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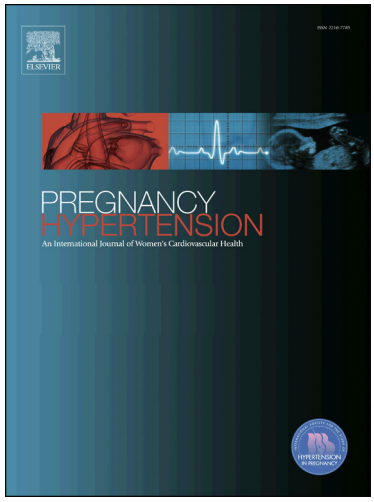

Prospective biomarkers in preterm preeclampsia: a review

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Title

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The authors have no competing interests to declare.

Abstract

Preterm pre-eclampsia (prior to 37 weeks' gestation) remains a major cause of maternal and fetal morbidity and mortality particularly in low to middle income countries. Much research has focused on first and second trimester predictors of pre-eclampsia with the aim of allowing stratification of antenatal care and trialling of potential preventative and therapeutic agents. However, none have been shown to be of benefit in randomised controlled trials.

In this literature review we critically evaluate predictive and diagnostic tests for preterm pre-eclampsia and discuss their clinical use and potential value in the management of preterm pre-eclampsia. We defined preterm pre-eclampsia as pre-eclampsia occurring prior to 37 weeks' gestation. Substantial progress has been made in the development of predictive screening tests for preterm pre-eclampsia, but further research is needed prior to their introduction and integration into routine clinical practice. The performance of diagnostic tests mainly utilising angiogenic and anti-angiogenic factors for determining time to delivery in later pregnancy currently hold more promise than first trimester predictive tests, possible reflecting the heterogeneity of pre-eclampsia.

Keywords

Hypertension, preterm pre-eclampsia, placental growth factor, pregnancy, screening.

Introduction

Pre-eclampsia is defined by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as hypertension and the coexistence of one or more of the following new-onset conditions; proteinuria (spot urine protein/creatinine >30 mg/ mmol or >300 mg/day or at least 1 g/L(2+) on dipstick testing); other maternal organ dysfunction including renal insufficiency, liver involvement, neurological complications, haematological complications; or evidence of uteroplacental dysfunction indicated by fetal growth restriction.[1] For the purposes of this review, preterm pre-eclampsia is defined as pre-eclampsia which occurs prior to 37 weeks gestation, though we acknowledge the current debate regarding this binary classification [2 3].

This hypertensive condition usually occurs during the second half of pregnancy, complicates 2% to 8% of pregnancies and remains a major cause of maternal and perinatal mortality and morbidity worldwide, accounting for 17-24% of all maternal deaths in low income settings.[4 5]

Preterm pre-eclampsia is thought to arise as a result of placental ischaemia which arises from impaired placentation (trophoblast invasion of the maternal uterine spiral arteries), which in turn leads to stimulation of sustained endoplasmic reticulum and

oxidative stress.[6-8] This pathophysiological cascade generates the characteristic systemic symptoms of the maternal disease; it is affected by genetic, behavioural or environmental influences and is mediated by release of factors into the maternal circulation.[9-12] In contrast, term pre-eclampsia is generally not associated with abnormal trophoblast invasion[7] and it is thought that other maternal constitutive factors or the pre-existing susceptibility of the maternal vasculature is responsible for the development of term pre-eclampsia[13].

There are currently few preventative strategies and no cure other than delivery of the placenta; for women presenting with disease preterm, this often results in iatrogenic delivery to prevent serious maternal or fetal adverse outcomes.

There have been wide ranging investigations into possible biomarkers for early identification of preterm pre-eclampsia [14]. The release of factors from the placenta into the maternal blood stream, or maternal generation of factors may precede clinical symptoms [14]. Therefore, there remains substantial interest in the use of these factors as potential biomarkers for subsequent disease. Initial research focused on angiogenic factors and placental-specific factors, whilst recent advances in the so called omics fields have led to further possible biomarkers [15 16]. Table 1 summarises the biomarkers discussed in this review. These biomarkers, alone or in combination with biophysical and sonographic findings, may allow development of a reliable and valid screening or diagnostic test for pre-eclampsia to enable risk stratification and timely use of

pharmacological interventions such as aspirin (or other novel therapies) to reduce the risk of pre-eclampsia.[17,18]

A difficulty when assessing studies which look at pre-eclampsia is that many do not subclassify pre-eclampsia into early onset and late onset. As noted in Wu et al, this results in a poorly defined phenotype of pre-eclampsia which may contribute to the low predictive value of studies to date. [19]Furthermore, as early and late onset pre-eclampsia are now believed to have pathogenetic mechanisms contributing in varying degrees, it is likely they are characterised by different biomarkers. This highlights the importance of stratifying by pre-eclampsia type in future studies of potential biomarkers.

Many proposed tests for the short and longer-term prediction of pre-eclampsia are currently under investigation, and some are now being offered in clinical practice. In this literature review we will critically evaluate predictive and diagnostic tests for pre-eclampsia and discuss their clinical use and potential value in the management of preterm pre-eclampsia. Studies are described in the text, with Table 1 providing further detail on statistical results.

Angiogenic factors

The placental hypoxia resulting from the impaired trophoblast invasion that occurs in pre-eclampsia results in an imbalance between pro- and anti-angiogenic factors, in particular, the anti-angiogenic soluble fms-like tyrosine kinase-1 (sFlt-1) and the pro-angiogenic vascular endothelial growth factor (VEGF) and placental growth factor (PlGF).[20-23] VEGF induces endothelial proliferation and vascular permeability by

inducing the expression of integrins on endothelial cells that are involved in angiogenic invasion.[24] In the placenta, it is involved not only in vascularisation, but also in trophoblast syncytialisation and proliferation of extravillous trophoblasts (as assessed in primary culture of trophoblasts and a cell line of human extravillous trophoblasts).[25,26]

The anti-angiogenic factor sFlt-1 is a splice variant of the VEGF receptor, Flt-1 that lacks both the transmembrane and cytoplasmic domains of the cell membrane bound receptor. sFlt-1 acts as a soluble receptor that binds PlGF and also binds and deactivates circulating VEGF.[27-30] Measurement of these angiogenic factors has resulted in several clinically commercially available assays.[29-32]

Chappell and colleagues previously investigated the diagnostic accuracy of low plasma PlGF concentration (using the Alere Triage assay) in women presenting with suspected pre-eclampsia between 20 and 35 weeks' gestation (and up to 41 weeks' gestation as a secondary analysis). Of 287 women recruited prior to 35 weeks’ gestation, PlGF <5th centile had high sensitivity (96%) and negative predictive value (98%) for pre-eclampsia leading to delivery within 14 days; specificity was lower (55%).[33]

For short term prediction of pre-eclampsia, a sFlt-1:PlGF ratio cut-off of 38 has been shown to have a negative predictive value for the development of pre-eclampsia in the subsequent week of 99.3% (95% CI, 97.9 to 99.9), with 80.0% sensitivity (95% CI, 51.9 to 95.7) and 78.3% specificity (95% CI, 74.6 to 81.7). The positive predictive value of an sFlt-1:PlGF ratio above 38 in women between 24 weeks and 36 weeks (and 6 days) of

gestation in whom pre-eclampsia was suspected, for a diagnosis of pre-eclampsia within four weeks was 36.7% (95% CI, 28.4 to 45.7), with 66.2% sensitivity (95% CI, 54.0 to 77.0) and 83.1% specificity (95% CI, 79.4 to 86.3).[32] A separate study demonstrated that PlGF (ROC 0.87, 95% CI 0.83–0.92), sFlt-1 (ROC 0.83, 95% CI 0.78–0.88) and endoglin (ROC 0.83, 95% CI 0.79–0.88) all have comparable results in the short term prediction for pre-eclampsia requiring delivery within 14 days in women with suspected preterm pre-eclampsia.[34]

Whilst the use of these tests for diagnosis is promising, predictive studies have had more mixed results. A prospective international multicentre study recruited 5121 participants between 2006 and 2009 who had risk factors for pre-eclampsia (nulliparity, diabetes, previous pre-eclampsia or chronic hypertension). Participants had their serum tested for sFlt-1, PlGF and endoglin concentrations and urine PlGF measured at fixed time-points of ≤20, 23-27 and 32-35 weeks' gestation. Of this cohort, 3.9% (198 women) developed pre-eclampsia, with 0.9% (47 women) developing pre-term pre-eclampsia. The median maternal serum concentrations of PlGF, sFlt-1, sFlt-1/PlGF and endoglin were significantly different in women who subsequently developed pre-eclampsia compared with those who did not. However, the areas under receiver operating characteristics curves at ≤20 weeks' gestation were poor for predicting any pre-eclampsia (0.52 and 0.59) or pre-eclampsia at less than 34 weeks' gestation (0.50 to 0.63)). The corresponding sensitivity, specificity and likelihood ratios were also poor.[35]

A further recent longitudinal prospective cohort observational study of angiogenic markers and Angiotensin II type 1 receptor antibodies (AT1AA) was conducted. In pre-eclampsia there is an enhanced vascular sensitivity to angiotensin II and norepinephrine resulting in vasoconstriction and hypertension. The enhanced sensitivity to angiotensin II may be secondary to increased bradykinin upregulation seen in preeclamptic patients. Hence it has been proposed as a potential biomarker.[36] Sequential recruitment of 351 women with a singleton pregnancy was undertaken. Plasma concentrations of women with a singleton pregnancy at 12, 18, 28, 36, 40 weeks' gestation and 6 weeks post-partum were assessed for levels of AT1AA, PlGF, sFlt-1 and endoglin. Women with pre-eclampsia had higher sFlt-1 from 28 weeks onwards (p= 0.003) and lower PlGF from 18 weeks (p=0.004). Endoglin and AT1AA concentrations did not vary over time or between groups.[37] PlGF compares favourably when used as a first trimester screening tool as evidenced by a recent systematic review which included a total of 103 studies and 432,621 participants. This reviewed the accuracy of serum biochemical markers PAPP-A, human Chorionic Gonadotropin, PlGF and Placental Protein 13 (PP13) in the first trimester to predict pre-eclampsia.[38] For pre-eclampsia, the best predictor was PlGF with a positive likelihood ratio of 4.01 (3.74, 4.28) and a negative likelihood ratio of 0.67 (0.64, 0.69).

Endothelial dysfunction

Endothelial cells are important in the systemic inflammatory response as well as mediating local inflammation by upregulating the secretion of cytokines and adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and E-selectin. Plasma concentrations of these adhesion cell molecules are significantly elevated in women with pre-eclampsia.[42-44] Elevations in these molecules have been demonstrated before clinical symptoms develop, suggesting they may serve as predictors of pre-eclampsia. One prospective study of samples from 57 pregnant women before and after 32 weeks' gestation demonstrated that VCAM-1 was significantly elevated in women developing hypertensive diseases as compared to normotensive women (pre-eclampsia: $p<0.001$; gestational hypertension: $p<0.05$; chronic hypertension: $p<0.001$).

Cell free fetal DNA

Cell free fetal DNA (cffDNA) refers to fetal DNA found in the maternal blood stream. This is now a minimally- invasive technique increasingly being used to screen for genetic anomalies. It has also been proposed as a biomarker for pre-eclampsia. A systematic review examined 13 studies of which 11 had found an increase in cffDNA among pregnant women who subsequently developed pre-eclampsia. In addition, all four studies examining early-onset or severe pre-eclampsia found significantly elevated

cffDNA levels prior to disease onset.[45] Additional recent case control studies examining first trimester cffDNA showed no association with first trimester cffDNA and subsequent development of pre-eclampsia.[46] However, these data conflict with a larger case control study supporting an association. In this study, compared with controls, the median levels and multiples of the median (MoM) values of HYP2, a cell free fetal DNA marker, were significantly higher in the pre-eclampsia and hypertensive disorders of pregnancy groups sampled at 6-14 and 15-23 weeks' gestation.[47] HYP2 is located on chromosome 13 and is hypermethylated in the placenta as well as in maternal blood cells. It has been studied as an epigenetic marker for total cffDNA [48].

Levine *et al* conducted a nested case control study within the Calcium for Pre-eclampsia Prevention trial cohort of healthy nulliparous women. One hundred twenty pairs were randomly chosen for analysis of serum cell-free fetal DNA (cffDNA), a marker of placental debris, and C-reactive protein (CRP), a marker of inflammation. Serum specimens were obtained from participants at 8 to 20 weeks (at enrolment in the trial), at 26 to 29 weeks, at 36 weeks if still pregnant, and when hypertension or proteinuria were noted and a diagnosis of pre-eclampsia confirmed. At 29 to 41 weeks of gestation, cffDNA concentrations were significantly higher after a diagnosis of pre-eclampsia was made than before (219 vs 112 genome equivalents [GE]/mL, P<.001). Before the diagnosis of pre-eclampsia was made, cffDNA levels in cases exceeded controls at 17 to 28 weeks (36 vs 16 GE/mL, P<.001). However, samples taken between 29 and 41 weeks were only significantly elevated within 3 weeks prior to the diagnosis of pre-eclampsia (176 vs 75 GE/mL, P<.001).[49] These data suggest that cffDNA may be useful

in the both early and late stage detection of preterm pre-eclampsia, as pre-eclampsia appears to be accompanied by a 2-stage elevation of fetal DNA.

Cotter *et al* reanalysed blood samples from women attending first antenatal clinics which were stored in a biobank. 88 women who subsequently developed pre-eclampsia (cases) and 176 matched controls were sampled at a mean gestation (+/-SD) of 15.7 +/- 3.6 weeks. The presence of fetal DNA in the maternal circulation in early pregnancy was associated with an 8-fold increased risk of developing pre-eclampsia.[50] This study did not differentiate between preterm and term pre-eclampsia.

Cell free RNA has also been proposed as a marker for pre-eclampsia. One study examined maternal plasma samples from 62 patients at a tertiary hospital in Indonesia from 15-20 weeks gestation who subsequently developed either early or late onset pre-eclampsia. These were compared with samples from 310 controls (unmatched subjects with a normal pregnancy) using a panel of messenger RNA markers. A receiver operating characteristic curve that was obtained with the estimated score for pre-eclampsia as a test variable yielded a detection rate of 84% (95% CI, 71.8-91.5) at a 5% false-positive rate with an area under the curve of 0.927 (P <0 .001).[51]

Immunological response, oxidative stress and inflammation

In pre-eclampsia, the systemic inflammatory response reported in uncomplicated normal pregnancies[52] becomes exaggerated resulting in abnormal activation of monocytes, platelets and the endothelium.[53] In addition, many acute phase inflammatory proteins increase including C-reactive protein, angiotensinogen, alpha-1-

acid glycoprotein, alpha-1-antitrypsin, caeruloplasmin and fibrinogen. Moreover, as part of the inflammatory response, microparticles (plasma membrane-derived vesicles) are released and elevated levels of circulating microparticles have been shown to correlate with the endothelial dysfunction documented in pre-eclampsia.[54,55]

During placentation, Natural Killer (NK) cells play an important role in the decidual inflammatory response. Decidual NK cells, which are a specialised subset of NK cells, are present pre-conceptually in the endometrium of the luteal phase[56]. Decidual inflammation contributes to the shallow placentation in pre-eclampsia by promoting excess decidual cell IP-10 (IFN-γ–induced protein 10), a chemokine. This has been shown to be elevated in the first trimester in a study of 90 women who subsequently developed pre-eclampsia.[57]

Oxidative stress is defined as an increase in the steady state levels of reactive oxygen species (ROS) and may occur as a result of increased free radical generation and/or reduced antioxidant activity. In pre-eclampsia there is an increase in the levels of placental oxidative stress and this oxidative stress may mediate endothelial cell dysfunction and contribute to the pathophysiology of pre-eclampsia.[58] In pre-eclampsia, oxidative stress may result from interactions between the maternal component (including pre-existing conditions such as obesity, diabetes, and hyperlipidemia) and/or the placental component that may involve secretion of lipid peroxides.[59] In a prospective observational study of 306 nulliparous women, markers of oxidative stress including cholesterol and caeruloplasmin levels taken at 14-16 weeks' gestation (sample 1) and

at 18-20 weeks (sample 2) were shown to correlate with the development of pre-eclampsia.[60]

Metabolomics and proteomics

Metabolomics, the scientific study of chemical processes involving metabolites, the intermediates and products of metabolism, has more recently been applied in the prediction of pre-eclampsia. Current metabolomic technologies have allowed the establishment of metabolic signatures of pre-eclampsia in early pregnancy. A two-phase discovery/validation metabolic profiling study from samples taken at 15 weeks' gestation demonstrated an odds ratio for developing pre-eclampsia of 36 (95% CI: 12 to 108), with an area under the receiver operator characteristic curve of 0.94 using a multivariate predictive model combining 14 metabolites.[61]

These findings were then validated using plasma obtained at 15 weeks' gestation from 39 women who subsequently developed pre-eclampsia and 40 similarly matched controls. In an independent case-control study the same 14 metabolites produced an odds ratio of 23 (95% CI: 7 to 73) with an area under receiver operator characteristic curve of 0.92. A phase IIa clinical study of predictive testing for pre-eclampsia to evaluate whether this leads to improved pregnancy outcomes through earlier detection is currently recruiting using this metabolomic platform.[16] The 14 metabolites included in this model were 5-Hydroxytryptophan, Monosaccharide(s), Decanoylcarnitine, Methylglutaric acid and/or adipic acid, Oleic acid, Docosahexaenoic acid and/or docosatriynoic acid, γ-Butyrolactone and/or oxolan-3-one, 2-Oxovaleric acid and/or oxo-

methylbutanoic acid, Acetoacetic acid, Hexadecenoyl-eicosatetraenoyl-sn-glycerol, Di-(octadecadienoyl)-sn-glycerol, Sphingosine 1-phosphate, Sphinganine 1-phosphate and Vitamin D3 derivatives.

Myers *et al* conducted a proteomics pipeline study which reported 8 validated models that predicted 50-56% cases of pre-eclampsia, centred on insulin like growth factor acid labile subunit [15]. A recent study suggested an association between predisposition to pre-eclampsia and cardiovascular disease, suggesting that this field may contribute to risk stratification of pregnant women for long term morbidities [62]. Pregnancy specific glycoproteins have also been investigated as potential predictors of early-onset pre-eclampsia.[63]

Placental-derived and other proteins

Other biomarkers proposed for pre-eclampsia include disintegrin and metalloprotease 12 (ADAM-12), inhibin-A and activin-A, pregnancy associated plasma protein A (PAPP-A) and placental protein 13 (PP-13).[64] PP-13 is a galectin that binds to proteins on the extracellular matrix between the placenta and the endometrium, and through its action as a lysophospholipase-A assists in placental implantation and maternal artery remodelling. PP-13 appears to be down-regulated in women with pre-eclampsia requiring early delivery.[65] A nested case control study of 446 controls and 44 cases who subsequently developed preterm pre-eclampsia (delivery prior to 35 weeks) yielded areas under the receiver operating characteristics curve for first trimester PP-13, PAPP-A and second trimester uterine artery pulsatility index respectively of 0.71 (95% CI,

0.63–0.79; P < 0.001), 0.59 (95% CI, 0.51–0.68; P = 0.076) and 0.86 (95% CI, 0.77–0.94; P < 0.001). Combining PP-13 and pulsatility index using logistic regression analysis yielded an area under the curve of 0.90 (95% CI, 0.84–0.96; P < 0.001) and a sensitivity of 0.79.[66] In one prospective study of early pregnancy activin A and inhibin A were significantly higher in preeclamptic patients than in the other groups (activin A: normotension: p<0.005; gestational hypertension: p<0.001; chronic hypertension: p<0.005) (inhibin A: normotension: p<0.005; gestational hypertension: p<0.001; chronic hypertension: p<0.01)[67] but these changes have not translated into a clinically applicable predictive or diagnostic test.

Misfolded proteins

Endoplasmic reticulum stress in the placenta, as in other cell types, leads to upregulation of the unfolded protein response pathway.[68-70] The unfolded protein response is a common cellular defence mechanism that promotes removal of unfolded or misfolded proteins to prevent potentially toxic accumulation. Buhimschi and colleagues recently proposed that the presence of elevated urine congophilia (which is thought to indicate the presence of amyloid protein, an aggregate of misfolded proteins) assessed using the Congo red 'dot' test was useful in the diagnosis of pre-eclampsia and the prediction of medically indicated delivery. In a prospective cohort study (n = 563) they demonstrated that Congo red retention (CRR) using a cut-off of ≥15% had a sensitivity of 85.9% (95% CI, 81.1 to 89.9), specificity of 85.0% (95% CI, 80.4 to 88.8), positive likelihood ratio of 5.7 (95% CI, 4.4 to 7.5), and negative likelihood ratio of 0.17

(95% CI, 0.1 to 0.2) in predicting pre-eclampsia requiring medically indicated delivery. The association of Congo red retention with medically indicated delivery remained significant after controlling for gestational age and maternal demographic characteristics in multiple logistic regression. Women with pre-eclampsia were sampled at a median of 34-36 weeks' gestation in this cohort.[71,72] However, a more recent study demonstrated that in addition to women with pre-eclampsia, women with chronic kidney disease without pre-eclampsia have elevated urine Congo Red retention compared to healthy pregnant women. Non-pregnant women with lupus nephritis also had elevated urine congophilia compared with healthy controls demonstrating that an elevated Congo Red retention may not be able to differentiate between these conditions.[73]

Combined screening

A recent systematic review and meta-analysis demonstrated the pooled sensitivity of all single biomarkers to be 0.40 at a false positive rate of 10%. The area under the Summary of Receiver Operating Characteristics Curve (SROC) was 0.786 . When a combination model was used, the predictive value improved to an area under the SROC of 0.893 .[19] A combination of biophysical, biochemical and ultrasound markers may provide a more useful screening test than a test of either component alone.

Screening by maternal characteristics and medical history alone identifies approximately 35% of all cases of pre-eclampsia and about 40% of pre-term pre-eclampsia cases, at a false-positive rate of 10%.[74] First trimester screening by maternal factors with biochemical (PAPP-A and B-hCG) and biophysical markers (blood pressure

and uterine artery Doppler) yields detection rates of all pre-eclampsia and pre-eclampsia requiring delivery before 37 and 34 weeks' gestation of 54%, 75% and 88%, respectively, at a false-positive rate of 10%.[75,76]

The Screening for Pregnancy Endpoints (SCOPE) study developed a predictive model that utilised clinical risk factors and putative biomarkers to predict pre-eclampsia in low risk nulliparous women [30]. A cohort of 5628 women was used to develop and validate a model to predict early onset pre-eclampsia, which included placental growth factor, mean arterial pressure, BMI at 14 to 16 weeks' gestation, the consumption of ≥3 pieces of fruit per day and mean uterine artery resistance index. The area under the ROC for this model in training and validation cohorts was 0.73 and 0.68 respectively, a modest prediction at best. This study concluded the model did not perform at a level acceptable for introduction to clinical practice, and that due to the low prevalence of pre-eclampsia, the test resulted in an unacceptable number of false-positive rates.

More recently, an analysis of a prospective cohort study of 35,948 women attending for their routine first hospital visit at 11-13 weeks gestation at two maternity hospitals in England was undertaken. . This cohort included 1058 pregnancies with pre-eclampsia (2.9%) demonstrated that prospective combined screening by maternal factors, uterine artery pulsatility index, mean arterial pressure, and placental growth factor predicted 75% (95% confidence interval, 70-80%) of preterm-pre-eclampsia and 47% (95% confidence interval, 44-51%) of term-pre-eclampsia, at a false-positive rate of 10%.[77]

Conclusion

Substantial progress in research has been made in recent years, which has considerably improved our understanding of the aetiology of pre-eclampsia. Findings from initial case-control studies require validation in prospective cohort studies and robust evaluation through randomised controlled trials (of a predictive or diagnostic test) before routine introduction into clinical practice. Increasingly, robust clinical studies are being performed assessing a variety of tools for the first and second trimester prediction of pre-eclampsia and point of care tests for assessing need for delivery. Measurement of angiogenic and anti-angiogenic factors remains the leading commercially available test to aid in the short-term diagnosis of pre-eclampsia requiring need for delivery within two weeks. Ongoing clinical trials will provide robust data on the clinical potential for first and second trimester prediction of pre-eclampsia.

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Table 1. Selected biomarkers and their clinical efficacy

	Clinical cutoff	Primary outcome	Sensitivity (95% CI)	Specificity (95% CI)	OR (95% CI)	ROC (95% CI)	+ LR (95% CI)	- LR (95% CI)	P value
Angiogenic markers									
PIGF [32]	<5th centile	Any PE diagnosis at 20-35/40 gestation	0.96 (0.89-0.99)	0.55(0.48-0.61)				0.98 (0.93-1.00)	
sFlt-1 : PIGF ratio [31]	Ratio>38	Prediction of PE in next 7 days	0.80 (0.52-0.96)	0.78 (0.75-0.82)				0.99 (0.98-1.00)	
sFlt-1, endoglin [33]		Prediction of preterm PE requiring delivery<14 days				0.83 (0.78–0.88) 0.83 (0.79–0.88)			
PIGF, sFlt, sFLT-1:PIGF, endoglin [34]	Combined predictor	Prediction of PE at <20/40 and <34/40 gestation				(0.52-0.59) (0.50-0.63)			
Endothelial dysfunction									

VCAM-1 [44]	Mean concentration	Comparison of women with hypertensive diseases to normotensive women, >32/40							<0.001
Cell free fetal DNA [48]	Mean concentration	Nested case control of women with PE, cffDNA concentrations at 29-41/40							<0.001
Cell free RNA [50]	Panel of mRNA markers, FPR 10%	Case control of women who developed early or late onset PE				0.93 DR 84 (71.8-91.5)			<0.001
Metabolomics									
14 metabolites* [60]	Predictive model	Discovery study, 15/40 gestation			36 (12-108)	0.94			
14 metabolites* [60]	Predictive model	Validation study, 15/40 gestation, case control			23 (7-73)	0.92			
Placental derived proteins									
PAPP-A [65]	Mean concentration	Nested case control, cases delivered<35/40 due to PE	.			0.59 (0.51–0.68)			0.076
PP-13 + UA pulsatility index [65]	Combined predictor	Nested case control, cases delivered<35/40 due to PE	0.79			0.90 (0.84–0.96)			<0.001
Misfolded proteins			.						
Urine congophilia [71]	Congo red retention >15%	Prediction of PE requiring medically indicated delivery, sampled at 34-36/40	85.9 (81.1 - 89.9)				5.7 (4.4-7.5)	0.17 (0.1-0.2)	
Combined screening			.	.					
Combined screening[+ 77]	FPR of 10%	Preterm PE; Term PE,	75.0 (70.0-80.0); 47.0 (44.0-51.0)	.					
Combined screening [$ 29]		Prediction of PE in low risk nulliparous women	.	.		0.68 (0.63-0.74)			

OR odds ratio; + LR positive likelihood ratio; - LR negative likelihood ratio; ROC area under the receiver operating characteristic curve; DR detection rate; PE: pre-eclampsia; *5-Hydroxytryptophan, Monosaccharide(s), Decanoylcarnitine, Methylglutaric acid and/or adipic acid, Oleic acid, Docosahexaenoic acid and/or docosatriynoic acid, γ-Butyrolactone and/or oxolan-3-one, 2-Oxovaleric acid and/or oxo-methylbutanoic acid, Acetoacetic acid, Hexadecenoyl-eicosatetraenoyl-sn-glycerol, Di-(octadecadienoyl)-sn-glycerol, Sphingosine 1-phosphate, Sphinganine 1-phosphate and Vitamin D3 derivatives; [+] maternal factors, uterine artery pulsatility index, mean arterial pressure, and placental growth factor; [$] placental growth factor, mean arterial pressure, BMI at 14 to 16 weeks' gestation, the consumption of ≥3 pieces of fruit per day and mean uterine artery resistance index

Highlights

- Preterm preeclampsia remains an important condition associated with substantial maternal and fetal morbidity and mortality, particularly in the developing world.
- Preeclampsia is thought to occur as a result of a two stage process: impaired trophoblast invasion resulting in placental ischemia, coupled and interacting with maternal constitutional factors including genetic, behavioural and environmental influences.
- Conflicting evidence exists regarding aetiology and potential biomarkers, which likely reflects the heterogeneity of the condition and the historical grouping of term and preterm preeclampsia in studies.
- The development of tests to date has focused on first or second trimester screening, and diagnostic tests to aid in the prediction of time to delivery.
- Placental growth factor and soluble fms-like tyrosine kinase-1 have provided the most promising results as a test to assist in determining need to delivery within a short time-frame.
- Placental growth factor in combination with clinical parameters is also useful in the first trimester prediction of preeclampsia; however, larger robust clinical trials are required.
- A phase IIa clinical study is currently being performed using a metabolomic platform. Robust clinical studies similar to this are required to examine other biomarkers.

- There is a need to address the translational gap between biomarkers which have shown promising results and the introduction using clinical trials into clinical practice.