PLGF – WHAT WE KNOW
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APEC PAPERS BY LEADING AUTHORS ON PRE-ECLAMPSIA

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About the Authors

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Andy is Professor of Obstetrics at King's College London, based at St. Thomas' Hospital, UK. He is clinical lead of the Maternal and Fetal Research Unit, and specialises in clinical trials in antenatal and intrapartum care. His research interests include interventions to prevent pre-eclampsia, preterm birth and the use of blood pressure monitoring. He chaired the Department of Health Committee on Blood Pressure Monitoring in clinical practice, and sits on the relevant committees for the International Standardisation Organization (ISO) and the British Hypertension Society (BHS). He also advises the World Health Organisation (WHO) on perinatal research. Andy was born in Malawi, grew up in Zimbabwe, and worked as an obstetrician in South Africa. His African roots and clinical experience has inspired his passion for improving global women's health, including the development of the CRADLE VSA. Andy is Chairman of Action on Pre-Eclampsia

Dr. Alice Beardmore Gray

Alice is a specialist trainee in Obstetrics and Gynaecology working at St Thomas' Hospital, London and is currently the Trial Coordinator for CRADLE 4. She has worked with vulnerable women in a variety of different settings including Sierra Leone, Tanzania and Ghana. She recently contributed to research into anti-retroviral adherence in Rwanda and spent 6 months working to improve emergency services in a South African hospital as an NHS Global Health Fellow. Her experiences have highlighted to her the unacceptably high burden of disease that women in resource-poor settings bear and she is determined to address this inequality. Alice sees research as being a fundamental tool in bringing about long term change and making a difference to women's lives. Pre-eclampsia is one of the top five preventable causes of maternal mortality worldwide and Alice has a keen interest in working out how we can prevent its devastating consequences.

Lay Summary

The use of PLGF based tests in women with suspected pre-eclampsia

Pre-eclampsia is a complication of pregnancy which can have serious consequences for both mother and baby if left unrecognised or untreated. It is thought to be related to problems with the development of the placenta and occurs in around 5% of pregnancies.

Some women are more at risk of developing pre-eclampsia than others. These include women with previous pre-eclampsia, underlying high blood pressure, obesity and twin pregnancies. Raised blood pressure and protein in the urine can often be the first signs of the disease. It is important to measure blood pressure and check the urine for protein as women often feel well and can't tell if they have a problem. Headaches, indigestion like pains, swelling or visual disturbances may be a sign but can also occur in uncomplicated pregnancies. Pre-eclampsia can also affect the baby's growth and in severe cases may cause stillbirth. The only way to cure the disease is to deliver the baby and therefore sometimes the baby will have to be delivered premature.

The signs and symptoms of pre-eclampsia are not reliable or consistent. Women will therefore often be admitted unnecessarily, or can have severe disease without being diagnosed. This is dangerous as severe problems can occur without warning (such as fits and uterine bleeds).

Because pre-eclampsia affects many different systems in the body and can present suddenly without warning, it can be difficult to diagnose. As well as monitoring blood pressure and checking the urine for protein, blood tests of liver, kidney and clotting function are used to check if the disease has started to cause organ damage in the mother as this can indicate severe disease needing immediate delivery. Scans of the baby are also done to ensure the pregnancy can continue safely. These tests are frequently normal and do not indicate whether pre-eclampsia exists or predict whether it will progress rapidly. It can therefore be difficult for the teams of doctors and midwives looking after women with pre-eclampsia to know who can be safely monitored out of hospital and which women have severe disease requiring admission and close surveillance, because there have previously been no tests which can confidently rule out the presence of pre-eclampsia.

A new blood test (known as PLGF based tests) indicates whether the women has the disease and when used in women with suspected pre-eclampsia can reliably indicate who will need delivery soon. It is a reliable test of whether the baby is at risk. This test can be used to indicate who needs admission and intensive monitoring to determine when delivery is required, or who can be discharged home. Many other tests can frequently be avoided and it is therefore cost effective.

There are currently 3 versions of these tests available in the UK. All of them measure PLGF, and one also measures SFlt, and uses it as a ratio with PLGF. The PLGF is not the same in each assay, as there are 4 subtypes (isoforms) and each assay measures them differently. Each test is equally valuable, but has different levels to indicate action.

These tests will be available in the NHS, and the clinicans will be aware of which values to use. If the test is normal in women with worrying features, it is unlikely that pre-eclampsia will develop. If the test is abnormal, then more monitoring can be instigated to both mother and baby and delivery arranged when appropriate. Blood pressure may be treated in the meantime and drugs (magnesium sulphate) given while delivery is being arranged.

At the moment PLGF based tested are not used to determine who needs delivery, or how severe the pre-eclampsia.

Technical Summary: The use of PLGF based tests in suspected pre-eclampsia

 Placental growth factor (PlGF) is a protein involved in placental angiogenesis (the development of new blood vessels). In pre-eclampsia, levels of PlGF can be abnormally low. As pregnancy progresses, PlGF levels should rise and peak around 26-30 weeks' gestation. When they do not rise as expected this may indicate a problem with the placenta, which is associated with pre-eclampsia. Soluble FMS-like tyrosine kinase-1 (sFlt-1) is a protein that is thought to block other proteins needed for the development of the placenta, such as PlGF. Higher levels of sFlt-1 are seen in women who develop pre-eclampsia compared to those without the disease. These tests can be used in early pregnancy to predict who will develop pre-eclampsia, and in women who present with suspected disease (for example with high blood pressure, or a small baby) to rule out developing pre-eclampsia. They are not yet used to risk discriminate in women with established pre-eclampsia, although that research is underway.

 PlGF-based tests measure the amount of PlGF in blood plasma or serum. In addition some tests measure the amount of PlGF relative to sFlt-1as a ratio. This means that any blood taken must be prepared by spinning before testing, and this is usually done in a laboratory. No test can be done on "whole blood" yet, so a simple test available by the bedside or in the clinic is not yet available. This would be valuable in suspected pre-eclampsia when women present sporadically, around the clock.

 There are currently 4 PlGF-based tests commercially available.

- o Triage PlGF test (Quidel)
- o Elecsys immunoassay sFlt-1/PlGF ratio (Roche diagnostics)
- o DELFIA Xpress PlGF 1-2-3 test (Perkin Elmer)
- o BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio (Thermo Fisher Scientific)

These tests are intended to be used alongside clinical assessment to help rule out pre-eclampsia in women suspected of developing the disease.

Currently only the Triage PlGF test and the Elecsys immunoassay sFlt-1/PlGF ratio are recommended for routine adoption in the NHS. DELFIA Xpress PlGF 1-2-3 test (Perkin Elmer) has been shown to be equivalent, and will be considered by NICE next year. Further research to show the clinical effectiveness of the BRAHMS test is needed but it is likely to be comparable. Although the tests are similar, each assay measures different components of PLGF and are not directly interchangeable i.e. they will have different levels on the same sample. This means that a threshold for action differs between each product.

<u>Triage PlGF test (Quidel)</u>

 This test is recommended for use in women with suspected pre-eclampsia between 20 weeks and 34 plus 6 days of gestation. It can be used to identify women unlikely to develop pre-eclampsia needing delivery within 14 days – *i.e. to rule out the development of pre-eclampsia in the next two weeks.* The test turnaround time is about 15 minutes. It uses a small benchtop analyser which can be installed in a small side-clinic or laboratory. The blood samples must be processed in a small centrifuge before they can be analysed. It produces a quantitative PlGF result with the following cut-off values recommended by the manufacturer:

Result	Classification	Interpretation
PlGF <12 pg/ml	Test positive – highly abnormal	Increased risk for preterm delivery
PlGF ³12 pg/ml and <100 pg/ml	Test positive - abnormal	Increased risk for preterm delivery
PlGF ³ 100 pg/ml*	Test negative - normal	Unlikely to progress to delivery within 14 days of the test

The PELICAN study demonstrated a result of ³100 to have a negative predictive value of 98% when used to rule out pre-eclampsia developing in the next 14 days *(i.e. you can be 98% sure the woman won't develop pre-eclampsia in the next two weeks if she has a level ³100).* The analyser costs £1,400 with an annual service charge of £259. The individual tests cost £55 and are supplied in a kit of 25 tests. The manufacturer recommends quality control testing on a monthly basis using liquid samples (available from the manufacturer), and an electronic quality control check every day using an electronic cartridge that is provided with the analyser. An economic evaluation performed by NICE showed a potential cost reduction of £2,896 per patient compared with standard clinical assessment.

<u>Elecsys immunoassay sFlt-1/PlGF ratio (Roche Diagnostics)</u>

 This test is recommended for use in women with suspected pre-eclampsia between 24 weeks' gestation up and 36 weeks plus 6 days. It can be used to rule-out pre-eclampsia developing in the next week. The turnaround time is about 18 minutes. It requires a laboratory analyser (typically a high-cost instrument installed in a pathology laboratory) to calculate the sFlt-1/PlGF ratio. The following cut-off values are recommended by the manufacturer:

Gestation	sFlt-1/PlGF ratio	Interpretation
24 weeks to 36 weeks plus 6 days	<38	Rule out pre-eclampsia
	>38*	Increased risk of pre-eclampsia

The PROGNOSIS study demonstrated that in women with suspected pre-eclampsia between 24 and 36 plus 6 days of gestation, a result of less than 38 had a negative predictive value of 99.3% when used to rule out pre-eclampsia developing in the next week (i.e. *you can be 99.3% certain that the woman will not develop pre-eclampsia in the next week if she has a level of <38*). This test requires daily quality control testing. The individual tests cost £57.23 plus similar price for daily quality testing and are supplied in a 100-test reagent pack which must be used before the expiry date (a few weeks). An economic evaluation performed by NICE showed a potential cost reduction of £2,488 per patient compared with standard clinical assessment.

*It is important to acknowledge these rule out thresholds are not identical (although similar) i.e. the Quidel threshold has slightly more favorable rule out characteristics at the expense of worse rule in (specificity). Overall the products are equivalent in value.

DELFIA Xpress PlGF 1-2-3 test

This test is recommended for use in women with suspected pre-eclampsia during the second and third trimesters of pregnancy. The turnaround time is 30 minutes. It uses the DELFIA Xpress random access analyser to produce a quantitative PlGF result. The manufacturer recommends that cut-off values are established by individual laboratories. However in the third trimester the manufacturer suggests that a fixed cut-off value of 184 picograms/ml can be used (a result above this level would indicate a decreased probability of pre-eclampsia developing). The COMPARE study was published in 2018 and demonstrated equivalent performance of this test compared with the Triage PlGF and Elecsys immunoassay sFlt-1/PlGF ratio which would support its use in ruling out pre-eclampsia before 35 weeks' gestation. The rule out threshold that is equivalent to Quidel is 150 picograms/ml. It was not included the economic evaluation performed by NICE but will be considered next year.

BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio

This test combines the results from two different assays, to provide a ratio. The assays are run at the same time using the BRAHMS Kryptor compact plus analyser. The analyser

produces results for each assay and the sFlt-1/PlGF ratio. The manufacturer recommends that individual laboratories should establish their own reference ranges. The turnaround time is 9 minutes for the sFlt-1 Kryptor assay and 29 minutes for the BRAHMS PlGF plus Kryptor assay. The only available study using this test was performed by Andersen et al. in 2015. The efficacy of the BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio was compared with the Elecsys immunoassay sFlt-1/PlGF ratio and performed comparably, however further evidence is needed before considering adoption into routine practice.

Table showing overall comparison between tests

Comparator	Triage PlGF test	Elecys immunoassay sFLt-1/PlGF ratio	DELFIA Xpress PlGF 1-2-3 test	BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio
Turnaround time (minutes)	15	18	30	9 and 29
Cost reduction per patient compared to standard clinical assessment (£)	2, 896	2, 488	Insufficient data available	Insufficient data available
QC testing	Monthly	Daily	Insufficient data available	Insufficient data available
Range of gestation intended for use (weeks)	20-34+6	24-36+6	2nd and 3rd trimester	Not specified
Negative result rules out pre-eclampsia for how many days	14	7	Insufficient data available	Insufficient data available
Recommended rule out threshold	100*picograms/ml	38* (Ratio)	183*picograms/ml	
Relevant study	PELICAN	PROGNOSIS	COMPARE	Andersen et al.

- Not equivalent in clinical prediction

Incorporating PlGF-based tests into routine clinical practice has the potential to optimise the way we currently manage women with suspected pre-eclampsia. The availability of a test which enables healthcare providers to confidently rule out the development of pre-eclampsia means that those women with a negative result can be reassured and monitored outside of hospital. This avoids the anxiety of an uncertain diagnosis as well as the significant cost associated with an inpatient hospital admission. This has the potential for cost-savings for the NHS and more importantly a better care pathway for women. In addition, it would enable clinicians to target those women at high risk of preterm delivery and focus resources on the increased monitoring and surveillance that these women need. It is important to state that although PlGF-based tests have yielded promising results when used to predict diagnosis of pre-eclampsia requiring early delivery, further evidence is still needed to support this. Their proposed use at present is in being able to confidently rule out those women who do not have the disease.

The commercially available PlGF-based tests all provide similar information to clinicians but provide it in different ways. The two tests currently recommended for adoption in the NHS, the Triage PlGF test and the Elecys immunoassay sFLt-1/PlGF ratio each have their own advantages and disadvantages. The Triage PlGF test has the advantage of using a 'bedside' analyser but still requires laboratory facilities for the centrifuge. The Elecys immunoassay sFLt-1/PlGF ratio requires a large high-cost laboratory analyser but would be beneficial in a high throughput setting. This is similar for the DELFIA Xpress PlGF 1-2-3 test. Both NICE recommended tests offer a cost saving per patient compared to standard clinical assessment.

It is also relevant to note that PlGF exists in 4 different isoforms. The most important are PlGF 1 and PlGF 2. The commercially available PlGF-based tests mainly measure PlGF-1 but there is a degree of cross-reactivity with PlGF-2. An assay with high cross-reactivity to PlGF-2 maybe less likely to be specific in predicting pre-eclampsia and this is an area which requires further research.