

## Preeclampsia: An Old Disease with New Tools for Better Diagnosis and Risk Management

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Preeclampsia (PE)<sup>8</sup> is a hypertensive disorder of pregnancy. PE is a common disease in pregnant women and is considered to be a major cause of preterm delivery. While PE is cured only by placental delivery, patients with stable disease can be managed up to 37 weeks of gestation by antihypertensive drugs. Successful management of PE greatly reduces maternal and fetal morbidity and mortality. Delayed treatment can lead to eclampsia and life-threatening complications such as stroke and pulmonary edema, which underscore the importance of early detection.

Several factors increase the risk of PE, including family history, but the role of genetics in PE is not well defined. Other risk factors include previous PE, chronic hypertension, diabetes, renal disease, and metabolic syndrome. Nulliparity is also linked to increased risk of PE, in which the mother's immune system is thought to play a role in the initiation or progression of PE. Patient stratification on the basis of risk factors may help in the early detection and better management of PE.

Currently there are no effective tools for the prediction or early detection of PE. Recent advances in PE research uncovered novel molecular events that contribute to the pathogenesis of this disease. PE patients were found to have high concentrations of antiangiogenic factors [e.g., fms-like tyrosine kinase 1 (sFlt-1)] and low concentration of angiogenic factors [e.g., placental growth factor (PLGF)], which initiates a state of angiogenic imbalance, placental insufficiency, and hypertension. Preliminary clinical studies showed that PLGF alone or the degree of imbalance between sFlt-1 and PLGF can be used in the prediction and early detection of PE. These biomarkers

are very promising, but their clinical utility awaits confirmation of their role in reducing maternal and fetal morbidity and mortality in PE patients.

PE is caused by multiple mechanisms. In addition to the angiogenic imbalance, placental immune intolerance, autoantibody-induced activation of the angiotensin II pathway, and hypoxia also participate in the pathogenesis of PE. This multiplicity of pathogenic mechanisms suggests the complexity and ambiguity of PE development. Thus, more studies are warranted to determine if these mechanisms act in isolation or together and whether their cooperation, if any, defines the severity of the disease. These studies are essential to better understand the pathogenesis of PE and to develop novel protective, therapeutic, and diagnostic strategies for this highly prevalent disease. In this Q&A we discuss the pathobiology and clinical aspects of PE with 3 experts.

### *Do you think there is a role of genetics in the pathogenesis of PE?*



**Ananth Karumanchi:** Yes, I believe there is a role for genetics in the pathogenesis of PE. Pregnant women with a family history of PE are approximately 2-fold more likely to develop the disease. Twin studies estimate the heritable component of PE to be >50%. Likewise, men who were the prod-

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<sup>8</sup> Nonstandard abbreviations: PE, preeclampsia; sFlt-1, fms-like tyrosine kinase 1; PLGF, placental growth factor; NK, natural killer; Tregs, T-regulatory cells; VEGF, vascular endothelial growth factor; sVEGFR-1, soluble VEGF receptor type 1; sEng, soluble endoglin.

uct of PE pregnancies are more likely to father a PE pregnancy. Taken together, these data suggest that both maternal and fetal genetic factors contribute to PE. With the availability of cheaper next generation sequencing technologies, I am hopeful that functional variants in disease pathogenesis will be discovered in the next 1–2 years.



**Andrew Shennan:** Genetics is clearly related since family history, for example in mother or sister, is one of the most potent risk factors for PE. PE depends on the fetal/placental unit inducing disease in the mother. It therefore requires the stimulus from the pregnancy, coupled with the mother being susceptible in manifesting the signs and symptoms of the disease. Although we are increasingly aware of data from studies showing that both mothers' and fathers' genetics contribute to PE risk, it is not clear if this relates to the baby or is just a factor in the mother making her susceptible to becoming unwell. Genetics, for example, may increase risk of clinical factors that are also risk factors for PE, such as diabetes, obesity, and hypertension. It is therefore likely that genetics will be complex and not a simple explanation of the disease. Even if a genetic test were available, how this would be used in clinical practice remains unclear.



**Robert Taylor:** PE is a familial disorder, involving multiple genes in many biological pathways. Family reports, twin studies, segregation analyses, linkage analyses, genome-wide association studies, and next generation sequencing all support a genuine genetic basis. Unfortunately, complex gene–gene interactions and epigenetic influences of the environment continue to obfuscate straightforward explanations of how a woman's DNA influences her likelihood to develop PE. Heritability studied in a Utah genealogy database revealed that both men and women who were the product of a pregnancy complicated by PE were significantly more likely than control men and women to have a child who was the product of a pregnancy complicated by PE. The coefficient of kinship was more than 30 SDs higher for offspring of PE cases than for controls. Genetics may,

in part, explain the higher rate of PE in African American mothers of all socioeconomic levels relative to the general population in the US.

*Some reports suggest the involvement of the immune system in the development of PE. What is the mechanism by which the immune system causes PE? Can we use this knowledge in the treatment, diagnosis, and early detection of PE?*

**Ananth Karumanchi:** Natural killer (NK) cells, which contribute to nearly 70% of the immune cells in the placenta during early pregnancy, are thought to play a critical role in the genesis of PE. The best evidence for this hypothesis comes from human epidemiological studies that suggest that the presence of certain NK-cell receptor haplotypes and their ligands are associated with a higher incidence of PE. Mouse studies have suggested that uterine NK cells play a role in spiral artery remodeling that occurs during physiological pregnancy, a process that is defective in PE. However, it is not yet known whether correction of NK-cell abnormalities will improve placental vascular abnormalities and cure or prevent PE. More recently, a role for T-regulatory cells (Tregs) in the development of PE has been proposed. It is not known if the alterations in Tregs are the cause or effect of the disease. Thus, while there are some exciting data for a pathogenic role for immune cells in PE, more data are needed before we can target NK cells or T cells for either prediction or treating PE today.

**Andrew Shennan:** The immune mechanism is largely hypothesized, although there is increasing research suggesting some immune pathways maybe responsible for the disease, but this research is not well developed. Given our observations of the clinical disease, an immune mechanism is plausible. Increased exposure to their partner seems to reduce risk in women, evidenced by less PE in multiparous women, and those who have cohabited longer, possibly inducing some immune-mediated suppression. However, treating immune diseases usually involves suppression or alteration of the immune response, and steroids and other immunosuppressants do not alter the disease onset. Indeed, an active immune response may be necessary for a successful pregnancy. Novel methods to treat the disease via this mechanism would need more basic science to further elucidate exact pathways.

**Robert Taylor:** Several epidemiological and clinical observations suggest that the immune system is intimately involved in the etiology of PE. First pregnancy, short duration of cohabitation, barrier contraception, donor gamete, and pregnancy with a new partner all support the hypothesis that unrecognized antigen exposure deriving from the conceptus predisposes to PE. Both the innate

and adaptive immune systems appear to contribute to these effects. Innate immunity constitutes a primitive and rapid response to danger signals. Of these, oxidative stress is one of the most relevant proinflammatory triggers in the setting of PE. Adaptive humoral factors also have been identified. Agonistic angiotensin type 1 receptor autoantibodies have been identified in the circulation of women with PE that stimulate vascular NADPH oxidase and reduce the bioavailability of nitric oxide by uncoupling its synthesis. Circulating endothelin-1 is increased in PE relative to normal pregnancy and indoleamine 2,3-dioxygenase, an enzyme that catabolizes tryptophan, is reduced, diminishing the inhibitory action of tryptophan depletion and resulting in immune cell activation. Tregs are a class of suppressor T cells. PE has been associated with a relative deficiency in Tregs, contributing to a proinflammatory environment with increased interleukin-17. To date, neither clinical quantification nor manipulation of these factors has led to new diagnostic or preventative therapies, but enthusiasm for their potential utility remains high.

***Angiogenic imbalance participates in the pathogenesis of PE, but the initiating event(s) of this imbalance are largely unknown. What are the potential triggers of this angiogenic imbalance?***

**Ananth Karumanchi:** Animal studies have demonstrated that placental ischemia is a major trigger for the induction of the angiogenic imbalance noted in PE. Placental ischemia leads to the upregulation of hypoxia-inducible transcription factors that control expression of sFlt1, a key antiangiogenic factor that is abnormally expressed in PE. In addition, several nonhypoxic mechanisms have been proposed, such as altered hemoxygenase expression and abnormal concentration of autoantibodies that activate the angiotensin II signaling pathway. These nonhypoxic pathways have been shown to upregulate sFlt1 in cell culture and animal models. However, because of the lack of longitudinal data, we do not know the exact trigger for the angiogenic imbalance in humans with PE.

**Andrew Shennan:** Immune and genetic susceptibility as a result of the maternal/paternal antigen interface is probably the initiating factor that alters placental production of these biomarkers. Research has suggested that the hypoxia generated by the placenta with the associated reperfusion can alter the production of angiogenic markers, but there may be more than this mechanism since biomarkers are altered early on in pregnancy, long before this pathogenesis can have any substantive effect.

**Robert Taylor:** Over the past decade there has been considerable interest in the balance of angiogenic and anti-

angiogenic proteins in PE. One of the most prominent of these factors is the circulating soluble vascular endothelial growth factor (VEGF) receptor type 1 (sVEGFR-1 or sFlt-1). Alternative splicing of the Flt1 gene product can result in the production of this truncated antiangiogenic receptor that antagonizes VEGF and PLGF by preventing the interaction of these ligands with full-length receptors. Systemic concentrations of sFlt1 in patients with PE are greatly increased before delivery and decrease to baseline 48–72 h after delivery. There is increasing evidence that increased sFlt1 plays a major pathogenic role in the endothelial dysfunction of PE. It is possible that syncytiotrophoblast is a direct source of sFlt-1. Syncytiotrophoblast microvesicles are shed in significantly increased amounts in PE relative to normotensive pregnancies, and these carry sFlt-1. Hence, shedding of microvesicle debris from the syncytial surface, in response to increased placental mass (e.g., twins) or oxidative stress (e.g., in women with chronic microvascular disease), might explain, in part, the effects of these risk factors on PE prevalence. Other circulating antiangiogenic factors have been described in PE, including endostatin (a circulating fragment of collagen XVIII), prolactin fragments (referred to as vaso-inhibins), and semaphorin 3B (a trophoblast-secreted antiangiogenic protein). Each of these proteins is potentially derived from the uteroplacental interface, suggesting that abnormal placentation may be the trigger for angiogenic imbalance.

***There are several factors that contribute to the pathogenesis of PE. Do these factors work together to initiate PE and would this cooperation define the severity of the disease?***

**Ananth Karumanchi:** There is evidence that synergistic factors such as soluble endoglin (sEng) cooperate with sFlt1 to induce the maternal syndrome of PE. It also appears that predisposing factors contribute to PE by sensitizing the maternal vascular endothelium to the antiangiogenic effects of sFlt-1. Such predisposing factors might include obesity, preexisting hypertension or renal disease, diabetes, and preexisting vasculitis.

**Andrew Shennan:** Risk factors can't be used to define severity, i.e., many women with no risk factors get severe disease and vice versa. How other factors work together remains an enigma. Certain groups of women are more susceptible to PE, so clearly their underlying conditions must contribute to the pathogenesis. Any treatment strategy is best directed at high-risk women, and using risk factors per se is a sensible approach to direct therapies. For example, the National Institute for Clinical Excellence in the UK recommends routinely prescribing aspirin to high-risk groups, and even to lower risk groups when risk factors are combined. For example, if a woman

is nulliparous and obese (body mass index >35), 75 mg aspirin is recommended until delivery. Twins are also more likely to “cause” PE, so fetal considerations must also be important. Women with multiple risk factors still represent only a small minority of the women who get PE.

**Robert Taylor:** PE is best understood as a multifactorial, polygenic, systemic maternal condition rather than as a Mendelian disease with a single allelic mutation directly causing the disease phenotype. Complex diseases are typically the result of many common variants or at multiple loci, as well as environmental and other susceptibility factors. It would seem likely that multiple biochemical pathways and many different biological molecules (and possibly some environmental influences) contribute to the continuum of phenotypes observed in PE, with those patients in whom some additive threshold is exceeded classified as having the disease.

*Recent studies showed that sFlt-1 and PLGF are promising biomarkers for the screening, diagnosis, and risk management of PE patients. Are there other emerging biomarkers that may enhance the diagnosis and early detection of PE in the future?*

**Ananth Karumanchi:** Discovery of biomarkers that are altered early and specifically in PE is an active area of research in academic laboratories as well as diagnostic companies. Emerging biomarkers that have shown promise include certain combinations of metabolites and complement factors, but data demonstrating clinical utility from large prospective studies are still lacking. Since large prospective studies are expensive, there is a move, largely pioneered by Dr. Jim Roberts and Dr. Annetine Staff, to create a global pregnancy biobank where researchers from several fields can collaborate and use established well-phenotyped samples to validate their discoveries. For early first-trimester screening, it is likely that multiple markers will be needed and, therefore, collaborative approaches are critical for advancing the field.

**Andrew Shennan:** There have been many markers investigated in the pathogenesis of PE. There is an argument that using a combination of pathways might enhance prediction, as those cases missed by one marker maybe identified by another using a different pathway. For the same reason good markers using the same pathway may not add value in combination, i.e., they are not independent. In reality, the prediction of some markers is so good that it is difficult to add value by combining additional ones. For example, at the time of suspected PE, low PLGF has high diagnostic sensitivity for women who will require delivery in the short term (<2 weeks). When diagnostic sensitivity is already 95% it is hard to add

value with other markers, and multiple markers have been directly compared to PLGF and not shown added value. Any benefit may be marginal and may not justify the creation of a delay from having to do additional assays, which may affect clinical decision-making.

**Robert Taylor:** A plethora of biophysical and biochemical biomarkers has been promulgated over the years to aid in the identification or management of women with PE. These have included candidate targets related to maternal vascular resistance (e.g., “roll-over” test, isometric exercise, and angiotensin II sensitivity tests), blood flow (transcranial and uterine artery Doppler velocimetry), and proteins reflecting the uteroplacental interface (e.g., human chorionic gonadotropin,  $\alpha$ -fetoprotein, estriol, inhibin A, activin A, and placental protein 13), maternal renal function (uric acid, microalbuminuria, kallikrein, and podocyturia), maternal endothelial cell function (cellular fibronectin, endothelin, homocysteine), and angiogenic/antiangiogenic factor balance (PLGF, VEGF, sFlt-1, sEng). Emerging biomarkers are likely to be identified through more agnostic approaches such as transcriptomics, proteomics, and metabolomics. A 2010 SCOPE (Screening for Pregnancy Endpoints) study used a 2-phase, metabolomic biomarker strategy to predict PE. Plasma samples analyzed by HPLC–mass spectrometry identified 14 metabolites with a diagnostic sensitivity of 73% at a fixed 90% diagnostic specificity. The same group validated 8 biomarker models with diagnostic sensitivities ranging from 53% to 67% at a fixed diagnostic specificity of 80% for predicting PE.

*sFlt-1 and PLGF are already on the test menu of several automated analyzers. Do you think this is the prime time for these biomarkers?*

**Ananth Karumanchi:** The greatest utility for sFlt1 and PLGF in the clinic today is to provide prognostic information in patients with suspected PE. Several prospective studies have provided compelling evidence that the concentrations of sFlt1 and PLGF closely correlate with the development of adverse maternal and fetal outcomes related to PE among patients presenting with suspected PE. This would be analogous to using proBNP (pro-brain natriuretic peptide) or troponin among patients presenting with cardiac symptoms in the emergency room. Since these markers correlate with the duration of pregnancy, obstetricians can use these assays as an aid during expectant management of preterm PE. Another utility for angiogenic markers is to distinguish PE from diseases such as diabetic kidney disease and/or lupus that mimic PE. It is my belief that we will not need invasive renal biopsies to differentiate PE from other kidney diseases. There is a lot of excitement in the field to use PLGF along with maternal risk factors and uterine artery flow data during

the first trimester to accurately screen patients at risk for development of the early-onset subtype of PE. However, not all studies have confirmed this approach, and we do not have evidence that accurate screening early in pregnancy will lead to improved maternal and/or fetal outcomes. It is worth noting that the majority of the clinical data for use of angiogenic markers in PE have come from Europe, where automated assays are available in the clinic. In the US, these assays are not yet approved by the US Food and Drug Administration, and hence experience is limited.

**Andrew Shennan:** Yes, these markers are clearly an excellent PE test in women with suspected disease and supersede all the blood tests we currently use to define and risk discriminate within suspected disease. Other tests are “downstream checking for damage” and nearly all cannot rule out the disease. Using these new markers in clinical practice will prevent a high amount of overmanagement and allow resources and interventions to be targeted to where they are really needed. The current data suggest that these markers may also be good discriminators of risk for the baby in suspected PE. Previously, few tests have shown good correlation with fetal risk. A more challenging question is, could these markers be useful in women who have established PE? Among women with PE, many will not have poor outcomes, particularly at later gestations, and further work needs to establish if the markers could be used in those presenting with the disease (rather than with suspected disease). The earlier promising studies need to be confirmed, and ideally tests introduced in a fashion that evaluates their economic and clinical impact, e.g., in a stepped-wedge randomized controlled trial.

**Robert Taylor:** In 2012 a systematic review and meta-analysis was published by Kleinrouweler et al. to assess the accuracy of angiogenic factors in the prediction of PE (*BJOG* 2012;119:778–87). Studies that measured PLGF, VEGF, sFlt-1, or sEng in serum or plasma of pregnant women before 30 weeks of gestation were considered. Data were assessed as diagnostic odds ratios (the

ratio of the odds of positive test results in women who developed PE relative to the odds of positive test results in those who did not develop PE). In 34 studies that met the inclusion criteria, overall concentrations of PLGF and VEGF were lower in those who subsequently developed PE, whereas concentrations of sFlt-1 and sEng were higher. Diagnostic sensitivities and specificities ranged from 18% to 95%. The authors concluded that these factors had a poor predictive accuracy for PE. In a recent nested case-control study of 63 women who developed PE and 252 unaffected controls, the diagnostic sensitivities of angiogenic factors varied from 26% to 45%. Overall, the predictive performance of maternal circulating concentrations of angiogenic factors, as a single test, is not clinically useful to predict PE as a whole, but ratios of angiogenic and antiangiogenic factors could be of value in the identification of women destined to develop early-onset PE.

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