Placental Growth Factor in Suspected Preterm Pre-eclampsia: A Review of the Evidence and Practicalities of Implementation

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Abstract

Despite extensive research, the pathophysiology and prevention of pre-eclampsia remain elusive, diagnosis is challenging, and pre-eclampsia remains associated with adverse maternal and perinatal outcomes. Angiogenic biomarkers, including placental growth factor (PlGF) and soluble fms-like tyrosine kinase 1 (sFlt-1), have been identified as valuable biomarkers for preterm pre-eclampsia, accelerating diagnosis and reducing maternal adverse outcomes by risk stratification, with enhanced surveillance for high-risk women. PlGF-based testing is increasingly being implemented into clinical practice in several countries. This review provides healthcare providers with an understanding of the evidence for PlGF-based testing and describes the practicalities and challenges to implementation.

Tweetable Abstract

Placental growth factor in pre-eclampsia: evidence and implementation of testing

Background

Pre-eclampsia is associated with increased adverse maternal and perinatal outcomes, as well as substantial costs for healthcare providers.[1] The schedule of antenatal care in the United Kingdom, similar to many other high-income settings, is designed for early detection of pre-eclampsia to minimise adverse outcomes. Maternal mortality has dramatically decreased in the United Kingdom over the last 70 years, likely due to the provision of free antenatal care and implementation of evidence-based guidelines, alongside adoption of recommendations from the landmark Confidential Enquiries into Maternal Deaths.[2] However, risks to the woman persist, child morbidity and mortality remain, and pre-eclampsia is the most common cause of iatrogenic preterm delivery.[3] The diagnosis of pre-eclampsia is evolving, particularly on a background of chronic medical co-morbidities. Proteinuria is not a pre-requisite for diagnosis, which can be made on the premise of new-onset or worsening of hypertension in association with neurological, biochemical or haematological abnormalities or fetal growth restriction.[4, 5] Hypertension alone may predict only 20% of adverse outcomes in pre-eclampsia, and therefore there is a need for better risk stratification and targeted surveillance.[6] Around 10% of women may present with suspected pre-eclampsia, often asymptomatic, even in the presence of severe disease.[7] At present, women are unnecessarily admitted to hospital with suspected pre-eclampsia, whilst more severe cases may go undiagnosed. The uncertain management of this group presents a considerable workload within maternity care.

Placental growth factor (PlGF) is an angiogenic protein, which is secreted by the syncytiotrophoblast and promotes placental angiogenesis. In a healthy pregnancy, PlGF concentrations increase as gestation advances, reaching a peak at 26 to 30 weeks' gestation, before decreasing towards term.[8, 9] Low concentrations of

PlGF precede the clinical onset of pre-eclampsia and abnormalities in angiogenic factors may predate the clinical syndrome by 10 weeks.[10] Soluble fms-like tyrosine kinase (sFlt-1) is a circulating anti-angiogenic protein which adheres to the receptor-binding domains of PlGF and vascular endothelial growth factor (VEGF), preventing their interaction with endothelial receptors and inducing endothelial dysfunction. sFlt-1 concentrations increase towards term in healthy pregnancies, but are prematurely elevated in women with pre-eclampsia.[8] Low PlGF concentrations in pre-eclampsia may reflect down-regulated expression as well as high levels of sFlt-1 reducing the bioavailability of PlGF.[11] Therefore, low PlGF and high sFlt-1 are secondary markers of placental dysfunction in pre-eclampsia, in contrast to hypertension and blood pressure, which are tertiary, downstream features.[12] This has focused research on whether angiogenic biomarkers may aid diagnosis of pre-eclampsia and reduce adverse outcomes.

The last decade has seen the development of fully automated, commercially available assays, replacing manual enzyme-linked immunosorbent assays. This has led to standardised, inexpensive measurements, with a high turnover and minimal sample handling. Some PlGF-based tests measure PlGF alone, whereas others quantify sFlt-1 and PlGF, presenting the results as a ratio. There are currently four commercially available PlGF-based assays. The thresholds associated with diagnosis are not interchangeable, as the assays have varying affinity to PlGF isomers and this has implications for clinical practice and implementation of PlGF-based testing. The National Institute for Health and Care Excellence has released specific diagnostics guidance relating to PlGF-based testing[13] and has recommended two tests for routine adoption into the NHS, to be used as rule-out tests for women with suspected preterm pre-eclampsia. These are the Triage PlGF test (Quidel) and the Elecsys sFlt-1/PlGF ratio (Roche Diagnostics).[5, 13]

Triage PlGF Test (Quidel)

The Triage PlGF test is a single-use, fluorescence immunoassay device, which is used with the CE-marked Triage MeterPro point-of-care analyser. Blood must be centrifuged, and plasma extracted before testing. The analyser can be installed either as a point-of-care test in a clinic or ward, or in a laboratory, and the assay takes 15 minutes. It detects PlGF-1 and quantifies concentration in the range of 12 to 3000 pg/ml. It is recommended by the National Institute for Health and Care Excellence as a rule-out test for suspected pre-eclampsia between 20 and 34^{+6}-weeks' gestation at a threshold of 100 pg/ml.[13]

Evidence for the Triage PlGF Test:

The PELICAN study was a prospective, multicentre, observational study investigating the diagnostic accuracy of PlGF in diagnosing pre-eclampsia.[12] This study showed that low PlGF had high sensitivity (0.96; 95% CI 0.89 – 0.99) and high negative predictive value (NPV 0.98; 0.93 – 0.995) in diagnosing pre-eclampsia necessitating delivery within 14 days, in women with suspected pre-eclampsia before 35 weeks' gestation.[12] The area under the receiver operating characteristic curve (AUC) for PlGF was greater than all other commonly used tests (systolic blood pressure, diastolic blood pressure, alanine transaminase, urate, dipstick proteinuria) for making a diagnosis in women presenting with suspected pre-eclampsia. Low PlGF was classified as $<5^{\text{th}}$ centile according to predetermined normal ranges.[9] However, for implementation in clinical practice a threshold of <100pg/ml was identified, independent of gestation, with test performance similar to the 5^{th} centile and retaining the same high sensitivity and NPV. When multiples of median were used for analysis, this reduced the predictive power of low PlGF (unpublished data). The study was conducted across seven sites in the United Kingdom and recruited 625 women; 346 developed pre-eclampsia, of whom 176 developed pre-eclampsia prior to 35 weeks' gestation. Therefore, this demonstrates high test performance across a broad range of clinical settings and population demographics.

PlGF testing has been investigated in the PETRA trial, a prospective observational study recruiting 1,112 women from 24 centres across North America.[14] This demonstrated that low PlGF concentration was strongly correlated with time to delivery in women with suspected pre-eclampsia before 35 weeks' gestation (753 women). The median time to delivery was 10 days for low PlGF (100pg/ml or lower) and 2 days for very low PlGF (less than 12pg/ml). The sensitivity for diagnosing pre-eclampsia and delivery within 14 days was 92.5% and the NPV was 90.3%. This lower NPV may reflect the higher prevalence of pre-eclampsia

in the study population, with 71% of participants (538 women) diagnosed with preterm pre-eclampsia. A secondary analysis of this study has also been reported.[15] This found a significant association between low and very low PlGF and composites of perinatal and maternal adverse outcomes. The sensitivity and NPV of low PlGF for adverse neonatal outcomes were 95.8% and 99.2%, and for adverse maternal outcomes 86.8% and 98.1%. They conclude that women with abnormal PlGF are significantly more likely to suffer adverse neonatal and maternal outcomes, and that PlGF is useful for risk stratification.

PlGF testing has been evaluated in the PARROT trial, a multi-centre stepped-wedge cluster-randomised controlled trial.[16]The trial design involved random and sequential transition of maternity units (representing clusters) from concealed to revealed PlGF testing, alongside a simple clinical management algorithm. 1023 women with suspected preterm pre-eclampsia were enrolled from 11 maternity units across the UK. The time to diagnosis was reduced from 4.1 to 1.9 days, with a significant reduction in a composite of severe maternal adverse outcomes from 5.4% to 3.8% (aOR 0.32, 95% CI 0.11 – 0.96). There was no difference in gestational age at delivery or perinatal adverse outcomes. The study strengths include its broad inclusion criteria and a population who were diverse from an ethnicity and sociodemographic perspective, from multiple sites, thereby enhancing generalisability of the findings to the real-world setting. Therefore, this study provided novel evidence that PlGF testing proves a valuable diagnostic adjunct in suspected pre-eclampsia, allowing targeted surveillance for those at highest risk of adverse outcomes.

Initial studies using economic modelling demonstrated that PlGF-based testing may afford a cost-saving of between £330 and £1032 per woman tested.[17, 18] In a more recent study describing their cost-effectiveness analysis of the PARROT trial, Duhig and colleagues found a total cost-saving of £149 per woman, based on £70 per Triage PlGF test.[19] Given that there were 646,794 births in England in 2017 and 10% of pregnant women have suspected pre-eclampsia, with 30% of these presenting prior to 37 weeks' gestation, PlGF testing could be performed in approximately 38,800 women per year. This represents a potential cost saving of £2,891,196 each year in England. These cost-savings are driven by a reduction in outpatient attendances for those with a normal, low risk result. There was an increased cost seen associated with inpatient admissions in those at higher risk. Despite this, overall PlGF testing was found to be cost saving, but the cost-effectiveness will depend on the cost of the test being performed. The authors conclude that their more conservative cost-saving is due to improved clinical risk stratification leading to appropriate redistribution of resources, rather than an overall reduction of resource use anticipated by hypothetical analyses.[19]

Elecsys Immunoassay sFlt-1/PlGF ratio (Roche Diagnostics)

The Elecsys sFlt-1/PlGF ratio is derived by combining the results from two CE-approved sandwich electrochemiluminescence immunoassays. The analysis turnaround time is 18 minutes. The test requires a large-scale laboratory analyser and is not a point-of-care test. The Elecsys sFlt-1 assay has a lower limit of detection of 10pg/ml and a range of 10 – 85,000pg/ml. The Elecsys PlGF assay has a lower limit of detection of 3pg/ml and a range of 3 to 10,000pg/ml. The National Institute for Health and Care Excellence recommends use as a rule-out test for suspected pre-eclampsia between 20 and 34^{+6} weeks' gestation, at a threshold of [?] 38.[9]

Evidence for the Elecsys Immunoassay sFlt-1/PlGF ratio:

Rana and colleagues performed a prospective observational study investigating the association between the Elecsys sFlt-1/PlGF ratio and adverse outcomes in suspected pre-eclampsia.[20] They found that in women presenting before 34 weeks' gestation, sFlt-1/PlGF ratio out-performed current approaches (systolic blood pressure, alanine transaminase, creatinine, urate) at predicting adverse outcomes, with an AUC of 0.89. A cut-off point of 85 was identified, with sensitivity of 72.9% and specificity of 94.0% for adverse outcomes, in women presenting before 34 weeks' gestation. sFlt-1/PlGF ratio was inversely correlated with time to delivery; delivery occurred within two weeks in 86.0% of women with a sFlt-1/PlGF ratio above 85. This was a single centre study, including 616 assessments of suspected pre-eclampsia, of which 81 were repeat evaluations.

The PROGNOSIS study was a multicentre observational study that analysed the predictive value of the Elecsys sFlt-1/PlGF ratio in 1050 women, with 500 women included in a development cohort to determine

a ratio cut-off and 550 women as a validation cohort.[21] This demonstrated that a ratio of [?] 38 had a negative predictive value (NPV) of 99.3% (95% CI 97.9 – 99.9), with a sensitivity of 80.0 (95% CI 51.9 – 95.7) for ruling out pre-eclampsia in less than one week. The positive predictive value for a diagnosis of pre-eclampsia within four weeks was 36.7% (95% CI 28.4 – 45.8), with a 66.2% sensitivity (54.0 – 77.0). Predictive performance of sFlt-1 and PlGF analysed individually were not superior to the ratio, with AUC of 78.2% for PlGF alone, compared to 88.4% for sFlt-1/PlGF.[21] They conclude that in clinical practice, high NPV is crucial in the evaluation of suspected pre-eclampsia, as failure to detect imminent disease could have important consequences for the woman or fetus. 199 women (19%) developed pre-eclamspia and this lower prevalence may explain the lower positive predictive value than the PELICAN study.[12]

The Elecsys sFlt-1/PlGF ratio has also been evaluated in the INSPIRE randomised controlled trial.[22] 370 women with suspected preterm pre-eclampsia were randomised on an individual level to revealed or non-revealed PlGF-based testing. Primary endpoint was hospitalisation within 24 hours of the test. The trial found no difference in the primary outcome but use of the test increased the proportion of high-risk patients admitted without influencing overall admission rate, which may reflect appropriate redistribution of resources. Overall, 85 women (23%) developed pre-eclampsia and all of those developing pre-eclampsia within seven days were admitted to hospital following the sFlt-1/PlGF test, demonstrating 100% sensitivity and 100% NPV for the defined primary endpoint. They concluded that larger trials are needed to assess whether the test could be used to mitigate adverse maternal and perinatal outcomes. The single centre nature of the trial, with 90% of participants of white ethnicity, limits its wider generalizability.

Comparison of Triage PlGF Test and Elecsys sFlt-1/PlGF Ratio

The two PlGF-based tests recommended by the National Institute for Health and Care Excellence have been subject to direct comparison.[23-25] McCarthy and colleagues (2019) compared the Triage PlGF test, Elecsys sFlt-1/PlGF ratio and the DELFIA Xpress PlGF 1-2-3 tests in 305 women, of whom 62 developed early-onset pre-eclampsia.[23] They found that the AUC were nearly identical for the Elecsys sFlt-1/PlGF ratio and the Triage PlGF test, with that for the DELFIA Xpress PlGF 1-2-3 test very similar. This suggests that the tests are similarly effective, and trade-off between sensitivity and specificity is dependent on the thresholds, which currently are slightly different. As more clinical data becomes available, appropriate and equivalent thresholds for clinical utility can be derived. It has been suggested that high sensitivity is a more useful attribute in early detection of pre-eclampsia than specificity because consideration of benefits, harms and costs indicates a much greater preference for minimizing false negatives than false positives, although the ideal would be to avoid both.[26]

In the COMPARE study,[23] equivalent clinical thresholds for PlGF rule-out differed by 50% and the DELFIA Xpress PlGF 1-2-3 has a rule-out threshold of 150 pg/ml, whereas the rule-out threshold for the Triage PlGF test is 100 pg/ml. This illustrates that they are not identical assays, nor are they interchangeable clinically. The differing thresholds are likely due to variable PlGF recovery and isomer cross-reactivity. PlGF is a homodimeric glycoprotein which exists in four isomers, derived from different splicing of primary gene transcripts.[27] PlGF-1 and PlGF-2 are the most abundant forms, and they are secreted in a strongly correlated manner. Isomer cross-reactivity is variable; one study found 12 to 19% cross-reactivity with PlGF-2 and 16 to 23% cross-reactivity with PlGF-3 for the Roche Elecsys PlGF assay.[28] This contrasts with the manufacturer reports; Roche Diagnostics quote less than 8% cross-reactivity and Triage quote 9.6% cross-reactivity with PlGF-2.[28]

There have been other interassay comparison studies.[24, 25, 28] Stepan and colleagues (2016) performed a prospective multi-centre case-control study of 569 women (178 with confirmed pre-eclampsia).[24] They found the Elecsys immunoassay had a higher specificity and lower sensitivity compared to the Triage assay at the thresholds selected, but used a cut off of [?]33 for the Elecsys sFlt-1/PlGF ratio. As the sensitivity and specificity are highly dependent on the thresholds selected, it is difficult to draw any conclusions from these comparative predictive statistics.

A smaller retrospective case-control study in 128 women (44 with confirmed preeclampsia) found that the

Triage assay had a higher sensitivity than the Elecsys ratio at only a small reduction of specificity (sensitivity 100% (95% CI 86-100) compared to 64% (95% CI 43-82), and specificity 96% (95% CI 85-99) compared to 100% (95% CI 93-100)).[24] This study also clearly demonstrated that PlGF concentrations were lower when measured on the Triage assay compared the Elecsys assay. They defined a positive test as PlGF $<5^{th}$ centile for a gestational-age dependent range, and >85 for the Elecsys sFlt-1/PlGF ratio.

In summary, both tests recommended by the National Institute for Health and Care Excellence have strong test performance with AUC outperforming currently used tests for diagnosing pre-eclampsia (see Table 1). The Triage PlGF test has a higher sensitivity when used with the current rule-out threshold of 100pg/ml, compared to the Elecsys sFlt-1/PlGF ratio threshold of 38, which has a higher specificity. These and other tests available on the market appear to be clinically similar in prediction, and other factors such as cost, and ease of use will dictate clinical uptake.

Practicalities of Implementation

The choice of which PlGF-based test to implement in a maternity unit is likely to be dictated by the unique practicalities of each platform, which are outlined in Box 1.

Despite the clinical and financial advantages of PlGF-based testing, there have been barriers to adoption encountered in a government-funded healthcare system such as the UK. These are outlined in Box 2. Therefore, each maternity unit will need to bring together all stakeholders in order to choose which PlGF-based test best suits their needs and setting. It is fundamental that the clinical, laboratory and financial teams work in partnership, so that every partner understands the implications of testing.

The Future of PlGF

The National Institute for Health and Care Excellence recommends a single Triage PlGF test or Elecsys sFlt-1/PlGF ratio as a rule-out test, concluding that the evidence is currently insufficient to recommend use as a rule in test.[13] Furthermore, the guidance is clear that testing should be used to aid diagnosis of pre-eclampsia, and not a wider syndrome of placental disease.

The role of repeat PlGF sampling has not been fully investigated and the impact of repeat testing on maternal and perinatal outcomes is unknown. This is particularly important in women in whom a clear risk trajectory or diagnosis is not reached at initial presentation, but ongoing suspicion of disease remains. One study has recently suggested that repeat PlGF testing retains high diagnostic accuracy, with a high sensitivity and NPV.[29] Another study demonstrated increasing sFlt-1/PlGF ratios in women who went on to develop pre-eclampsia or adverse perinatal outcomes.[30]However, before repeat testing is recommended, clinical and cost-effectiveness need to be established, given as an explicit research recommendation in the diagnostic guideline.[13] This is being addressed by the PARROT-2 trial, a multicentre randomised controlled trial of repeat revealed PlGF-based testing compared to usual care with repeat concealed testing (ISRCTN85912420).

The role of PlGF in the assessment of pre-eclampsia in multi-fetal pregnancy is uncertain, as normal ranges and interpretation have not been defined. There are a few small studies to date, with conflicting results.[31-33] The largest of these studied 79 women, presenting with suspected pre-eclampsia and found elevated sFlt-1/PlGF ratio was associated with adverse outcomes.[31] Although a smaller population of women, use of PlGF-based testing remains an important challenge due to the higher prevalence of pre-eclampsia in multi-fetal pregnancy.

PlGF-based testing in the assessment of early-onset fetal growth restriction warrants further study and there is conflicting evidence. One study of 213 pregnancies showed that low PlGF had a sensitivity of 98.2% with a NPV of 99.2% for placental fetal growth restriction (confirmed on placental histology).[34] A large prospective cohort study of 4,512 women found that addition of the sFlt-1/PlGF ratio to ultrasonic assessment improved the positive likelihood ratio for delivering a small-for-gestational-age infant.[35] Another study demonstrated that PlGF outperformed both ultrasound and numerous other biomarkers.[36] However, a multicentre study including 592 women demonstrated that PlGF performed no better than estimated fetal weight $<5^{th}$ centile in predicting delivery of a small-for-gestational-age infant.[37]

Other outstanding issues include the ability of PlGF-based tests to risk stratify in established disease, as well as the role of PlGF in low-resource countries. By far the greatest burden of morbidity and mortality due to pre-eclampsia is borne by women and their infants in low and middle-income settings. Initial evidence of the impact of PlGF-based testing in this setting is promising.[38, 39]

Finally, now that value in diagnosis of pre-eclampsia has been proven, PlGF and sFlt-1 may prove informative to guide treatments that influence the outcome or ameliorate development of the disease.[40]

Summary

There is a growing body of evidence that PlGF-based testing improves diagnosis and reduces adverse maternal outcomes in the assessment of suspected preterm pre-eclampsia, by appropriate risk stratification and resource redistribution. The Triage PlGF test and Elecsys sFlt-1/PlGF ratio are both recommended by the National Institute for Health and Care Excellence but are not interchangeable. Both assays have similar predictive properties but also unique advantages and disadvantages. The test to be implemented needs to be carefully chosen for the setting with involvement from all relevant parties.

Disclosure of Interests

AS has received funds from Perkin Elmer, paid to the university, to evaluate angiogenic markers. Other authors declare that they have no competing interests.

Contribution to Authorship

Conceptualisation – AS; writing – original draft outline – AS, LC, LW, AH; writing – original draft preparation – AH; writing – review and editing – AH, ABG, KD, LW, LC, AS. Supervision – AS. All authors have given final approval of the version to be submitted.

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Table 1: Test performance of PlGF-based tests

	Triage PlGF test	Elecsys sFlt-1 /PlGF ratio	DELFIA Xpress PlGF 1-2-3 test	BRAHMS sFlt-1/ PlGF plus ratio
Recommended rule-out threshold	[?] 100 pg/ml	[?] 38	[?] 150 pg/ml	>55
Sensitivity	96%	80%	87.5%	
Negative predictive value	98%	99.3%	97.2%	
Suggested rule in threshold	<12 pg/ml	> 85	<50 pg/ml	>188
Relevant study	PELICAN[12] PARROT[16]	PROGNOSIS[21] INSPIRE[22]	COMPARE[23]	Cheng *et al.*[28]

Box 1: Practical aspects of implementing PlGF-based testing.

Barriers	Inconsistent cross-discipline awareness of the impact of testing Lack of clinical coding for suspected pre-eclampsia
Facilitators	National guidance now recommending adoption of testing Momentum of clinical community moving towards u

Box 2: Barriers and facilitators to implementation of PlGF-based testing in UK healthcare setting

Barriers	Inconsistent cross-discipline awareness of the impact of testing Lack of clinical coding for suspected pre-eclampsia
Facilitators	National guidance now recommending adoption of testing Momentum of clinical community moving towards u