnostic kits (18).

Increased levels of sFlt-1 and decreased levels of PlGF in maternal serum have been observed from early pregnancy in women with PE, suggesting a blockade of PlGF action by sFlt-1 (15-20,33,38-59) (Table I). In 2003, Maynard *et al* (38) demonstrated almost 5-fold higher placental and serum sFlt-1 levels in women with PE compared to normotensive pregnant women. Of note, the sFlt-1 levels decreased in preeclamptic women 48 h after delivery, suggesting its placental origin (38). Moreover, decreased levels of free serum VEGF and PlGF were found in patients with PE compared to normal controls, which was proportionate to the rise in serum sFlt1 levels in these patients (38). Another two studies demonstrated significantly higher serum levels of sFlt-1 (an almost 6-fold increase) and lower free PlGF levels in women with PE than those from non-pregnant women (16,17).

In 2004, Levine *et al* (18) demonstrated an increase in serum sFlt-1 concentrations 5 weeks prior to the onset of PE with a parallel decrease in the free PlGF and VEGF levels, which may have been due to sFlt-1 binding. In addition, they demonstrated an association between serum sFlt-1 levels and the severity of the disease (18). In normotensive pregnancy, the serum sFlt-1 levels were increased and PlGF levels were decreased during the last 2 months of pregnancy (18). Their study suggested the relevance of these markers to the early identification of PE and the prediction of its severity (18).

Thadhani *et al* (39) suggested that the combination of first trimester serum sFlt-1 and PlGF levels can identify women who are at a high risk of developing PE. A subsequent study concluded that the plasma sFlt-1 concentration began to increase 6-10 weeks prior to the clinical manifestations of PE with a more pronounced increase at 2-5 weeks before the diagnosis, as well as at clinical presentation (40). Furthermore, it was observed that the plasma sFlt-1 concentration increased both in early- and late-onset disease, although in EOPE the elevation occurred earlier than in LOPE; it was thus suggested that the optimal time for the determination of plasma sFlt-1 concentrations for diagnostic purposes was at 28-32 weeks of gestation for EOPE and at 30-34 weeks of gestation for LOPE, or ~1 month before its clinical diagnosis (40).

Buhimschi *et al* (41) observed that the urinary sFlt-1-to-PlGF (uFP) ratio had a high sensitivity and specificity in differentiating women with severe PE from normotensive controls, as well as other hypertensive disorders; it was suggested that the uFP ratio would be a better indicator for defining the severity of the disease (41).

Table I. PIGF and sFlt-1 in PE.

Authors/year of publication	Patient population	Sample size	Findings	(Refs.)
Maynard *et al*, 2003	Mild and severe PE	11 women with mild PE, 10 women with severe PE, 10 normal pregnant after 30th week of pregnancy	Serum sFlt-1 level was 5-fold higher in patients with severe PE than in normotensive pregnant women sFlt-1 levels felt in preeclamptic women 48 h after delivery. Decreased levels of free serum VEGF and PlGF in PE patients compared to normal controls	(38)
Koga *et al*, 2003	PE	31 women with PE between 18-40 weeks of pregnancy, 52 nonpregnant women	Serum sFlt-1 concentrations in women with PE were >6-fold higher than non-pregnant women	(16)
Tsatsaris *et al*, 2004	Severe PE/IUGR	60 pregnant women (19 with severe PE, 10 with IUGR infant, 31 with no complicated pregnancy used as controls)	Significantly higher serum sFlt-1 levels and lower free PlGF levels in pregnancies with PE and IUGR, compared with normal pregnancies at term or matched for gestational age	(17)
Levine *et al*, 2004	Mild and severe PE	120 women with PE (80 had mild and 40 had severe PE), 120 normotensive controls [calcium for preeclampsia prevention (CPEP) trial]	sFlt-1 concentrations begin to increase 5 weeks before the onset of PE with a parallel decrease in the free PlGF and VEGF levels; association between serum sFlt-1 levels and the severity of PE as women with pre-term PE or PE and IUGR infant had higher serum sFlt-1 levels and lower PlGF levels at 21-32 weeks and at 33-41 weeks than those with an onset of PE at term or PE without an IUGR infant, respectively. Increased sFlt-1 levels in normotensive pregnancy and decreased PlGF levels during the last 2 months of pregnancy	(18)
Thadhani *et al*, 2004	PE with gestational hypertension/ IUGR	40 women who developed PE, 40 women with gestational hypertension, 40 women with an IUGR infant, 80 normal pregnant women in the 1st trimester of pregnancy	The combination of 1st trimester serum levels of sFlt-1 and PlGF identify women who are at a high risk of developing PE	(39)
Chaiworapongsa *et al*, 2005	PE	44 patients with PE, 44 normal pregnant women	Elevation of serum plasma sFlt-1 6-10 weeks prior to clinical signs of PE Plasma sFlt-1 concentration raised both in early-onset and late-onset disease, but in early-onset PE plasma sFlt-1 concentration was elevated earlier than the late-onset. The optimal time forthe determination of plasma sFlt-1 concentrations for diagnostic purposes is 28-32 weeks of gestation (mean, 30 weeks) for EOPE and 30-34 weeks of gestation (mean, 32 weeks) for LOPE, or ~1 month before its clinical diagnosis	(40)

Table I. Continued.

Authors/year of publication	Patient population	Sample size	Findings	(Refs.)
Buhimschi *et al*, 2005	PE/pregnant hypertensive/ proteinuric women	17 women with severe PE, 21 pregnant hypertensive and pro teinuric women who did not meet criteria for severe PE, 16 healthy pregnant control, 14 non-pregnant reproductive age	Increased urinary levels of sFlt-1 but decreased urinary PlGF expression in hypertensive pregnant women Urinary sFlt-1-to-PlGF (uFP) ratio has highsensitivity and specificity in differentiating women with severe PE from normotensive controls and even more could discriminate severe PE from other hypertensive disorders	(41)
Hirashima *et al,* 2005	PE	148 women (4 with PE who delivered at <37 weeks of gestation, 2 with PE who delivered at ≥37 weeks of gestation and 142 normal pregnant women) at 10, 18, 28, and 37 weeks of gestation	In normal pregnancies, the concentration of serum sFlt-1 decreased from 8-12 weeks to 16-20 weeks, gradually increased at 26-30 weeks and rapidly increased at 35-39 weeks of gestation Serum free PlGF concentration increased from 8-12 weeks to 26-30 weeks and then decreased at 35-39 weeks of gestation Decrease in serum free PlGF levels in women with PE in both the first and the second trimester before the onset of PE Higher serum sFlt-1 levels after 21 weeks of gestation, but not before 21 weeks	(27)
Ohkuchi *et al*, 2007	Severe EOPE (<32 weeks)/ severe LOPE (≥32 weeks)	14 women with EOPE (<32 weeks) severe PE, 20 women with LOPE (≥32 weeks) severe PE, 65 normotensive controls (28-34 weeks of gestation)	Decreased serum PlGF and increased serum sFlt-1 levels in EOPE and LOPE women with PE compared to normotensive controls at 28 and 37 weeks Decreased serum PlGF with concomitant increased sFlt-1 levels were more pronounced in EOPE than in LOPE The sFlt-1/PlGF ratios at around 28 weeks of gestation before the onset of severe preeclampsia were increased in 83% of cases	(42)
Levine *et al*, 2006	Term PE/preterm PE/gestational hyper-ten sion/IUGR	120 women with term PE, 72 women with preterm PE, 120 with gestational hypertension, 120 normotensive women with an IUGR infant, 120 normal pregnant women	sFlt-1/PlGF ratio increases in women with PE beginning 2 to 3 months before the onset of the disease	(37)
Stepan *et al*, 2007	Abnormal uterine perfusion	63 sec trimester pregnant women with abnormal uterine perfusion: 25 developed a later complication (12 with PE, 11 with IUGR and 2 with intrauterine death), 38 had a normal course of pregnancy	Significantly higher serum sFlt1 and lower PlGF levels in pregnancies with adverse pregnancy outcome compared with those with normal outcomes Alterations were more pronounced in pregnancies with subsequent PE compared with IUGR and early-onset diseases (delivery <34 weeks) compared with late-onset diseases)	(43)

Table I. Continued.

Authors/year of publication	Patient population	Sample size	Findings	(Refs.)
De Vivo *et al*, 2008	EOPE (<37 weeks) and LOPE (≥32 weeks)	26 women with EOPE (<37 weeks) and 26 women with LOPE (≥32 weeks), 52 healthy pregnant women collected between 24-28 weeks of gestation	Increased sFlt-1/PlGF ratio during gestation in both preeclamptic and control group in both trimesters, but in the control group the increase was moderate (51%), while in the preeclamptic group the increase was notable (285%) Serum PlGf levels decreased in the 3rd trimester in this group In the 2nd trimester, the sFlt-1/PlGF ratio was the optimal predictor of PE with a specificity, a sensitivity, a diagnostic accuracy, a positive predictive value and a negative predictive value of 88.5% using a cut-off of 38.47	(44)
Romero *et al*, 2008	PE/IUGR	144 singleton pregnant women (46 women with uncomplicated pregnancies who delivered appropriate for gestational age neonates, 56 women who delivered an IUGR neonate but did not develop PE, 42 women patients who developed PE)	Changes in the maternal plasma concentration of s-Eng, sFlt-1 and PlGF precede the clinical presentation of PE, while only changes in s-Eng and PlGF precede the delivery of an IUGR neonate	(45)
Ohkuchi *et al*, 2010	PE	144 normal pregnant women at 19-25, 27-31 and 34-38 weeks of gestation, from 34 women with PE	The sFlt-1/PlGF ratio was shown to have the optimal diagnostic power for both EOPE and LOPE The cut-off value of 85 for the sFlt-1/PlGF ratio might assist in the diagnosis of preeclampsia, especially for EOPE	(46)
Verlohren *et al*, 2010	PE	351 patients (71 patients with PE and 280 gestational age-matched control subjects) from 5 European study centers	sFlt-1/PIGF ratio had an area under the receiver operating characteristic curve (ROC) of 0.95 and the optimal performance was obtained in the identification of early-onset PE in an area under the curve of 0.97	(47)
Sunderji *et al*, 2010	PE	457 subjects (409 without PE and 48 with PE) at 20-36 weeks of gestation	A new system clearly separated normotensive women from those with pre-term PE with excellent sensitivity and specificity (95% for each biomarker) with the sFlt-1/PlGF ratio exhibiting the optimal performance	(48)
Chaiworapongsa *et al*, 2011	PE	87 patients presenting to the to the obstetrical triage area with the suspicion of PE (divided into four groups based on clinical severity of PE and gestational age at delivery-term or preterm) at <37 weeks of gestation, 180 women with uncomplicated pregnancies	Plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in the obstetrical triage area in identifying patients with severe PE requiring preterm delivery within 2 weeks	(49)

Table I. Continued.

Authors/year of publication	Patient population	Sample size	Findings	(Refs.)
Rana *et al*, 2012	PE	616 plasma samples from women presenting to obstetrical triage <34 weeks of gestation with suspected PE	Plasma sFlt1/PlGF ratio >85 at presentation was predictive of adverse outcomes occurring within 2 weeks and in these women this marker had better results than other laboratory tests currently used	(50)
Moore *et al*, 2012	Pregnancy complications	276 pregnant women (78 with complications, 198 without complications) after 20 weeks of gestation	Increased serum sFlt1/PlGF ratio was associated with an increased odds of complications among women presenting <37 weeks Multivariable model combining the sFlt1:PlGF ratio with clinical variables was more predictive of complications (AUC, 0.91; 95% CI, 0.85-0.97) than a model using clinical variables alone (AUC, 0.82; 95% CI, 0.79-0.90)	(51)
Verlohren *et al*, 2012	PE/GH/CH	630 women (388 singleton pregnancies with normal pregnancy outcome, 164 singleton pregnancies with PE outcome, 36 subjects with gestational hypertension (GH), 42 patients with chronic hypertension	Patients with PE had significantly elevated sFlt-1/PlGF ratios as compared with controls and with patients with chronic and gestational hypertension in <34 weeks and ≥34 weeks of gestation Patients with a sFlt-1/PlGF ratio in the highest quartile (P<0.001) had a significantly reduced time to delivery In the <34 weeks PE group, the early identification of high risk for delivery women was strongly associated with maternal and fetal morbidity and mortality as and the timely referral to an intensive care unit alone could reduce perinatal morbidity and mortality by 20%	(52)
Herraiz *et al*, 2014	Fetal growth restriction/PE or HELLP/ PE/HELLP/ fetal growth restriction	171 women with singleton pregnancies, complicated by fetal growth restriction (n=27), PE or HELLP (n=105) or PE or HELLP and fetal growth restriction (n=39), 171 gestational age matched healthy pregnant women	Increased sFlt-1/PlGF ratios in cases with fetal growth restriction, PE or HELLP, and preeclampsia or HELLP and fetal growth restriction than control pregnancies both <34 weeks and ≥34 weeks	(53)
Chaiworapongsa *et al*, 2014	Severe PE	85 patients who presented to the obstetrical triage area at 20-36 weeks with a diagnosis of 'rule out PE' were included in the study (37 remained stable until term (group I), 48 developed severe PE requiring preterm delivery (group II)	Maternal plasma concentrations of angiogenic/anti-angiogenic factors have prog nostic value for patients presenting with suspected PE before 34 weeks of gestation	(54)

Table I. Continued.

Authors/year of publication	Patient population	Sample size	Findings	(Refs.)
Rana *et al*, 2013	PE/non-angiogenic PE/angiogenic PE	97 women presented at GA <37 weeks in triage who deve loped PE within 2 weeks; 46 of the 97 women had non-angiogenic PE (sFlt1/PlGF ratio <85), 51 had angiogenic PE (sFlt1/PlGF ratio ≥85)	Women with non-angiogenic form of PE had a very low risk of adverse outcomes	(55)
Gómez-Arriaga *et al*, 2014	PE	51 singleton pregnancies withearly-onset PE	Mean uterine artery pulsatility index (UtA-PI) and sFlt-1/PlGF ratio in combination with gestational age are useful for the prognostic assessment of perinatal complications at the time of diagnosis of early-onset PE, but not of maternal complications sFlt-1/PlGF ratio >655 is closely related to the need to deliver within 48 h	(56)
Garcia-Tizon Larroca *et al*, 2014	PE	2,140 women that developed PE and 83,615 that were unaffected by PE	Screening by biophysical and biochemical testing at 30-33 weeks could identify most pregnancies developing PE and requiring delivery within the subsequent 4 weeks	(57)
Verlohren *et al*, 2014	PE	234 women with PE and 915 controls	The use of individual two cut-off values, one for EOPE and one for LOPE allows maximized accuracy of diagnosis For EOPE, between 20^{+0} and 33^{+6} weeks of gestation, the cut-offs at ≤33 was negative and at ≥85 was positive for PE/HELLP syndrome with a sensitivity/specificity of 95/94 and 88/99.5%, respectively For LOPE, ≥34 weeks, the cut-offs at ≤33 and ≥110 resulted in lower sensitivity/specificity of 89.6/73.1 and 58.2/95.5%, respectively	(58)
Schoofs *et al*, 2014	PE/IUGR	43 women with PE including nine samples with EOPE <34 weeks, 24 with IUGR and 244 controls	Repeated measurements of the sFlt-1/PlGF ratio along with or in addition to calculating the slope between two measurements seems to be superior inpredicting preeclampsia to a single measurement of the sFlt-1/PlGF ratio alone	(59)

PE, preeclampsia; IUGR, intrauterine growth retardation; EOPE, early-onset PE; LOPE, late-onset preeclampsia; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; HELLP, hemolysis, elevated liver enzymes, and low platelets.

Hirashima *et al* (27) established the reference values for serum sFlt-1, free PlGF and the sFlt-1/PlGF ratio with a 90% confidence interval (90% CI) throughout pregnancy, useful for identifying pregnant women who are at a high risk of developing PE. In normal pregnancies, the serum concentration of sFlt-1 decreased from 8-12 weeks to 16-20 weeks, gradually increased at 26-30 weeks and rapidly increased at 35-39 weeks of gestation, while the serum free PlGF

concentration increased from 8-12 weeks to 26-30 weeks and then decreased at 35-39 weeks of gestation, implying that the cut-off value for PE should be changed according to the gestational period (27). Furthermore, in women with PE, they indicated a decrease in serum free PlGF levels in both the first and the second trimester prior to the onset of PE, and they reported that higher serum sFlt-1 levels after 21 weeks of gestation, but not before 21 weeks, was probably associated with an increased risk of developing PE (27).

Using the newly developed reference values, Ohkuchi *et al* (42) observed that women with EOPE and LOPE exhibited decreased serum levels of PlGF and increased serum levels of sFlt-1 compared to normotensive controls at 28 and 37 weeks, with more pronounced changes in EOPE than in LOPE. In addition, they found that the sFlt-1/PlGF ratios at ~28 weeks of gestation prior to the onset of severe PE were increased in 83% of cases, suggesting its role as s putative marker for the prediction of both early- and late-onset PE (42). Levine *et al* (37) concluded that the sFlt-1/PlGF ratio in women with PE began to increase 2 to 3 months prior to the onset of the disease and was more strongly predictive of PE than were individual biomarkers.

In 2007, Stepan *et al* (43) demonstrated significantly higher serum levels of sFlt1 and lower levels of PlGF in pregnancies with adverse pregnancy outcomes compared with those with normal outcomes, with more noticeable alterations in pregnancies with subsequent PE compared with IUGR and in early-onset diseases (delivery <34 weeks) compared with late-onset diseases. Moreover, they concluded that the concurrent measurement of uterine perfusion with Doppler sonography and angiogenic factors may be a useful tool for the prediction of early-onset pregnancy complications, particularly PE (43).

De Vivo *et al* (44), found that the serum sFlt-1/PlGF ratio increased during gestation in both the PE and control group in both trimesters; however, in the control group, the increase was moderate (51%), while in the PE group, the increase was prominent (285%) due to the significant decrease in serum PlGF levels in the third trimester in this group. Romero *et al* (45) demonstrated that women who delivered an IUGR neonate had changes in the plasma concentration of pro- and anti-angiogenic factors from the first trimester of pregnancy onwards, indicating that differences in their response to intrauterine insults may determine whether a patient will deliver an IUGR neonate, develop PE, or both (45).

All the aforementioned studies relied exclusively on ELISA kits and their results could not be used in clinical practice. The need for a rapid and reliable diagnostic test led to the introduction of automated, commercially available systems for the determination of sFlt-1 and PlGF levels. In 2010, Ohkuchi *et al* (46) introduced automated electrochemiluminescence immunoassay systems; they demonstrated in only 18 min, that the sFlt-1/PlGF ratio had the optimal diagnostic power for both EOPE and LOPE and that a cut-off value of 85 may assist in the diagnosis of PE, particularly for EOPE (46). In addition, Verlohren *et al* (47) evaluated the newly developed automated Elecsys (Roche Diagnostics, GmbH) assay and they confirmed the results of the aformentioned study, demonstrating that the cut-off value of 85 for the sFlt-1/PIGF ratio had a 82% sensitivity and 95% specificity for diagnosing PE. Notably, for EOPE, the same cut-off value had a 89% sensitivity and 97% specificity, indicating the usefulness of this platform for the establishment of a reliable test that could be used as a diagnostic tool in obstetrics. In a subsequent study, Sunderji *et al* (48), using a novel automated immunoassay (Beckman Coulter), revealed that the sFlt-1/PlGF ratio was the optimal biomarker for the separation of normotensive women from those with pre-term PE. They also demonstrated the potential of the markers to differentiate pregnant women with chronic hypertension and PE from those with chronic hypertension only (48).

Chaiworapongsa *et al* (49) demonstrated that the plasma concentrations of angiogenic/anti-angiogenic factors are of prognostic value in the obstetrical triage area in identifying patients with severe PE requiring pre-term delivery within 2 weeks, strengthening their clinical value in obstetrics for better management of at-risk patients. Subsequent studies confirmed the usefulness of the sFlt-1/PlGF ratio measurement in the triage. Rana *et al* (50) indicated that a plasma sFlt1/PlGF ratio >85 at presentation was predictive of adverse outcomes occurring within 2 weeks and that in these women, this marker had better results than other laboratory tests currently used to predict such outcomes. In another study, Moore *et al* (51) demonstrated that an increased serum sFlt1/PlGF ratio was associated with an increased risk of complications among women presenting <37 weeks and that multivariable model combining the sFlt1:PlGF ratio with clinical variables was more predictive of complications than a model using clinical variables alone.

In a following multicenter study, Verlohren *et al* (52) performed serum sFlt-1 and PlGF measurements using the fully automated Elecsys system and they reported significantly elevated sFlt-1/PlGF ratios in patients with PE compared to the controls and to patients with chronic and gestational hypertension at <34 weeks and ≥34 weeks, thus allowing the discrimination between different types of pregnancy-related hypertensive disorders. Moreover, it was shown for the first time that patients with a sFlt-1/PlGF ratio in the highest quartile ($P<0.001$) had a significantly reduced time to delivery. It was noted that particularly in the <34 weeks PE group, the early identification of high risk for delivery women was strongly associated with maternal and fetal morbidity and mortality as and the timely referral to an intensive care unit alone could reduce perinatal morbidity and mortality by 20% (52). Herraiz *et al* (53) observed increased serum sFlt-1/PlGF ratios in cases with fetal growth restriction, PE or HELLP, and PE or HELLP and fetal growth restriction than control pregnancies both <34 weeks and ≥34 weeks of gestation.

In 2014, Chaiworapongsa *et al* (54) demonstrated that the maternal plasma concentrations of angiogenic/anti-angiogenic factors have prognostic value for patients presenting to the obstetrical triage area with suspected PE before 34 weeks of gestation and that these biomarkers allow for the prospective categorization of patients requiring pre-term delivery or who are at risk of adverse maternal and/or neonatal outcomes. Rana *et al* (55) observed that women with a non-angiogenic form of PE (sFlt-1/PlGF ratio <85) had very low risk of adverse outcomes.

Gómez-Arriaga *et al* (56) demonstrated that the mean uterine artery pulsatility index (UtA-PI) and sFlt-1/PlGF

ratio in combination with gestational age were useful for the prognostic assessment of perinatal complications at the time of diagnosis of EOPE, but not of maternal complications. Furthermore, they proposed that a serum sFlt-1/PlGF ratio >655 was closely related to the need to deliver within 48 h (56). Garcia-Tizon Larroca *et al* (57) concluded that screening by biophysical and biochemical testing at 30-33 weeks could identify the majority of pregnancies developing PE and requiring delivery within the subsequent 4 weeks.

In 2014, Verlohren *et al* (58) demonstrated that the use of individual two cut-off values, one for EOPE and one for LOPE allowed for the maximized accuracy of diagnosis. For EOPE, between 20^{+0} and 33^{+6} weeks of gestation, the cut-off value at ≤33 was negative and at ≥85 was positive for PE/HELLP syndrome with a sensitivity/specificity of 95/94% and 88/99.5%, respectively and for LOPE, ≥34 weeks, the cut-off values at ≤33 and ≥110 resulted in a lower sensitivity/specificity of 89.6/73.1 and 58.2/95.5%, respectively. Schoofs *et al* (59) indicated that repeated measurements of the sFlt-1/PlGF ratio along with or in addition to calculating the slope between two measurements appeared to be superior in predicting PE to a single measurement of the sFlt-1/PlGF ratio alone.

4. sFlt-1/PlGF ratio as second and third trimester diagnostic biomarkers for the prediction of PE

Hypertension and proteinuria are currently the classical clinical criteria used to diagnose PE, which however, develop after 20 weeks of pregnancy and their positive predictive value (PPV) for detecting severe adverse maternal and perinatal outcomes was only 20% (60,61). Aspirin administration commenced in early pregnancy before 16 weeks of pregnancy has been proven to reduce the risk of developing PE by ~50% (62). Therefore, the development of an effective screening test to identify women who are at a high risk of developing PE early in pregnancy is of utmost importance; this would prevent pre-term birth, facilitating both maternal and fetal outcomes and decreasing healthcare costs associated with hospitalization. Women who are at a high risk would benefit from often and intensive surveillance, and drug administration for the optimal birth time.

Extensive research has provided evidence that angiogenic factors and their receptors may be used as biomarkers, either alone or in combination with other markers, for predicting PE (Table II). The sFlt-1/PlGF ratio appears to be the optimal predictive tool and several national societies, including the German Society of Obstetrics and Gynecology, the American College of Obstetrics and Gynecology, the National Institute for Care and Health Excellence, the Italian Advisory Board, the Swiss Society for Gynaecology and Obstetrics, have published a guidance regarding PlGF-based diagnostic testing for suspected PE and how clinicians should implement this testing in order to improve patient safety and to deliver benefits to the healthcare system (63-65). The recommended cut-off values for the Elecsys immunoassay sFlt-1/PLGF ratio in these guidelines are of 33, 38, 85 and 110, with 38 and 85 being the mostly used (63-65).

The PRediction of short-term Outcome in preGNant wOmen with Suspected preeclampsIa Study (PROGNOSIS) was the first clinical study designed to demonstrate the utility of the sFlt-1/PlGF ratio in the short-term (up to 4 weeks) prediction of PE using Elecsys immunoassays for sFlt-1 and PlGF (66). PROGNOSIS was conducted between 2010-2013 in 14 countries and demonstated that among pregnant women at <37 weeks of gestation, an sFLT-1:PIGF ratio ≤38 can accurately rule out the likelihood of developing PE in the subsequent week, with a negative predictive value (NPV) of 99.3%, with 80% sensitivity and 78.3% specificity. It was also demonstrated that an sFLT-1:PIGF ratio >38 can accurately predict PE/HELLP within 4 weeks, with a PPV of 36.7%, with 66.2% sensitivity and 83.1% specificity (66,67).

The Preeclampsia Open Study (PreOS) was the first prospective, multicenter study in pregnant women with suspected PE aiming to evaluate the clinical utility of the fully automated Elecsys sFlt-1/PlGF test in the diagnosis of PE and how it influences their clinical management (68). Its results demonstrated that the use of sFlt-1/PlGF ratio influenced clinical decision-making towards appropriate hospitalization in a considerable proportion of women with suspected PE (69).

Perales *et al* (70) performed the Study of Early Pre-eclampsia in Spain (STEPS) study in order to evaluate the sFlt-1/PlGF ratio at 20, 24 and 28 weeks as a predictive marker for EOPE (<34 weeks). They found that the sFlt-1/PlGF ratio was significantly increased in women with EOPE compared to those with LOPE and the controls, and they developed a prediction model for EOPE combining sFlt-1/PlGF ratio with considerably increased specificity and sensitivity compared with using UtA-PI or sFlt-1/PlGF ratio alone (70).

Herraiz *et al* (71) designed a study to analyze the usefulness of the sFlt-1/PlGF ratio measurement at 24-28 weeks for the prediction of early (requiring delivery <32 weeks), intermediate (delivery at 32 to <36 weeks) and late (delivery ≥36 weeks) PE/IUGR. Their results demonstrated the sFlt1/PlGF ratio measurement was useful in previously selected women to predict mostly early PE/IUGR, with optimal diagnostic accuracy for values >95th centile as the cut-off (71).

Sabriá *et al* (72) evaluated the effectiveness of N-terminal pro-B natriuretic peptide (NT-proBNP), which is released from cardiac myocytes in response to myocardial stretch or ischemia and is increased in women with PE, uric acid and the sFlt-1/PlGF ratio >38 for the prediction of delivery within 1 week. They observed that the addition of NT-proBNP assessment yields superior results for the prediction of delivery with PE in the subsequent week compared with the use of the sFlt-1/PlGF ratio alone (72). Lafuente-Ganuza *et al* (73) conducted a study for identifying and validating cut-off values for the sFlt-1/PlGF ratio and NT-proBNP predictive model of EOPE and they demonstrated that two sFlt-1/PlGF ratio cut-off values of 23 and 45 can rule out and rule in EOPE at any time between 24 and 33^{+6} weeks of gestation.

In the Pregnancy Outcome Prediction (POP) study, Sovio *et al* (74) demonstrated that an sFlt-1:PlGF ratio >38 at 28 weeks had a 32% PPV for PE and pre-term birth, with a similar PPV in both high- and low-risk women and at 36 weeks, it had 20% PPV for severe PE in high-risk women and 6.4% in low-risk women. At 36 weeks, an sFlt-1/PlGF ratio >110 had a PPV of 30% for severe PE, and the PPV was similar comparing low- and high-risk women (74). Among low-risk women at 36 weeks, an sFlt-1/PlGF ratio ≤38 had a NPV value for severe PE of 99.2%, indicating that the sFlt-1/PlGF

Table II. sFlt-1/PlGF ratio as second and third trimester diagnostic biomarkers for the prediction of PE.

Authors/year of publication	Patient population/sample size	Findings	(Refs.)
Hund *et al*, 2014; Zeisler *et al*, 2016	PROGNOSIS study with 1,050 pregnant women (24 weeks 0 days to 36 weeks 6 days of gestation)	Among women at <37 weeks, an sFLT-1:PIGF ratio ≤38 can accurately rule out the risk of developing PE in the subsequent week, with a NPV of 99.3% (95% CI, 97.0-99.9), with 80% sensitivity and 78.3% specificity An sFLT-1:PIGF ratio >38 can accurately predict PE/HELLP within 4 weeks, with a PPV of 36.7, with 66.2% sensitivity and 83.1% specificity	(66,67)
Hund *et al*, 2015; Klein *et al*, 2016	192 women with a gestational age of ≥24 weeks with suspicion of PE	The use of sFlt-1/PlGF ratio influenced clinical decision making towards appropriate hospitalization in a considerable proportion of women with suspected PE	(68,69)
Perales *et al*, 2017	729 women at risk for PE at <34weeks	The sFlt-1/PlGF ratio was significantly increased in women with EOPE compared to LOPE and controls	(70)
Herraiz *et al*, 2018	5,601 consecutive singleton pregnancies (4.3% women were selected for intensive monitoring by combining maternal history and second trimester uterine artery Doppler data)	The sFlt1/PlGF ratio measurement is useful in previously selected women to predict mostly early PE/IUGR, with optimal diagnostic accuracy for values >95th centile as the cut-off	(71)
Sabriá *et al*, 2018	495 women (24^{+0} to 36^{+6} weeks of gestation with clinically suspected PE and evaluated the effectiveness of NT-proBNP	The addition of NT-proBNP assessment yields superior results for the prediction of delivery with PE in the subsequent week compared with the use of sFlt-1/PlGF ratio alone	(72)
Lafuente-Ganuza *et al*, 2020	309 women in the development phase and 276 in the validation phase between 24 to $33^{=6}$ weeks of gestation with suspected PE	Two sFlt-1/PlGF ratio cut-off values of 23 and 45 can rule out and rule in EOPE at any time between 24 and 33^{+6} weeks of gestation	(73)
Sovio *et al*, 2015	4,099 nulliparous women at 20, 28, and 36 weeks of gestation	An sFlt-1:PlGF ratio >38 at 28 weeks had a 32% PPV for PE and preterm birth, with a similar PPV in both high- and low-risk women and at 36 weeks, it had 20% PPV for severe PE in high-risk women and 6.4% in low-risk women At 36 weeks, an sFlt-1/PlGF ratio >110 had a PPV of 30% for severe preeclampsia, and for low- and high-risk women Among low-risk women at 36 weeks, an sFlt-1/PlGF ratio ≤38 had a NPV value for severe PE of 99.2%	(74)
Zeisler *et al*, 2019	Exploratory post-hoc analysis of data from the PROGNOSIS study	A sFlt-1/PlGF ratio ≤38 can rule out the onset of PE for up to 4 weeks in women with suspected PE with a NPV of 94.3% (95% CI, 91.7-96.3%) and that repeat testing of the sFlt-1/PlGF ratio in these women could further elucidate the risk of developing PE	(75)
Bian *et al*, 2019	PROGNOSIS Asia study with 764 pregnant Asian women [20^{+0} (18^{+0} days in Japan) to 36^{+6} weeks of gestation]	The NPV for ruling out preeclampsia within 1 week using an sFlt-1/PlGF ratio of ≤38 was 98.6% (95% CI, 97.2-99.4%), with 76.5% sensitivity and 82.1% specificity and the PPV ofa sFlt-1/PlGF ratio >38 for ruling in preeclampsia within 4 weeks was 30.3% (95% CI,23.0-38.5%), with 62.0% sensitivity and 83.9% specificity	(76)

Table II. Continued.

Authors/year of publication	Patient population/sample size	Findings	(Refs.)
Cerdeira *et al*, 2019	370 women between 24^{+0} and 37^{+0} weeks of gestation with suspected PE	A sensitivity of 100% and a NPV of 100% compared with a sensitivity of 83.3% and NPV of 97.8% with clinical practice alone	(77)
Cerdeira *et al*, 2022	Post hoc analysis of the INSPIRE trial	The sFlt-1/PlGF-ratio at the cut-off of 85 predicts preeclampsia within 4 weeks with a PPV of 71.4%	(78)
Perry *et al*, 2020	302 pregnant women with hypertension >20 weeks of gestation	sFlt-1/PIGF ratio could predict a PE-related delivery within 1 and 2 weeks, particularly in gestational ages <35 weeks	(79)
Dröge *et al*, 2021	1,117 women (>20^{+0} weeks of gestation) with symptoms of PE	The addition of sFlt-1/PlGF ratio to a multi-marker model including maternal characteristics and routine clinical examination improves the predictive validity	(64)
Peguero *et al*, 2021	86 women with confirmed EOPE (<34 weeks)	Longitudinal changes in maternal angiogenic factors levels improve the prediction capacity for EO-PE of adverse outcome and time interval to delivery	(80)
Dathan-Stumpf *et al*, 2022	283 singleton pregnancies with suspected PE	Positive associatino between the sFlt-1/PlGF ratio and severity of placental dysfunction and a negative association with time to delivery	(81)
Hughes *et al*, 2023	222 women (20^{+0} and 36^{+6} weeks gestation) from New Zealand	In participants < 37 weeks, an sFlt-1/PlGF ratio ≤38 ruled out PE in the subsequent week with a NPV of 96.2% (95% CI, 92.3-98.2) and ruled in PE within 4 weeks with a PPV of 75% (95% CI, 65.0-82.9)	(65)
Kifle *et al*, 2022	Pregnant women between 24^{+0} to 37^{+0} weeks of gestation with PE clinical suspicion	Models using continuous values of sFlt-1 only or sFlt1/PIGF ratio had better predictive performance compared to a PIGF only or the model with sFlt-1/PIGF ratio as a cut-off at 38	(82)

PE, preeclampsia; IUGR, intrauterine growth retardation; PROGNOSIS, PRediction of short-term Outcome in preGNant wOmen with Suspected preeclampsIa Study; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; HELLP, hemolysis, elevated liver enzymes, and low platelets; LOPE, late-onset preeclampsia; PPV, positive predictive value; NT-proBNP, N-terminal pro-B natriuretic peptide

ratio provides clinically useful prediction of the risk of the most important manifestations of preeclampsia in a cohort of unselected nulliparous women (74).

An exploratory post hoc analysis of data from the PROGNOSIS study by Zeisler *et al* (75) demonstrated that a sFlt-1/PlGF ratio ≤38 can rule out the onset of PE for up to 4 weeks in women with suspected PE (24^{+0} to 36^{+6} weeks' gestation) with a NPV of 94.3% and that repeat testing of the sFlt-1/PlGF ratio in these women could further elucidate the risk of developing PE.

PROGNOSIS Asia was a prospective, multicenter study conducted at 25 sites in Asia designed to investigate the value of the sFlt-/PlGF ratio for predicting adverse outcomes (76). The NPV for ruling out preeclampsia within 1 week using an sFlt-1/PlGF ratio of ≤38 was 98.6%, with 76.5% sensitivity and 82.1% specificity and the PPV of a >38 sFlt-1/PlGF ratio for ruling in preeclampsia within 4 weeks was 30.3% with 62.0% sensitivity and 83.9% specificity (76).

Cerdeira *et al* (77) performed the Interventional Study Evaluating the Short-Term Prediction of Preeclampsia/Eclampsia In Pregnant Women With Suspected Preeclampsia (INSPIRE) study, the first randomized clinical trial for assessing the use of angiogenic biomarkers sFlt-1/PlGF using a ratio cut-off of 38. They yielded a sensitivity of 100% and a NPV of 100% compared with a sensitivity of 83.3% and NPV of 97.8% with clinical practice alone, indicating that the sFlt-1/PlGF ratio in combination with standard clinical practice both identifies and leads to correct admission of women with increased risks of preeclampsia without changing the admission rate (77). Cerdeira *et al* (78) performed a post hoc analysis of the INSPIRE trial and their finding that the sFlt-1/PlGF-ratio at the cut-off of 85 predicts PE within 4 weeks with a PPV of 71.4% confirmed the predictive utility of this cutoff and suggested that combining this cut-off of 85 with the rule out cut-off of 38 could improve the management of patients with suspected PE.

Table III. sFlt-1/PlGF ratio in first trimester prediction models for PE.

Authors, year of publication	Patient population/ sample size	Findings	(Refs.)
Crovetto *et al*, 2014	5,759 women (28 cases of early PE, 84 cases of late PE, 84 controls)	For early PE, the prediction model observed 77.8 and 88.9% detection rates for 5 and 10% FPR, respectively, and for late PE, the detection rates were 51.2 and 69% at 5 and 10% FPR, respectively (AUC, 0.888; 95% CI, 0.840-0.936)	(84)
Crovetto *et al*, 2015	9,462 pregnant women undergoing routine pregnancy care	For early PE, the prediction model observed 87.7 and 91.2% detection rates for 5% and 10% FPR, respectively and for late PE, the detection rates were 68.3 and 76.4% at 5 and 10% of FPR, respectively	(85)
Lamain-de Ruite *et al*, 2019	External validation of the above studies in a Dutch study including 3,736 women with 87 (2.3%) affected by preeclampsia	Showed suboptimal calibration and discrimination for PE	(86)
Tsiakkas *et al*, 2016	7,066 cases of PE at 11-13 weeks, 8,079 cases at 19-24 weeks, 8,472 at 30-34 weeks and 4,043 at 35-37 weeks	Confirmed the superior performance for the detection of early, compared to late, PE and improvement with advancing gestational age at screening. The integration of sFlt-1 measurement at 11-13 weeks did not improve the prediction of PE achieved by maternal factors alone	(87)
Diguisto *et al*, 2017	226 women with a high risk of PE	The optimal prediction model was the combination of UAD and serum PlGF, while the combination of UAD and sFlt-1 did not significantly improve the prediction of preeclampsia or other outcomes compared with serum PlGF alone	(88)

PE, preeclampsia; FPR, false-positive rate; sFlt-1, soluble fms-like tyrosine kinase 1; UAD, uterine artery Doppler; PlGF, placental growth factor.

Perry *et al* (79) demonstrated that the combination of the sFlt-1/PIGF ratio and maternal characteristics could predict a PE-related delivery within 1 and 2 weeks, particularly in gestational ages <35 weeks, and they emphasized the superior performance of a continuous scale of sFlt-1/PlGF ratio in the model.

Dröge *et al* (64) performed the first retrospective real-world study in order to evaluate the clinical use of serum sFlt-1/PlGF ratio cut-off values of 38 and 85 alone or integrating into a multi-marker model for the prediction of adverse maternal of fetal outcomes. They observed that the addition of sFlt-1/PlGF ratio to a multi-marker model including maternal characteristics and routine clinical examination improved the predictive validity (64).

Peguero *et al* (80) measured the levels of PlGF, sFlt-1 and the sFlt-1/PlGF ratio from admission and before delivery at fixed time points, and demonstrated that longitudinal changes in maternal angiogenic factors levels improved the predictive capacity for EOPE with adverse outcomes and time interval to delivery.

Dathan-Stumpf *et al* (81) in a real-world study with sFlt-1/PlGF ratio measurements at admission and follow-up measurements before delivery and confirming previous studies they observed a positive correlation between the sFlt-1/PlGF ratio and severity of placental dysfunction and a negative association with time to delivery.

Hughes *et al* (65) evaluated the sFlt-1/PlGF value for predicting PE and they demonstrated that in participants at <37 weeks of gestation, an sFlt-1/PlGF ratio ≤38 ruled out PE in the subsequent week with a NPV of 96.2% and ruled in PE within 4 weeks with a PPV of 75%; these results were comparable to those reported in international trials, indicating the predictive value of the sFlt-1/PlGF ratio in PE and emphasizing its incorporation into national guidelines.

Kifle *et al* (82) designed a secondary analysis of INSPIRE trial in order to compare the prognostic utility of models using the continuous values of sFlt-1, PIGF, sFlt-1/PIGF ratio or sFlt-1/PIGF ratio as a cut-off at 38 for predicting PE within 7 days of screening among women with suspected PE. They observed that models using continuous values of sFlt-1 only

or sFlt-1/PIGF ratio had a better predictive performance compared to a PIGF only or the model with sFlt-1/PIGF ratio as a cut-off at 38 (82).

5. sFlt-1/PlGF ratio in first trimester prediction models for PE

Considerable efforts have been made to develop first trimester prediction models for PE, which need to be evaluated and undergo external validation in an independent population with different demographics and geographic settings than those of the original models (Table III) (83). Thus far, the Fetal Medicine Foundation (FMF) first trimester prediction model (namely the triple test), which combines maternal factors, biophysical parameters (MAP and UtA-PI) and serum pregnancy-associated plasma protein A (PAPP-A) has undergone successful internal and external validation (83). The FMF triple test has detection rates of 90 and 75% for the prediction of early and pre-term PE, respectively, with a 10% false-positive rate (FPR) (83).

Crovetto *et al* (84) explored the independent and combined integration of VEGF, PlGF, sFlt-1 along with maternal characteristics, biophysical parameters and biochemical measurements in first trimester predictive models of early and late PE. For early PE, the model achieved 77.8 and 88.9% detection rates for 5 and 10% FPR, respectively, and for late PE, the detection rates were 51.2 and 69% at 5 and 10% FPR, respectively (area under the curve, 0.888; 95% CI, 0.840-0.936) (84).

In 2015, the same scientific group (85) performed a study aiming to confirm, in a substantially larger sample size, their previous results and to develop the optimal first trimester screening model for PE based on the combination of maternal characteristics, biophysical parameters and biochemical markers, including PAPP-A, PlGF and sFlt1 in a low-risk population. The optimal model for early PE achieved 87.7 and 91.2% detection rates for 5 and 10% FPR, respectively and for late PE, the detection rates were 68.3 and 76.4% at 5 and 10% of FPR, respectively, indicating that the inclusion of angiogenic factors in existing predicting models for PE can substantially improve their detection rate with high accuracy in general low-risk obstetric populations (85). However, the aforementioned models had undergone external validation in a Dutch study including 3,736 women with 87 (2.3%) affected by PE and suboptimal calibration and discrimination for PE was observed (86).

Tsiakkas *et al* (87) examined the combined screening with maternal factors, medical history and serum sFlt-1 and their results confirmed the superior performance for the detection of early, compared to late, PE and improvement with advancing gestational age at screening. Moreover, they demonstrated that the integration of sFlt-1 measurement at 110-13 weeks did not improve the prediction of PE achieved by maternal factors alone (87).

Diguisto *et al* (88) examined whether first-trimester Uterine artery Doppler (UAD) combined with angiogenic markers could help to predict PE and other adverse outcomes. They found that the optimal prediction model was the combination of UAD and serum PlGF, while the combination of UAD and sFlt-1 did not significantly improve the prediction of PE or other outcomes compared with serum PlGF alone, confirming the poor performance of sFlt-1 in first-trimester screening of PE (88).

Recently, Verlohren *et al* (89) published an article following a meeting of international experts with the aim of providing clinicians guidance for the use of sFlt-1/PlGF ratio in the management of women with PE and improving clinical care, as well as suggestions for further research on their clinical utility in various circumstances.

6. Conclusions and future perspectives

The present review summarized the role of the sFlt-1/PlGF ratio in the prediction and diagnosis of PE. The sFlt-1/PlGF ratio represents an additional and advanced diagnostic tool for PE, independent of blood pressure or laboratory markers of HELLP syndrome, to identify patients who develop PE or develop severe PE requiring pre-term birth. Estimated maternal/fetal complications are highly desirable and are urgently required. Furthermore, the economic impact of the routine clinical use of the sFlt-1/PlGF ratio has been demonstrated in a number of studies, as the sFlt-1/PlGF ratio is easy to be measured and its use results in shorter hospital stays (90). The use of highly specific tests, such as sFlt1 and PlGF, risk stratification and the management of patients with suspected PE will reduce unnecessary investigations, introductions and even pre-term births and at the same time, will provide better focus on patients who are at an increased risk of adverse outcomes. In addition, there is a double benefit: Tailor resources to women at highest risk, while minimizing overestimation and intervention for women at lower risk. It is therefore a useful tool for individual risk stratification and further studies/larger trials are warranted in order to improve its clinical applicability and to provide guidance for its global use in order to obtain a better homogeneous clinical management of women with PE.

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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