The Promise of Angiogenic Markers for the Early Diagnosis and Prediction of Preeclampsia

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BACKGROUND: An imbalance in circulating factors that regulate blood vessel formation and health, referred to as angiogenic factors, plays a central role in the pathogenesis of preeclampsia.

CONTENT: Several studies have demonstrated a strong association between altered circulating angiogenic factors and preeclampsia. These factors include circulating antiangiogenic proteins such as soluble fms-like tyrosine kinase 1 and soluble endoglin and proangiogenic protein, placental growth factor. Abnormalities in these circulating angiogenic factors are not only present during clinical disease, but also antedate clinical signs and symptoms by several weeks. These alterations are particularly prominent in patients who present with preeclamptic signs and symptoms prematurely and/or in patients with severe preeclampsia. The availability of automated platforms for the rapid measurement of circulating angiogenic proteins in blood samples has now allowed researchers and clinicians to evaluate the utility of these assays in the diagnosis of the disease, in the stratification of patients in clinical trials, or in the monitoring of therapies. In this review we highlight the various studies that have been performed, with a focus on large validation studies.

SUMMARY: Measurement of circulating angiogenic proteins for the diagnosis and prediction of preeclampsia is still at an early stage but is rapidly evolving. Standardization across the various automated platforms and prospective studies that demonstrate clinical utility are needed.

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Preeclampsia is a serious and potentially life-threatening condition complicating 3%–5% of all pregnancies worldwide (1–3). Preeclampsia is characterized by sustained new-onset hypertension and proteinuria typically developing after 20 weeks of gestation. The disorder is associated with significant morbidity and mortality in both mother and fetus (4). Even today, preeclampsia is still a leading cause of maternal mortality, with an estimate of >60,000 maternal deaths per year (5). In more developed countries, pregnancies are terminated early by caesarean delivery. Therefore, the burden of the disease rests primarily on the fetus with prematurity, low birth weight, respiratory distress syndrome, and other complications of early delivery. After preeclampsia in pregnancy, women are characterized by an increased risk of cardiovascular and renal disease in future years (6, 7).

Diagnosis of preeclampsia remains a challenge because it relies on nonspecific signs of the disease, such as progressive proteinuria and hypertension. In particular, the diagnostic value of these 2 classic features is limited and not useful when women have preexisting hypertension and/or proteinuria (e.g., chronic renal disease). Recent exciting work identified novel soluble angiogenic factors that are related to the pathogenesis of the disease (8). In this review we summarize the role of circulating angiogenic proteins in the diagnosis and prediction of preeclampsia.

Angiogenic Factors in the Pathogenesis of Preeclampsia

Evidence suggests that preeclampsia is primarily an endothelial disease (9) in which endothelial cells lose their typical flat morphology and function. As a result, the endothelial cell layer becomes leaky. This loss of morphology and function is most evident in the glomerular endothelium, which shows the classic histological finding of endothelial cell swelling, called endotheliosis, and microvascular obstruction (10). As a consequence of endothelial dysfunction, patients develop hypertension, proteinuria, and progressive edema. Intriguingly, these same symptoms and morphologic changes of endothelial cells are seen in cancer patients treated with vascular endothelial growth factor

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VEGF antagonists, which suggests that endothelial damage in preeclampsia may be the result of altered VEGF signaling in endothelial cells (11–13).

Several years ago, a number of reported studies demonstrated increased concentrations of the soluble fms-like tyrosine kinase-1 (sFlt-1) (or sVEGFR1), a splice variant of VEGF-receptor 1 in the serum/plasma of preeclamptic patients (14–17). The sFlt-1 protein is a truncated form of the VEGF receptor Flt-1, lacking the transmembrane and the intracytoplasmic domains and therefore secreted extracellularly (18). sFlt-1 is produced in the placenta and secreted into the bloodstream, where it is thought to bind and neutralize VEGF, and placental growth factor (PIGF), with high affinity (19). VEGF and PIGF bound to sFlt-1 are no longer available to their innate receptors on endothelial cells, and VEGF signaling is disrupted (Fig. 1). In preeclampsia, sFlt-1 concentrations are increased, whereas free concentrations of PIGF and VEGF are decreased. This angiogenic imbalance correlates with severity of signs and symptoms of preeclampsia (14, 20). After delivery of the placenta, sFlt-1 levels rapidly decrease within 48 h. The cardinal manifestations of human preeclampsia can also be generated in gravid rats, mice, and baboons by experimental manipulations to over-

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**Nonstandard abbreviations:** VEGF, vascular endothelial growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; PIGF, placental growth factor; sEng, soluble endoglin.
express sFlt-1 (14, 21–25). In humans, abnormalities in these circulating angiogenic proteins are not only present during clinical preeclampsia, but also antedate clinical symptoms by at least 5–6 weeks (26–28).

Soluble endoglin (sEng), another antiangiogenic protein, has also been implicated in the pathogenesis of preeclampsia (29). Acting as an antiangiogenic protein, sEng disrupts transforming growth factor-β signaling in the vasculature. Overexpression of sFlt1 and sEng through an adenoviral expression system in rats led to a severe preeclampsia-like phenotype with features of HELLP (H: hemolysis (the breakdown of red blood cells); E: elevated liver enzymes; L: low platelet count) syndrome and fetal growth restriction (29). In human pregnancy, alterations in sEng antedated clinical symptoms of preeclampsia by several months (30). Alterations in sFlt1, PlGF, and sEng are more pronounced in patients with severe preeclampsia and eclampsia (31). These findings support the theory that preeclampsia and its complications may be due to an imbalance in circulating angiogenic factors (8, 32).

Angiogenic Factors in the Prediction of Preeclampsia

There is a large body of literature demonstrating that maternal serum concentrations of sFlt-1 and PlGF correlate with preeclampsia disease activity and are increased well before the onset of clinical signs and symptoms (16, 28, 33, 34). In a cross-sectional nested case control study, Levine et al. compared gestational age-matched women with active preeclampsia and those with a normal pregnancy and revealed that concentrations of sFlt-1 were significantly higher in the former group (28). They also showed that concentrations of this antiangiogenic protein were significantly increased 5–6 weeks before the detection of hypertension and proteinuria (28, 30). More recently, other investigators have evaluated sFlt-1 concentrations longitudinally throughout gestation in women with normal pregnancies and those with preeclampsia and noted that sFlt-1 concentrations seemed to be increased throughout gestation in women destined to develop preeclampsia, a significant difference usually detectable 5–6 weeks before disease presentation (35). In addition, circulating concentrations of sFlt-1 were found to be increased in conjunction with decreased free PlGF in the bloodstream at the time of disease presentation. Increased sFlt-1 and depressed PlGF are more dramatically altered in preterm preeclampsia and/or in preeclampsia complicated by fetal growth restriction (30, 36). Serum concentrations of PlGF tend to be lower in women who go on to develop preeclampsia from the first or early second trimester (33, 37). The sFlt-1/PlGF ratio has been proposed as an index of antiangiogenic activity that reflects alterations in both biomarkers and is a better predictor of preeclampsia than either measure alone (30, 35, 38) (Fig. 2). A number of studies estimated the predictive value of sFlt-1 and PlGF as well as other angiogenic factors such as sEng and transforming growth factor-β1 during the second and third trimester as single markers. Values for diagnostic sensitivity and specificity ranged between 80% and 90% (27, 39); however, these are likely overestimated. More recent studies (40, 41) have demonstrated that sFlt-1 measurement alone gives diagnostic specificity and sensitivity of up to approximately 80% and approximately 70%, respectively, for the prediction of preeclampsia during the second trimester. Examining the ratio of sFlt-1 (rising in preeclampsia) and PlGF (falling in preeclampsia) improved prediction of the condition remarkably (40–42). Free VEGF concentrations are not useful for clinical purposes because the circulating concentrations are below the detection limit of most commercially available ELISA kits during pregnancy.

Another promising strategy is urine screening with a PlGF assay followed by blood confirmation with sFlt-1/PlGF ratio (38). In the absence of glomerular damage, sFlt-1 is too large a molecule to be filtered into the urine, whereas PlGF is readily filtered and can thus be measured and used as a potential predictive test. In a nested case-control study by Levine et al., urinary PlGF was measured in 120 normotensive controls and women who subsequently developed preeclampsia (38). This study revealed that a low concentration of PlGF in the urine at midgestation was strongly associated with the subsequent development of preterm preeclampsia. The adjusted odds ratio for the risk of developing preterm preeclampsia in those women who had low urinary PlGF concentrations (<118 pg/mL) at 21–32 weeks was 22.5.

Data on maternal first-trimester circulating concentrations of sFlt-1 are inconsistent. Several studies showed increased concentrations of sFlt-1 during the first trimester (43, 44), whereas the majority of studies did not find increased concentrations (33, 45). In general, it has been shown that testing for placental disease, including preeclampsia before the second trimester, is of limited use (46). The intended benefit of early identification of patients at risk is overrun by the low precision of the test currently available in first-trimester screening. However, because PlGF alterations occur early in the first trimester (33), PlGF has been tested alone and in combination with other biomarkers as a potential predictive test. In a large prospective clinical study involving nearly 8000 women, Poon et al. demonstrated that a combination of angiogenic factors (PlGF), pregnancy-associated plasma protein A, and uterine artery Doppler velocimetry in the first trimester can predict the subsequent development of early
onset preeclampsia in a low-risk population with a diagnostic sensitivity of 93% at a 5% false-positive rate (47). This finding suggests that screening for early onset preeclampsia in the general population is possible and that 1 in 5 pregnant women with positive screening results will develop preeclampsia. More prospective studies are needed to confirm this observation.

More recently, higher concentrations of sEng at 13–20 weeks and at 21–32 weeks were reported be associated with subsequent preterm preeclampsia.
Interestingly, a composite measure incorporating sFlt-1, PlGF, and sEng was more predictive of preterm preeclampsia than the individual biomarkers alone (30). Women with isolated SGA (small for gestational age) pregnancies were also characterized by early sustained increases in sEng (30, 36), suggesting that sEng may be a marker for placental insufficiency.

Because circulating concentrations of angiogenic factors change with gestational age, it has been proposed that sequential changes in concentrations of sFlt-1, PlGF, and sEng could be more informative in assessing the risk for preeclampsia than are time-point measurements. Rana et al. and Vatten et al. reported that sequential changes in angiogenic factors from the first to the second trimester differ in women destined to develop preeclampsia (45, 48). A small increase in PlGF and a high increase in sFlt-1 were strong predictors of preeclampsia, and the risks were higher for sequential change than for each measurement alone. Interestingly, the combination of the lowest quartile of PlGF change and the highest quartile of sFlt-1 change was associated with an OR of 35.3 (95% CI 7.6 – 164.2) for preterm preeclampsia, and an OR of 3.2 (95% CI 1.4 – 7.0) for term preeclampsia. Recently, Kusanovic et al. reported a remarkable performance of PlGF/sEng ratio, from early pregnancy and midtrimester, with a positive likelihood ratios of 55.6 (95% CI 36.4 – 55.6) and 89.6 (95% CI 56.4 – 89.6), respectively, for predicting early onset preeclampsia. Overall, the diagnostic accuracy of the PlGF/sEng ratio was better than that of any individual factors (49).

Moore Simas et al., using linear mixed-modeling to compare gestational change of angiogenic factors, calculated the rate of rise of serum sFlt-1 for individual patients and illustrated differences in intercept and slope between healthy pregnancy and early and late-onset preeclampsia (50). As for all screening tests the positive predictive value of angiogenic factor screening depends on the population tested. Higher positive predictive values can be achieved by testing a population at risk. Risk factors screened included pregestational diabetes mellitus, chronic hypertension, chronic kidney disease, maternal age 18 years or younger, obesity, systemic lupus erythematosus, antiphospholipid syndrome, and prior history of preeclampsia (50). With this approach, Moore Simas et al. studied the high-risk population and achieved excellent diagnostic accuracy, calculating areas under the ROC curve for the sFlt-1/PlGF ratio as 0.97 and for sFlt-1 as 0.90 for the prediction of early onset preeclampsia. Perni et al. demonstrated in a longitudinal study of women with chronic hypertension that alterations in sFlt-1, PlGF, sFlt-1/PlGF, and sEng were dramatically altered as early as 20 weeks of gestation in women who developed subsequent early onset preeclampsia (51). Results have been more modest in other studies using angiogenic markers in high-risk populations (52, 53). However, in the high-risk population the diagnosis of preeclampsia may not be straightforward, and therefore it may be important to study the utility of these biomarkers in the context of preeclampsia-related adverse maternal and fetal outcomes.

Several recent prospective studies investigated the use of angiogenic factors in a high-risk group identified by abnormal uterine artery Dopplers. The combination of early second-trimester ultrasound (including measurements of pulsatility index) with determination of angiogenic factors, sFlt-1 and PlGF, largely improved the predictive value of ultrasound screening alone (54 – 58). According to current data, the combination of Doppler ultrasound measurements with laboratory testing for angiogenic factors is a strong tool for the prediction of preterm preeclampsia. Unfortunately, metaanalyses of these trials are limited owing to studies with limited sample size for early onset preeclampsia, heterogeneity in gestational age at blood sampling, and ultrasound examination.

**Angiogenic Factors in the Diagnosis of Preeclampsia**

In addition to being useful in the prediction of preeclampsia before the onset of clinical symptoms, angiogenic factors may also prove useful in diagnosing the disease and in distinguishing it from other hypertensive disorders of pregnancy, such as gestational hypertension and chronic hypertension. The clinical utility of serum concentrations of angiogenic proteins in differentiating among hypertensive disorders of pregnancy has been evaluated. The diagnostic sensitivity and specificity in differentiating preeclampsia from gestational and chronic hypertension were 84% and 95%, respectively, for sFlt-1 (59). Clinically, sFlt-1 concentrations have been observed to be directly proportional to severity of proteinuria, but inversely correlated with platelet count, gestational age, and neonatal birth weight adjusted for gestational age (60, 61). In women with preeclampsia, concentrations of sFlt-1 are higher in those with early onset (<37 weeks), more severe disease (14, 28, 30, 62), and SGA (small for gestational age) neonates (28, 30, 60). Urinary angiogenic markers have also been reported to be useful in the diagnosis of preeclampsia (63).

Wikström et al. found that alteration in plasma levels of angiogenic factors are more pronounced in early preeclampsia during clinical disease compared to late-onset preeclampsia (62). In this study the authors evaluated alterations of angiogenic factor plasma concentrations in patients with early and late-onset preeclampsia compared to controls. They demonstrate 43
times higher median plasma concentrations of sFlt-1 in early onset compared to a 3-fold increase in late-onset preeclampsia and 21 times lower median plasma concentrations of PlGF in early onset disease compared to a 5-fold decrease in late-onset disease (62). This correlation between more pronounced alterations in angiogenic-factor plasma concentrations with early vs late-onset preeclampsia were confirmed in several prospective studies (64–68). In a cross-sectional study, Tripathi et al. additionally estimated the diagnostic sensitivity and specificity of sFlt-1 plasma concentrations in differentiating between normal pregnancy and early onset preeclampsia as well as between normal pregnancy and late-onset preeclampsia. Consistent with previous findings, sFlt-1 measurements showed 89% diagnostic sensitivity and 90% specificity in early preeclampsia compared to 55% diagnostic sensitivity and 58% specificity in late preeclampsia. This observation supports the hypothesis that in early onset preeclampsia placental pathology predominates, whereas late preeclampsia is predominantly maternal disease or disturbed maternal response to (unknown) factors and underlines the role of angiogenic factors in the diagnosis of early onset preeclampsia. Taken together, these data indicate that sFlt-1 and/or the sFlt-1/PlGF ratio may be ideal laboratory parameters to diagnose early onset preeclampsia (preeclampsia at <34 weeks) or preterm preeclampsia (preeclampsia at <37 weeks).

Several recent studies have evaluated the use of automated assays for the measurement of the various angiogenic factor analytes and found them to be useful for routine diagnosis of preeclampsia, particularly in women who presented early, at <37 weeks (42, 69–73). Current assessment relies on detection of angiogenic factors by manual ELISA, which is suitable for research purposes but not for widespread use in the clinical setting. Automated tests would allow a fast and easy-to-implement assessment of angiogenic factors in the routine clinical context. However, standardization of these various analytes across the different platforms is needed before the impact of these markers in clinical practice can be investigated.

**Clinical Relevance of Preeclampsia Prediction and Diagnosis**

Although it has been shown that altered angiogenic factors are strongly associated with the development of preeclampsia (74), clinical applications for these factors have been debated. Patients who have a positive angiogenic factor test early in pregnancy may be referred to high-risk specialists and screened more intensively to determine the onset of clinical preeclampsia. Early risk stratification would enable risk-adapted management of pregnancy. Selection of the proper place for delivery and early referral to a tertiary perinatal center in case of a high risk of early preeclampsia and subsequent premature delivery may reduce neonatal morbidity as well as mortality (75–77).

Identification of patients at risk for subsequent preeclampsia could also be of tremendous utility in prevention trials (such as with L-arginine) to limit the exposure of all pregnant women to potentially toxic medications. A recent metaanalysis demonstrated that aspirin has a “moderate but consistent” effect of decreasing the likelihood of a preeclamptic complication (68). Because the impact of aspirin can be observed only when administration begins as early as possible (ideally before the second trimester), angiogenic factors may help to identify those women who may have the greatest benefit from aspirin. Clinical therapeutic trials targeting sFlt-1 will also greatly benefit from the ability to use these angiogenic biomarkers to assist in recruitment of study participants, determination of dosage, and monitoring of therapies such as the use of statins or dextran sulfate apheresis in preterm preeclampsia (79, 80).

It has also been shown that the sFlt-1/PlGF ratio is particularly useful for differentiating the various hypertensive disorders of pregnancy (81, 82). In clinical settings this tool can be used to confirm or exclude preeclampsia in women with suspected preeclampsia who may present with atypical features. Early confirmation of preeclampsia is particularly useful in patients who present very prematurely, in whom accurate diagnosis is critical for management. In this regard, 2 recent studies demonstrated not only that the measurement of angiogenic factors is useful in the triage setting for diagnosing preeclampsia, but also that it may be useful for the identification of patients at risk for adverse outcomes and preterm delivery (83, 84).

**Conclusions**

Screening for preeclampsia before the onset of clinical disease is important for identifying at-risk patients who might benefit from close follow-up and, potentially, treatment. There is evidence that altered placentation and subsequent placental ischemia plays a critical role in disease development. Whether release of antiangiogenic factors is a cause or effect of this placental process remains unclear. Altered placentation occurs early in the first trimester. At this stage of pregnancy, however, serum concentrations of antiangiogenic factors do not show significant differences in healthy pregnant women compared to those who later develop preeclampsia. In contrast, low PlGF in the first trimester in combination with uterine artery Doppler abnormalities may be partic-
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References


