Angiogenic factors: potential to change clinical practice in pre-eclampsia?

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Pre-eclampsia is a complex disease with significant maternal and fetal morbidity and mortality. Its syndromic nature makes diagnosis and management difficult. The field is rapidly evolving with the definition of pre-eclampsia being challenged by some organisations, with proteinuria no longer being essential in the presence of other features. In the last decade, angiogenic factors, in particular soluble fms-like tyrosine kinase 1 (sFlt-1), have emerged as important molecules in the pathogenesis of pre-eclampsia. Here we review the most recent evidence regarding the potential of these factors as biomarkers and therapeutic targets for pre-eclampsia.

Keywords Angiogenic factors, fms-like tyrosine kinase 1, National Institute for Clinical Excellence, placental growth factor, pre-eclampsia, pregnancy.

Tweetable abstract A review of angiogenic factors, sFlt-1 and PlGF, in the diagnosis, prediction and management of pre-eclampsia.

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Introduction

Pre-eclampsia (PE) is a pregnancy-specific syndrome, defined by new onset hypertension and proteinuria or biochemical abnormalities after 20 weeks of gestation. It complicates 2–8% of pregnancies and is a leading cause of maternal and fetal morbidity and mortality worldwide.[1]

Pre-eclampsia is caused by the placenta and is specifically a disorder of dysfunctional syncytiotrophoblast, the interface between the mother and the fetus.[2] Early PE (<34 weeks) is characterised by more intense fetal growth restriction (FGR) compared with late PE (≥34 weeks).[3] FGR is not increased in term PE. Early PE is thought to result from deficient placentation associated with maladaptation of the spiral arteries and restricted trophoblast invasion at 8–18 weeks, which causes uteroplacental malperfusion. At this stage there are no signs of PE, which evolve later in pregnancy, as described in the widely accepted 'two-stage' model.[2] The later PE form involves a complex interaction between 'stressed' syncytiotrophoblast (thought to be caused by uteroplacental malperfusion; not from spiral artery disease but from diffuse compression of the intervillous space caused by the growing placenta and increasing fetal and intervillous hypoxaemia) and a 'susceptible' maternal cardiovascular system,[4,5] although some believe the cardiovascular system plays a more important role.[6]

The classical clinical features of pre-eclampsia are essentially secondary or tertiary to the placental problems. They are grouped to allow recognition of the syndrome but are neither specific nor precise, with the classic diagnostic hallmarks, raised blood pressure and proteinuria, having a positive predictive value of only 20%.[7] Diagnostic criteria are thus evolving to reflect the heterogeneity of the condition in an attempt to apply more disease specificity to what is essentially a syndrome. Currently, the American College of Obstetricians and Gynecologists (ACOG),[8] the International Society for the Study of Hypertension in Pregnancy (ISSHP)[9] and others[10,11] no longer require the presence of proteinuria as a diagnostic criterion and thus PE can now be diagnosed by the presence of new onset hypertension after 20 weeks, associated with proteinuria or evidence of end-organ dysfunction (i.e. thrombocytopenia, severe headache, renal insufficiency, impaired liver function, heart and lung dysfunction). When PE is suspected, patients are often

admitted to the hospital for further monitoring/investigation and discharged only when PE has been ruled out. Given the nonspecific presentation of the syndrome, many patients are admitted unnecessarily, and some cases may be missed. Furthermore, as of 2017, there is no effective treatment for PE and the only cure is removal of the diseased placenta, i.e. delivery, which can cause substantial iatrogenic prematurity of the neonate.[12]

A biomarker that could improve diagnosis, prediction or prognosis of PE would be a major advance, but only if it directly reflected syncytiotrophoblast stress. In the latter case it would be involved in the pathogenesis of the disease and could be a target for therapeutic intervention.

Work from the Karumanchi laboratory showed that PE is an anti-angiogenic state. Anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) are overexpressed by a disordered syncytiotrophoblast and released into the maternal circulation.[13,14] Serum or plasma sFlt-1 is elevated in patients with pre-eclampsia in comparison with healthy pregnant controls. These changes are on average present before the PE manifests and are correlated with severity.[15,16] sFlt-1 is a shorter version of the full-length membrane-bound, vascular endothelial growth factor (VEGF) receptor Flt-1, arising from alternative RNA processing of the FLT-1 gene. Flt-1 can bind VEGF and placental growth factor (PlGF), an angiogenic molecule released by the placenta. sFlt-1 lacks the transmembrane and intra-cytoplasmic domains and therefore circulates freely, and acts as a decoy receptor for VEGF and PlGF in the circulation. This is thought to cause the widespread endothelial dysfunction and clinical manifestations of the disease. Alongside increased levels of sFlt-1, women with PE have decreased levels of free VEGF and free PlGF when compared with healthy pregnant controls.[15,17,18] PlGF may also decline as a result of syncytiotrophoblast stress.[19]

Another anti-angiogenic factor is soluble Endoglin (sEng), derived from a cell surface co-receptor for transforming growth factor-β (TGF-β),[13,20] which is also a product of syncytiotrophoblast. The maternal levels of sEng are also raised, on average, prior to the onset of PE[13,16]

Because they more closely reflect the primary pathology of syncytiotrophoblast stress, these factors are promising biomarkers for prediction, diagnosis and prognosis of the disease.[17,18] Importantly, sFlt-1 and/or PlGF assays are available for clinical use.[21,22] Recently, they have been recommended by the National Institute for Clinical Excellence (NICE) to rule out PE in patients presenting with suspicion of the disease.[23]

Aid in diagnosis

Several studies have reported that angiogenic factors may be useful in diagnosing PE[24–26] and superimposed PE, and distinguishing them from other diseases such as gestational hypertension, renal disease, chronic hypertension, gestational thrombocytopenia and immune thrombocytopenic purpura.[18,27–34] Recently, Bramham et al.[34] showed that in women with chronic kidney disease (CKD), chronic hypertension (CHTN) or both, low levels of PlGF had high diagnostic accuracy for superimposed PE requiring delivery within the next 14 days. Importantly, PlGF levels were similar between healthy controls and women with CKD or CHTN who did not develop superimposed PE. The diagnostic performance of sFlt-1 in this study was also high.[34] This large study echoed findings from several previous studies.[18,30,32,33] We identified two small studies that found no difference and the reason for this is unknown.[35,36]

Verlohren et al.[24] provided reference ranges and cut-offs for the diagnosis of early- and late-onset PE. They followed 1149 pregnancies and used a ratio of the levels of sFlt-1 and PlGF (sFlt-1/PlGF ratio) to calculate risk. For each group, i.e. late-onset (≥ 34/<37 weeks) and early-onset (<34 weeks) PE, separate lower and higher cut-offs were defined. For early-onset PE, the target was to reach a sensitivity of $\geq 95\%$ at the lower cut-off and a specificity of $\geq 95\%$ at the higher cut-off. The target for late-onset PE was to achieve a specificity of minimum 95%. sFlt-1/PlGF ratios ≥ 85 (20–33^{+6} weeks) or ≥ 110 (34–36^{+6} weeks) were highly suggestive of PE. The positive likelihood ratio (LR$^+$) was 176 (95% CI 24.88–1245) and 13 (95% CI 7.34–23.0), respectively. In addition, a ratio ≤ 33 performed well for the exclusion of PE in both the gestational age groups, with a negative likelihood ratio (LR$^-$) of 0.05 (95% CI 0.02–0.13) and 0.14 (95% CI 0.09–0.24) for <34 and ≥ 34 weeks, respectively. In other words, the combination of a sFlt-1/PlGF ratio ≤ 33/≥ 85 at <34 weeks of gestation, resulted in 95% sensitivity and 99.5% specificity and an sFlt-1/PlGF ratio ≤ 33/≥ 110 at ≥ 34 weeks resulted in a sensitivity of 89.6% and a specificity of 95.5% for the diagnosis of pre-eclampsia.

Similarly, PlGF has been used as a diagnostic marker (using a point-of-care platform). In these studies, diagnostic cut-off levels were defined as either PlGF <5th centile for gestational age[37,38] or <36 pg/ml.[37] Using PlGF <5th centile for gestational age, Benton et al.[37] revealed a sensitivity of 100% (95% CI 86–100) and specificity of 96% (95% CI 85–99) for early-onset PE, and a sensitivity of 47% (95% CI 24–71) and specificity of 95% (95% CI 81–99) for late-onset PE. They proceeded to use a cut-off of <36 pg/ml (derived from ROC curves) as a diagnostic cut-off and in this analysis they showed a sensitivity of 84% (95% CI 64–95) for early onset and a specificity of 100% (95% CI 92–100). In late-onset PE, use of the <36 pg/ml cut-off yielded a sensitivity of 95% (95% CI 74–100) and a specificity of 73% (95% CI 56–86). Knudsen et al. examined PlGF at different gestational ages and showed that at

<35 weeks the AUC (area under the curve) was 0.994, whereas at ≥35 weeks, the AUC was 0.895.[38] Values below the limit detection (<12 pg/ml) were deemed to represent a high risk of having PE. Similarly values >100 pg/ml have been suggested as capable of ruling out PE.[39]

Stepan et al.[40] compared the diagnostic performance of sFlt-1/PlGF ratio and PlGF alone, using the above-mentioned cut-offs for sFlt-1/PlGF[24] a cut-off of <36 pg/ml[37] for PlGF, for the diagnosis of PE.[40] The two tests showed a similar sensitivity but the specificity was slightly higher for the sFlt-1/PlGF ratio. Calculated AUC for sFlt-1/PlGF ratio was 0.941 (95% CI 0.9188–0.9632) and for PlGF alone 0.9172 (95% CI 0.8918–0.9426). Both assays performed better before than after 34 weeks of gestational age.[40]

Prediction of disease

The angiogenic factors, alone or in combination with other features, have been evaluated for the prediction of pre-eclampsia with some conflicting results, in particular in the first half of pregnancy.[41]

Poon et al.[42] proposed a dual-stage strategy for identification of at risk pregnancies which included screening (using biomarkers and ultrasound) in the first trimester followed by risk assessment in the third. This first trimester screening appears to have clinical utility, as reflected in a large trial where an algorithm incorporating PlGF was used to identify high-risk patients who ultimately benefitted from low dose aspirin.[43] Other researchers found that a significantly low concentration of PlGF in early 2nd trimester was highly associated with subsequent development of PE.[44–47] Another study linked the 1st and 2nd trimester levels of s-Eng, sVEGFR-1 and PlGF in maternal plasma with an increased risk of developing PE later in pregnancy.[48] Conversely, a recent large study assessing the value of angiogenic factor measurement at 20 weeks for prediction of PE showed that angiogenic factors in the first part of pregnancy did not perform well in predicting PE later in pregnancy.[49]

Given that the angiogenic factors change more significantly within 5–6 weeks of the onset of clinical features,[15] it may be that the accuracy of the markers to predict disease or adverse outcomes decreases over long time frames and, because of this, at earlier gestations we believe the additional screening and risk assessment in the form of algorithms show better performance than angiogenic factors alone.[42,43,50,51] These algorithms will require specialised training and widespread dissemination as well as assessment of cost-effectiveness before they can be widely implemented.

At later gestations (>20 weeks), angiogenic factors have been reported to perform better for detection of early-onset (< 34 weeks) than late-onset PE (≥34 weeks).[17,22,38,52] This has been attributed to a greater severity of early-onset

cases, or, as suggested by some, that late onset PE might be a different subtype.[3,4,6,53,54] We have suggested an alternative explanation,[5] namely that at term syncytiotrophoblast stress is increasing in all pregnancies, (accompanied by increasing stillbirth and pre-eclampsia rates), that all women would get pre-eclampsia if spontaneous delivery did not intervene, so that the background of normality allowing clear diagnosis simply does not exist. Changes in the biomarker of control 'normal' pregnancies at term are entirely consistent with this interpretation.[5]

The reported high negative predictive values of these biomarkers are important to rule out PE accurately. Accurate exploitation of this property could decrease the number of unnecessary admissions or interventions and allow a better allocation of resources.

During the second half of pregnancy, angiogenic markers seem to be particularly useful in patients presenting with suspicion of pre-eclampsia. Recently, a large study PROG-NOSIS (Prediction of Short term Outcomes in Pregnant Women with suspected PE Study) prospectively enrolled 1273 pregnant women across 14 different countries, who presented to medical centres between 24^{+0} and 36^{+6} weeks with suspected PE. The primary objectives of the study were to determine whether low levels of sFlt-1/PlGF ratio could rule out the development of PE, eclampsia or HELLP syndrome in the following 7 days, and whether high levels of sFlt-1/PlGF ratio could rule-in the development of PE, eclampsia or HELLP syndrome in the following 4 weeks.

A sFlt-1/PlGF ratio of 38 or lower was able to rule out PE in the following 7 days with a negative predictive value of 99.3% (95% CI 97.9–99.9) with 80% sensitivity (95% CI, 51.9–95.7) and 78.3% specificity (95% CI, 74.8–81.7). This high NPV, indicating that the woman is unlikely to develop PE, can allow the physician both to rationalise treatment and simultaneously reassure the patient.

The study had several strengths including its large sample size, prospective nature, it was multi-centred, and utilised development and validation cohorts. However, it was conducted in a high-risk singleton population and therefore results cannot be extrapolated to the general pregnant population, twin pregnancies, women presenting outside the 24- to 37-week window, or women of diverse ethnic background.

Further studies are warranted to test these biomarkers in twin pregnancies and in PE patients who present after 37 weeks. The latter group constitute most PE cases. Current NICE guidelines recommend delivery at 37 weeks for patients with PE. Given the problems related to the workload associated with arranging induction at 37 weeks, further studies are needed to explore the role of the sFlt-1/PlGF ratio to help prioritise which deliveries may safely be deferred. This strategy might alleviate pressure on the delivery suite and potentially improve neurodevelopmental

outcome, as has been noted in normal pregnancy.[55] Twin pregnancies have an increased risk of developing PE *per se*, and may have different cut-offs for the diagnosis of the disease. In many studies[56–59] it has been found that the levels differ in twin compared with singleton pregnancies and appropriate cut-off values need to be determined. Similarly, the reported sensitivities and specificities in the presented studies are specific for the study populations, which may not reflect each and every single ethnicity and region in the world. A wide external validation in other populations would be desirable. Currently, substantial data are being generated worldwide, including in low-income countries, which will hopefully help to address this matter.[60,61]

Klein et al.[62] conducted a multi-centre, prospective non-interventional study (PreOS) of the application of the sFlt-1/PlGF ratio in decision-making in women with suspected PE, The results suggested that use of angiogenic markers significantly impacts both the decision to hospitalise patients appropriately and stratifies the risk, and may help to guide the required intensity of patient management.[62]

Prediction of outcomes

In an extension of the PROGNOSIS study, the authors demonstrated that the sFlt-1/PlGF ratio is a predictor of pregnancy duration, with a low ratio (≤38) being associated with longer pregnancy duration independently of the presence of PE.[63] Women who had an sFlt-1/PlGF ratio >38 in their first visit, had a shorter time to delivery (median 17 vs. 51 days) and a 2.9-fold greater likelihood of imminent delivery. Importantly, this was a reflection of iatrogenic delivery and there was no association of the sFlt-1/PlGF ratio with spontaneous preterm labour.[63]

Other authors have also shown previously that in women who present with suspicion of PE, sFlt-1 and PlGF, alone or in combination, were useful to predict the development of poor outcomes within 2 weeks and correlated with time to delivery,[17,22,64] particularly in relation to adverse outcomes in early onset PE (<34 weeks). Rana et al.[64] divided the women with PE into angiogenic PE and non-angiogenic PE depending on the level of angiogenic factors (sFlt-1 and PlGF). They found that the women with non-angiogenic PE (sFlt-1/PlGF ratio < 85) had no serious adverse outcomes within 2 weeks, whereas the women with angiogenic PE (sFlt-1/PlGF ratio > 85) developed abruption, pulmonary oedema, eclampsia, small-for-gestational-age babies, elevated liver function tests or low platelet counts. The sFlt-1/PlGF ratio performed better for severe outcome prediction compared with elevated blood pressure and proteinuria alone.[17,64]

In patients who were admitted for evaluation of PE, the rate of increase of the sFlt-1/PlGF ratio over time correlated with the development of adverse outcomes. Patients who did not develop adverse outcomes had a persistently low sFlt-1/PlGF over time compared with patients who went on to developed adverse outcomes.[65]

Given the above, it is possible that angiogenic factors could be used not only to rule out disease and, more importantly, disease-related adverse outcomes in patients with a low ratio (given its remarkable negative predictive value), but also to help unravel a population at high risk of developing adverse outcomes (those with a high ratio). The former could allow a step down in the intensity of monitoring, decrease anxiety, potentially prevent iatrogenic early intervention and consequently safely prolong pregnancy; the latter would highlight a population that could benefit from a higher level of care/monitoring and, importantly, new therapeutic interventions that are now promising to reach the clinical setting.

Angiogenic factors are currently used in clinical practice in Germany as an aid in diagnosis and have been approved in England as per the NICE guidance. NICE has now recommended the use of angiogenic factors (sFlt-1 and PlGF or PlGF alone, depending on the manufacturer) to rule out PE in women between 20 and 34^{+6} weeks of pregnancy. Currently the factors are not recommended for diagnosing or ruling in the disease.

Economic modelling/potential economic impact

The use of biomarkers and other models as screening tools to predict PE has been considered economically ineffective and of no clinical benefit.[66,67] However, the use of angiogenic factors in high-risk populations (women who present with signs or symptoms of PE) may confer significant economic benefit.[68,69] Various studies have suggested savings using the sFlt-1/PlGF ratio of £334/patient (UK),[68] £945/patient (UK); €670/patient (Italy),[70] and using PlGF alone of £35 087/1000 patients (UK).[71]

Therapeutic intervention

Angiogenic factors can be used as surrogate markers of response to new therapies in clinical trials, given that they directly reflect abnormal placental function.[72] Furthermore, given the role of these angiogenic factors in the pathogenesis of PE, they are regarded as potential therapeutic targets themselves, the rationale being to correct the anti-angiogenic imbalance either by replenishing circulating VEGF or PlGF or by depleting sFlt-1. Administration of VEGF or PlGF into animal models of PE ameliorates the syndrome.[73–76] PlGF seems more promising as it has less off-target effects compared with VEGF. Other strategies include the use of small compounds, RNA interference, statins or other means of modulating sFlt-1 or PlGF expression.[77–81]

A promising study conducted in humans was the extracorporeal removal of sFlt-1. Thadhani et al. used a negatively charged dextran sulphate column for extracorporeal removal of sFlt-1 from the maternal circulation.[82] Using this apheresis system, they were able to deplete sFlt-1 in the maternal circulation and safely prolong the gestation by ~15 days in women who were treated compared with 3–5 days in untreated pre-eclampsia controls.[83]

Conclusion

These are exciting times in the field of pre-eclampsia. The recent NICE approval sets the stage for large-scale analysis of the clinical impact of these circulating angiogenic factors. Time will tell whether angiogenic factors will have a profound impact on clinical practice and whether they will help improve maternal and perinatal outcomes.

Disclosure of interests
Full disclosure of interests available to view online as supporting information.

Contribution to authorship
ASC and MV structured the manuscript. ASC, SA, ACS, CWR and MV contributed to writing and critically appraising the manuscript. All authors approved the final version.

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