

Redefining Preeclampsia Using Placenta-Derived Biomarkers

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Preeclampsia affects 3% to 8% of all pregnancies.¹ Acute maternal complications include eclampsia, stroke, placental abruption, disseminated intravascular coagulation, HELLP (hemolysis, elevated liver enzymes, low platelets), liver hemorrhage or rupture, pulmonary edema, adult respiratory distress syndrome, acute renal failure, and death.² Preeclampsia complications account for more than 50 000 maternal deaths annually.^{2,3} In developing countries, where lack of access to appropriate maternal care is a major problem, maternal death rates are as high as 15% as compared with 0% to 1.8% in industrialized countries.² Perinatal consequences include stillbirth, preterm delivery, fetal growth restriction (FGR), neonatal complications, and later sequelae.⁴ Long-term maternal risks include chronic hypertension, diabetes mellitus, coronary artery disease,⁵ neurological deficit, and premature death.²

Here, we argue that the classic definitions of preeclampsia, based on concepts that are now more than 50 years old, have become outdated and that the definition could be modernized to take account of our current understanding of disease pathophysiology. We propose a first step that incorporates the placental biomarker placenta growth factor (PlGF), but we allow for the possibility that the definition may need to be expanded to include other factors, such as the antiangiogenic factors, soluble fms-like tyrosine kinase-1 (sFLT1) or soluble endoglin (sENG),⁶ in due course. This is intended as an exploratory rather than a final development.

Definition and Diagnosis of Preeclampsia

Diseases may be defined and classified by cause, pathogenesis, or by clinical findings. Clear definitions and classification are difficult when pathogenesis is unknown. As a result, diagnostic labels may reflect only a set of symptoms and signs, defining a syndrome. Syndromes are never precise, because the features are multiple, nonspecific, and therefore may have diverse causes. Preeclampsia is a syndrome of new onset hypertension and proteinuria in the second half of pregnancy, that was defined more than half a century ago.⁷ Despite advances in the understanding of preeclampsia, our current definitions remain substantially unaltered.

The definitions of preeclampsia and other hypertensive disorders of pregnancy⁸⁻¹⁰ are based on thresholds of blood

pressure and proteinuria before and during the second half of pregnancy. The current definition of preeclampsia from the American College of Obstetricians and Gynecologists⁹ specifies de novo hypertension (>140/90 mmHg) and proteinuria (>0.3 g per 24 hours) after 20 weeks of gestation, and the definition of preeclampsia of the International Society for the Study of Hypertension in Pregnancy is similar.⁸ Both these definitions are under revision. The Australasian Society for the Study of Hypertension in Pregnancy and the Society of Obstetricians and Gynaecologists of Canada has adopted a broader approach to encompass the variability and multisystem involvement of preeclampsia.^{11,12} In essence, the syndrome as defined by these groups is extended to include new onset hypertension not only with new proteinuria, but also new maternal or fetal features (≥ 1), such as renal insufficiency, hepatocellular dysfunction, or FGR. This was the first time that fetal involvement was included as a possible component of the syndrome. The definitions of preeclampsia may be used either for clinical practice (in which case clinical judgment contributes substantially) or for research, where objective criteria are mandatory to ensure uniform application by different investigators. In this study, we use the term classic preeclampsia when we mean the usual definition of new onset hypertension and proteinuria.⁹

Preeclampsia has been subclassified by clinical severity determined by maternal and fetal characteristics.⁹ For example, classic preeclampsia is graded as severe if complicated by FGR, because it has higher perinatal morbidity and mortality than preeclampsia without FGR.¹⁰ Also, a clinical subclassification into early and late onset of preeclampsia is widely used (see Heterogeneity of Preeclampsia below).

As with all syndromes, there can be no single gold standard by which the merits of different definitions can be judged. Also, above the thresholds of blood pressure and proteinuria that define preeclampsia, further increments do not correlate well with more severe adverse maternal and perinatal outcome.¹³ In fact, there are cases of normotensive preeclampsia,¹⁴ with unaffected blood pressure, but similar placental pathophysiology to classic preeclampsia. It is well known that both eclampsia and the HELLP syndrome may occur without premonitory hypertension or proteinuria.^{12,15,16}

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Pathogenesis of Preeclampsia

Maternal Systemic (Vascular) Inflammatory Responses: Normal Pregnancy and Preeclampsia

Compared with the nonpregnant state, normal pregnancy is characterized by a low-grade systemic inflammatory response, which is further enhanced in preeclampsia.¹⁷ It is important to recognize that inflammation is a multisystemic integrated response which, when manifested in the circulation, activates endothelial cells as well as inflammatory leukocytes and platelets, inducing changes in coagulation proteins and complement^{18,19} as well as changes in circulating proinflammatory cytokines.^{20,21} Oxidative stress is both a cause^{22,23} and consequence of inflammation. Systemic inflammation induces other metabolic adaptations, including increased insulin resistance.²⁴ All of these changes are characteristic of preeclampsia and have been reviewed elsewhere.^{24–28}

Role of the Placenta

The placenta is both necessary and sufficient for the development of preeclampsia. A fetus is not required, because preeclampsia occurs with hydatidiform mole,²⁹ and preeclampsia is also reported for pregnancy implanted outside the uterus.³⁰ The syndrome resolves after delivery or attrition of the placenta. How the placenta contributes to the syndrome seems to vary and has led to the broad concepts of placental or maternal preeclampsia. The former is hypothesized to reflect an interaction between an abnormal placenta and a normal maternal vasculature; the latter is hypothesized of a normal placenta in the setting of an abnormal maternal vasculature. Throughout this paper, maternal and placental preeclampsia refer to concepts that are hypothetical rather than explicit diagnoses. The dichotomy is also somewhat artificial, because in most cases there is varying contribution of both causes. The possible synergistic interactions between the maternal and placental preeclampsia have been previously presented from a theoretical analysis.³¹ In fact, maternal–placental preeclampsia, when both processes are clearly evident, are associated with the most severe early forms and early onset of the disorder. Intrinsic to these concepts is the evidence that even a normal placenta is an inflammatory burden to the mother. This burden increases with advancing gestation as the placenta grows. Hence, from this perspective, preeclampsia is not a fundamentally different state from normal pregnancy, but one where placental-induced changes are exaggerated to the point of decompensation.¹⁷

Maternal perfusion of the placenta depends on ≈30 to 60 uteroplacental spiral arteries,³² which may be affected by 3 related, relevant pathologies: poor placentation, acute atherosclerosis, and thrombosis, which will be described below.

Placentation and Placental Preeclampsia

During placentation, between weeks 8 and 18, the placental bed and its spiral arteries are invaded by mononuclear extravillous fetal cytotrophoblasts. This invasion into the decidua (the endometrium of pregnancy) and the inner third of the myometrium occurs either interstitially or via the blood vessels.³³ The endovascular cytotrophoblast enters the lumina of the spiral arteries, which are extensively remodeled in their inner myometrial and terminal decidual segments. The invaded

segments lose their smooth muscle and become widely dilated, which reduces the velocity, pressure, and pulsatility of uteroplacental flow, while spiral artery blood volume flow is calculated to increase only modestly.³² In preeclampsia, endovascular trophoblast invasion is restricted to the peripheral, decidual segments of the spiral arteries,³⁴ which are incompletely remodeled³³ and retain their smooth muscle and elastic lamina. These incompletely remodeled spiral arteries remain more tortuous, thick-walled, and less dilated than the normally transformed arteries. Burton proposed that this dysfunctional flow may not cause chronic placental hypoxia, *per se*,³² but that the retention of vasoactive smooth muscle in the vessels results in intermittent hypoperfusion leading to oxidative stress. Furthermore, the intervillous space is hydrodynamically stressed by high-velocity perfusion, secondary to the minimally dilated terminal segments of the spiral arteries. The release of a number of trophoblast-derived factors is stimulated by this placental stress, and these factors contribute to the exaggerated maternal inflammatory response seen in preeclampsia.⁶ Such placental-derived factors include the antiangiogenic proteins, sFLT1 or soluble vascular endothelial growth factor (VEGF) receptor 1³⁵ and sENG,³⁶ as well as proangiogenic PIGF or PGF. The maternal circulating concentrations of sFLT1 and sENG are elevated in preeclampsia, whereas the circulating concentrations of free PIGF are lower (see Circulating PIGF in Normal and Complicated Pregnancies) compared with normotensive pregnancies. Current assays lack sensitivity enough to measure the low levels of free plasma VEGFA in pregnancy^{37,38} or cannot measure the relevant isoforms.³⁹ The net result of this angiogenic imbalance is speculated to increase maternal vascular inflammation with generalized endothelial dysfunction⁴⁰ and induce the maternal signs of preeclampsia, including *de novo* onset of hypertension and preeclampsia.

In contrast, maternal preeclampsia is not necessarily associated with abnormal placentation and inadequate perfusion. Maternal endothelial dysfunction is already present, because of preexisting vascular dysfunction, and is further exacerbated as a result of the physiological burden of pregnancy.⁴¹

The effect of poor placentation has been incorporated into a 3-stage model of preeclampsia.⁴² Incomplete tolerization to the allogenic fetus, presumed to occur very early in pregnancy (Stage 1), is thought to underlie incomplete placentation with reduced remodeling of maternal uteroplacental spiral arteries (Stage 2). Enhanced placental oxidative and endoplasmic reticulum stress ensues, with release of diverse placental factors into the maternal circulation that cause excessive systemic inflammation, endothelial dysfunction, and the signs of preeclampsia (Stage 3).⁴³

Acute Atherosclerosis and Spiral Artery Thrombosis

Acute atherosclerosis is a lesion confined to the distal ends of spiral arteries that are not remodeled more proximally.³² Acute atherosclerosis consists of subendothelial foam cells (lipid-filled, CD68-positive macrophages), fibrinoid necrosis, and perivascular lymphocytic infiltration.⁴⁴ Systemic vascular inflammation, combined with a proatherogenic profile of plasma lipids, is present in normal pregnancy, but exaggerated in preeclampsia.^{45,46} Not all preeclamptic women develop acute

atherosis,^{45,47} and some pregnancies without preeclampsia show acute atherosclerosis, especially when complicated by FGR.⁴⁸ It has also been documented in first trimester decidual vessels with the antiphospholipid syndrome,⁴⁹ at a time when preeclampsia by definition cannot occur. These observations are consistent with a localized inflammatory response in the uteroplacental spiral arterial wall, similar to the processes also occurring in atherosclerosis.^{40,50}

Acute atherosclerosis would be expected to have a major impact on intervillous blood flow, as it substantially reduces vessel caliber.^{32,51} The lesion predisposes to local thrombosis and complete arterial obstruction, leading to placental infarction downstream of the occluded spiral artery.^{52,53} Thus, dysfunctional perfusion of the placenta attributable to poor placentation is likely to be exacerbated by acute atherosclerosis.

Other Placental Pathology: Trophoblast Oxidative Stress and Necrosis

In the proposed model of abnormal spiral artery remodeling in preeclampsia, syncytiotrophoblast (the multinucleated placental microvillous epithelium in direct contact with maternal blood) is subjected to oxidative stress and may show focal necrosis. In severe cases, there are substantial morphological changes in syncytiotrophoblast,⁵⁴ and evidence of endoplasmic reticulum⁵⁵ and oxidative stress.^{25,26,56–62}

Among the stress responses of syncytiotrophoblast, the most relevant to preeclampsia include increased apoptosis,⁶³ and secretion of antiangiogenic⁶⁴ and proinflammatory products,⁶⁵ which together contribute to the maternal syndrome. Circulating syncytiotrophoblast–derived extracellular vesicles are increased in preeclampsia and are likely to further amplify these maternal inflammatory responses.⁶⁶

Heterogeneity of Preeclampsia

Preeclampsia is heterogeneous in its presentation as well as in its association with long-term consequences for mother and child. Two broad types of preeclampsia have been suggested: placental and maternal.⁴¹ It is generally agreed that poor placentation is strongly associated with FGR, even in the absence of preeclampsia, but it is less clearly documented in association with preeclampsia and normal fetal growth. Thus, when Ness and Roberts pointed out that 70% of infants of preeclamptic women do not show FGR, this suggested that abnormal placentation is not likely to be associated with the majority of cases of preeclampsia.⁴¹ It later emerged that FGR is a feature of early, not term, disease,^{67,68} suggesting that poor placentation is more likely to underlie this presentation.

Most preeclampsia occurs at term, that is, after 37 weeks' gestation.^{69,70} Although term preeclampsia is less often associated with placental dysfunction, severe maternal complications can still occur. For example, 20% of cases with HELLP syndrome⁷¹ and 55% of cases with eclampsia¹⁵ occur at term. In other words, term preeclampsia is not benign just because the fetus is less threatened by FGR, such as in early-onset preeclampsia.

Long-Term Health After Preeclampsia

After a preeclamptic pregnancy, both offspring and mothers have increased risks of long-term cardiovascular risk.⁷² In a

systematic review and meta-analysis, Bellamy et al⁷³ found a relative maternal risk of 3.7 for hypertension, 2.2 for ischemic heart disease, 1.8 for stroke, and 1.8 for venous thromboembolism, 5 to 15 years after preeclampsia. The association between preeclampsia and subsequent cardiovascular mortality and morbidity strengthens with more severe preeclampsia, including early onset, recurrent disease, and neonatal morbidity.^{74–78} The risk for coronary heart disease, stroke, and other cardiovascular events is the highest among women who develop both maternal signs of preeclampsia (hypertension and proteinuria) and manifest abnormal placentation function, such as FGR, especially with preterm delivery.⁷⁹ There are also substantial consequences for the child in later life. Preeclampsia may lead to premature delivery, FGR, or both. Although the risk of later hypertension is increased with preterm delivery, with or without preeclampsia, the underlying endothelial dysfunction differs.⁸⁰ However, even delivery at term after maternal preeclampsia also confers increased risks of later arterial disease. The complexities are well reviewed recently by Davis et al.⁸¹

Diagnosing Subtypes of Preeclampsia

Maternal preeclampsia is suggested to be driven by an exaggerated maternal response to pregnancy and a normal functioning placenta, as occurs in the well-defined states of systemic inflammation associated with chronic hypertension, obesity, and type 2 diabetes mellitus, or with the metabolic syndrome, when these conditions coexist.^{82–84} During the second half of pregnancy, the combination of the normal systemic inflammation of pregnancy and preexisting vascular inflammation may be excessive and generate the clinical features of the preeclampsia syndrome (Stage 3).⁴² If true, then this condition, on its own, would not be associated with abnormal placentation and placental perfusion, such as FGR or markers of syncytiotrophoblast stress.

The view of dichotomous placental and maternal preeclampsia is likely simplistic. It is probable that the impact of preexisting systemic inflammation would not be confined to the end of pregnancy. For example, it is not known whether pregestational systemic inflammation (as with obesity) or insulin resistance (as with pregestational type 2 diabetes mellitus) could affect uteroplacental spiral artery remodeling and placentation, contributing to mixed types of preeclampsia. Or if systemic inflammation were reflected in decidual tissue at the time of conception and subsequent placentation, then increased local production of inflammatory cytokines, such as tumor necrosis factor- α , could also possibly inhibit trophoblast invasion and thereby adversely affect placentation,⁸⁵ leading to the development of preeclampsia. It is more likely that preeclampsia is predominantly placental or predominantly maternal.

Early- and late-onset preeclampsia have different attributes and are now generally accepted as subtypes of preeclampsia.¹⁰ The Table summarizes the clinical differences to help illustrate the points that we make subsequently in this study. A threshold of 34 weeks is usually used to distinguish the 2; more reliably defined as the time of delivery, not the time of onset. The former is objective, the latter is subject to bias, especially that

of the availability of adequate clinical observations before the time of diagnosis.

Although early-onset preeclampsia is considered primarily placental, there is also evidence for a much greater risk of later life maternal cardiovascular disease. Hence, early-onset preeclampsia includes both placental and maternal components. Late-onset preeclampsia appears to have a weaker placental component. Nonetheless, there is still evidence for an increased incidence of placental pathology and abnormal spiral artery remodeling compared with normal pregnancy.⁹⁸ Thus, mixed placental–maternal disease may also be a feature in the late-onset disease, but with a smaller placental component.

Why Redefine Preeclampsia?

The current definition of preeclampsia is a relic of the past when the disorder was not well understood. Now that circulating trophoblast–derived biomarkers of preeclampsia are recognized, placental components of the syndrome can be separately identified and incorporated into its definition. We propose that these components could be used to define subtypes of preeclampsia. This approach would acknowledge that there appears to be 2 routes to Stage 3 of the syndrome.⁴² The first is primarily driven by poor placentation (early Stages 1 and 2) and the second by underlying maternal abnormalities. An updated definition of preeclampsia directed by more exact pathophysiological understanding of the various forms of the syndrome would be helpful for improved clinical management of the mother and fetus during pregnancy, for targeting intervention, and for appropriate follow-up after pregnancy. Thus, a redefinition may contribute to better maternal and offspring health in the short and long terms.

Redefining Preeclampsia Using Circulating Trophoblast–Derived Biomarkers

One of the objectives of this study is to propose that preeclampsia can be redefined on the basis of placental contributions to the syndrome. Given current evidence, we suggest that

levels of circulating PIGF could be considered for this purpose, where a redefinition of preeclampsia includes low-circulating PIGF in pregnancy as a biomarker for poor placental function (section 6). In the following sections, we describe the biological source of PIGF, significance in pregnancy, evidence for its use as a marker of placental preeclampsia, problems with its measurement, its use in a new definition of preeclampsia, and further research that is needed.

We do not know how well PIGF will serve as a single marker for preeclampsia, or whether performance might be enhanced in combination with other biomarkers (eg, sFLT1 or sENG). This is a first, not a final step to a new definition. New data should emerge when we have completed meta-analyses across cohorts in the CoLab Angiogenic Factor study (see Future Research). Nevertheless, several arguments favor a primary focus on PIGF. First, the placenta is the predominant source of circulating PIGF, whereas endothelial cells, peripheral blood mononuclear cells, and even adipose tissue also are sources of circulating sFlt1.^{99–101} Second, alterations in circulating PIGF concentration can be detected in the first trimester of pregnancy in women destined to develop preeclampsia, before notable changes in sFlt1.¹⁰² Finally, in most studies PIGF is a more sensitive and precise predictor of preeclampsia and FGR than any other single biomarker.^{103–106}

Biomarkers that are predominantly maternal in origin (such as inflammatory cytokines or angiotensin II Type I receptor-autoantibodies; see Other Potential Biomarkers for Redefinition of Preeclampsia) would be expected to reflect maternal not placental pathophysiology of preeclampsia and for this reason are not the focus of this review.

Cellular Sources and Regulation of Circulating PIGF in Nonpregnancy and Pregnancy

PIGF was discovered >20 years ago,¹⁰⁷ but its biological importance is still unclear.¹⁰⁸ It is highly expressed during pregnancy in the placenta, in particular by the syncytiotrophoblast. In nonpregnant individuals, low levels are normally produced by the heart, lung, thyroid, skeletal muscle, and adipose tissue.¹⁰⁹ The cellular sources within these tissues include endothelium, inflammatory cells,¹¹⁰ and cardiomyocytes.¹¹¹ PIGF is inducible in fibroblasts,¹¹² where it can stimulate wound healing.¹¹³ Similarly, after myocardial injury, it can enhance repair via local angiogenesis, vasculogenesis, and cardiomyogenesis.¹⁰⁸ Circulating levels of PIGF in normal, nonpregnant individuals are low, if not undetectable with present assays.¹¹⁴ However, increased circulating PIGF has emerged as a prognostic marker for clinical outcomes in nonpregnant patients with acute coronary syndromes.^{115,116} Loss or inhibition of PIGF appears to have little effect on normal health, but PIGF is necessary to stimulate angiogenesis in pathological settings.¹¹⁷ PIGF has been recognized as a mediator of the angiogenic switch in malignant, inflammatory, and ischemic disorders.¹⁰⁷ The important role of PIGF in angiogenesis has recently been reviewed elsewhere.¹¹⁸

In uncomplicated pregnancy, PIGF can be detected in the maternal circulation from 8 weeks gestation, with a substantial increase in concentration until 29 to 32 weeks gestation. Levels decline thereafter until delivery.^{38,102,119} Fetal trophoblasts are the primary source of circulating PIGF in

Table. Clinical Features of Early-Onset Preeclampsia

	Reference(s)
More Common in Early-Onset Preeclampsia	
More fetal growth restriction	67
Higher perinatal mortality	86
More placental pathology (see comments in text)	87–89
Higher maternal mortality	76,90
Higher recurrence rate	91
Lower nulliparity rate	92
Higher rate of remote postpartum medical complications after premature preeclampsia	
Higher remote risk of maternal cardiovascular disease, including chronic hypertension	76,93
More often maternal remote metabolic syndrome	94
More often maternal remote renal disease	95
Increased risk of maternal death of cardiovascular disease	76
Higher remote risk of cardiovascular disease in the offspring	81,96,97

pregnancy. Cultured cytotrophoblast and syncytiotrophoblast isolated from normal pregnancies contain PIGF mRNA and secrete PIGF.^{120,121}

Regulation of PIGF Release and its Actions

The molecular regulation of *PIGF* gene expression in placental villi and trophoblast cells is not entirely clear, but can be mediated via the protein kinase A pathway¹²² and via a transcription factor called glial cells missing-1.¹²³ The promoter for the human *PIGF* gene contains nuclear factor κ B-binding sites, and overexpression of nuclear factor κ B or hypoxia-activated nuclear factor κ B increases *PIGF* mRNA in some cells.^{122,124}

VEGF mRNA is upregulated by hypoxia and hypoxia inducible factors, but the *PIGF* gene responds variably to these factors. It contains, however, other recognition sequences for the cAMP responsive-binding protein,¹²² nuclear factor κ B, and the hypoxia-induced metal transcription factor 1 (summarized in Reference 117). In many tissues, PIGF is induced by hypoxia, but not in trophoblasts where it is repressed,¹²⁵ although contrary changes have been reported.¹²⁶ We have observed that in trophoblasts, the VEGF gene promoter is activated by the hypoxia inducible factor mimetic CoCl_2 , whereas in the same cells the *PIGF* promoter is downregulated by CoCl_2 (C. Depoix and R.N. Taylor, unpublished results). Intervillous pO_2 tends to decrease with advancing gestational age in normal pregnancy.¹²⁷ This may, at least in part, account for the falling circulating levels of PIGF from early in the third trimester. In contrast, in preeclampsia, these trends are likely to be exaggerated associated with lower circulating PIGF.

The balance between pro- and antiangiogenic factors and their receptors in the maternal circulation in pregnancy is complex and dynamic.^{125,126} There are many interactions involving PIGF in the circulation and pericellular environment that have the potential to dramatically alter PIGF function and the ensuing angiogenic response. The simplest model is that homodimeric PIGF is released from a variety of cell types (including trophoblast) and binds to its cognate cell surface receptor (VEGF receptor 1, also known as FLT1). Ligand binding induces dimerization of cell surface FLT1, activates its intrinsic intracellular kinase causing signals that stimulate proliferation or migration. A soluble splice variant form of FLT1 (sFLT1), acting as a decoy receptor, competitively inhibits binding of free PIGF to its activating cell surface receptors. However, free sFLT1 can also associate with monomeric forms of cell-bound FLT1 or KDR (VEGF receptor 2) and block their signaling. In this way, soluble FLT1 can act as a dominant-negative and inhibit both PIGF and VEGFA signaling.¹²⁸ As PIGF only binds to VEGF receptor 1, its presence can alter the partitioning of VEGFA between FLT1 and KDR and increase its bioavailability to KDR. Furthermore, there is evidence that, in cells that synthesize both VEGFA and PIGF, these growth factors can heterodimerize. Because PIGF only interacts with FLT1, the heterodimers will only be able to act with FLT1.

An additional complexity is that PIGF, like VEGFA, is expressed in multiple isoforms, derived from differential splicing of the primary gene transcripts. PIGF-2 and -4 contain basic domains with high affinity for heparan sulfate proteoglycans. This is also true of FLT1, hence all these interacting

moieties can be adsorbed to heparan sulfate proteoglycans on the cell surface without eliciting a signaling response.^{129,130} It is likely that such immobilized molecules also interact with other binding partners (growth factors, transmembrane or soluble receptors, respectively) and sequester them from the local cellular environment. As suggested above, different splice variants (of PIGF, VEGFA, and sFLT1) interact with heparan sulfate with differing affinities. Thus, there are multiple equilibria among PIGF, VEGFA, sFLT1, membrane-bound receptors, and charged extracellular matrix molecules, which are affected by the binding kinetics and the affinities and concentrations of each of the components. Local concentrations are extremely difficult to determine, and therefore local cellular consequences of binding are hard to predict. Single-point measurements in serum or plasma of any 1 of these components will be influenced by all of these complex interactions. Specific assays may be influenced by ≥ 1 of these interactions, making robust predictions based on such assays problematic.

In circulation, it is postulated that PIGF and other angiogenic factors regulate maternal vascular function during pregnancy. VEGFA is a key survival factor for the vascular endothelium, and current understanding is that VEGFA works in concert with PIGF to maintain endothelial homeostasis, such as through VEGFA-dependent activation of endothelial NO synthase).^{35,131} PIGF may increase the bioavailability of VEGFA in circulation by altering its partitioning between the VEGF receptors.

Circulating PIGF in Normal and Complicated Pregnancies

The past 2 decades of research have highlighted significant changes in circulating PIGF levels (as well as in sFLT1 and sENG levels) in pregnancies complicated by preeclampsia compared with normal pregnancy.^{35-37,98,119,132-147} Figure 1

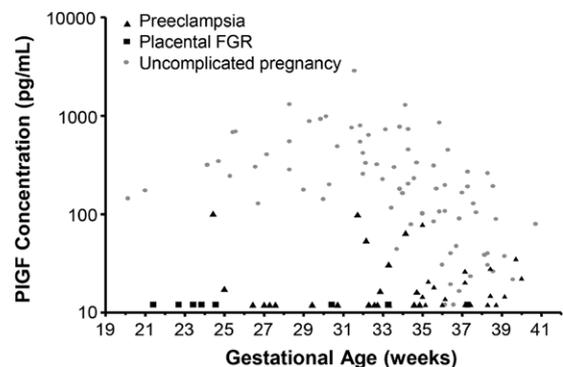


Figure 1. Placental growth factor (PIGF) concentrations in the circulation of women with normal and complicated pregnancies. Maternal plasma was collected at the time of preeclampsia diagnosis (black triangles) or fetal growth restriction (FGR; black squares). The FGR cases were selected, because they also had placental histopathologic abnormalities indicative of poor perfusion. Uncomplicated pregnancies (grey circles) serve as references. As seen from the figure, most women with uncomplicated pregnancies have higher circulating PIGF than women with the classic definition of preeclampsia. In addition, normotensive women with placental FGR had abnormally low PIGF, validating the concept that the biomarker detects this placental problem. There are, however, cases of preeclampsia with high PIGF as well as normotensive controls with low PIGF. The discrimination between cases and controls is better before 35 weeks with early-onset disease.

demonstrates circulating PIGF across gestation in normotensive pregnancies. Free PIGF concentrations gradually increase in the maternal circulation from early pregnancy until 29 to 33 weeks gestation, followed by a gradual decrease until delivery. In pregnancies complicated by preeclampsia, PIGF concentrations are significantly lower relative to those of normal pregnancies at the same gestational age. Moreover, women who develop preeclampsia before 35 weeks gestation appear to have significantly lower concentrations of PIGF in circulation before the onset of disease and at the time of diagnosis. Preeclampsia diagnosed beyond 35 weeks gestation does not appear to be associated with such dramatic decreases in circulating PIGF nor in placental tissue levels.¹⁴⁸ In addition, cases with FGR associated with placental pathology indicative of poor placental perfusion had similarly low PIGF before 35 weeks, validating that this measure is one of placental function, but not of the maternal response to abnormal placental function. In addition, circulating levels of sFLT1 and sENG are also altered in preeclampsia. Significant elevations in both factors occur at the time of preeclampsia diagnosis and before the onset of disease,¹⁴⁹ although test accuracies of the individual markers are too poor for accurate prediction of preeclampsia.¹⁵⁰ Mechanistically, it has been suggested that lower oxygen levels, or more likely fluctuation in oxygenation in the placenta, may lead to lower PIGF production by trophoblasts and altered release into maternal circulation¹⁵¹ (see Cellular Sources and Regulation of Circulating PIGF in Nonpregnancy and Pregnancy above).

Does Low Circulating PIGF in Preeclampsia Reflect Abnormal Placentation?

Circulating free PIGF levels are lowest in women who develop early-onset forms of the preeclampsia syndrome, which are associated with more placental pathology and FGR.^{38,119,133,152} Women who develop preeclampsia with FGR have further decreased levels of PIGF compared with women who develop preeclampsia without FGR.³⁸ Most interestingly, low-PIGF concentrations are also observed in the circulation of women with growth restricted fetuses without preeclampsia.^{38,133,153}

Smallness for gestational age can be used as a crude proxy to assign FGR outcomes, which will include pregnancies complicated by poor placentation. Hence, studies have found women who deliver a small for gestational age infant more likely to have lower circulating PIGF concentrations.^{38,133,154} When placental pathology is used to define an FGR outcome, a stronger association with lower circulating PIGF is observed.¹⁵³ These findings lead us to postulate that low maternal circulating PIGF concentration reflects placental dysfunction resulting in clinical manifestations of preeclampsia and FGR.

Extending the Definition and Diagnosis of Preeclampsia Using PIGF as a Placental-Derived Biomarker to Identify Specific Disease Subsets

We propose that the definition of preeclampsia can be redefined on the basis of placental contributions to the syndrome. Using current evidence, we suggest that low PIGF in pregnancy indicates poor placentation with its clinical correlate of FGR with or without preeclampsia. Current definitions of preeclampsia depend on the clinical signs of hypertension and proteinuria, which are tertiary, maternal features of the placental disease, whereas trophoblast-derived markers are upstream, closer to the origins of placental preeclampsia and should be more sensitive and specific diagnostically, at least for the subtypes of preeclampsia that they can identify.

Figure 2 depicts different pathways that lead to a diagnosis of preeclampsia, and where measurements of circulating PIGF might distinguish whether there is placental pathophysiology or not. The exact threshold for low PIGF (or other dysregulated circulating placental-derived factors) would be gestational-age specific, because circulating PIGF varies across gestational weeks (see Circulating PIGF in Normal and Complicated Pregnancies above; Figure 1). An ongoing global collaboration among researchers involving the meta-analysis of PIGF across gestational ages, analytic platforms, and pregnancy phenotypes will establish normative distributions and could provide information for defining such thresholds (see Future Research below).

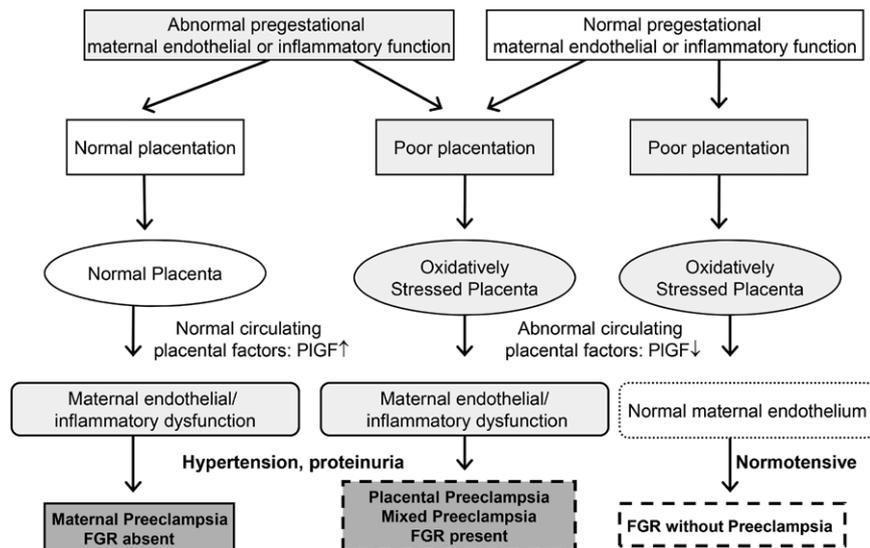


Figure 2. A model of extended definition of preeclampsia on the basis of placenta-derived biomarkers. The figure illustrates the main alternative pathways during pregnancy leading to same diagnosis of preeclampsia, with or without fetal growth restriction (FGR), or to development of FGR without preeclampsia. The pathways are differentiated by maternal circulating placenta growth factor (PIGF) as a biomarker of placental dysfunction. At present, it is not clear whether the addition of other placenta-derived biomarkers (such as soluble fms-like tyrosine kinase-1 and soluble endoglin), or even maternally derived biomarkers (such as inflammatory cytokines), should be added to this flow-chart to improve the definition and subclassification of preeclampsia types.

It is not likely that any single marker for the maternal type of preeclampsia will be identified. It is likely there are several maternal pathways to preeclampsia. It will also be difficult to separate causal maternal factors from components of maternal response. For example, a marker that reflects maternal endothelial dysfunction (final Stage 3) would be expected to be elevated in the preeclamptic syndrome, regardless of its cause (maternal or placental).

It is not clear why FGR, secondary to poor placentation,^{153,155} can yield similar changes in circulating proangiogenic and antiangiogenic factors without stimulating maternal hypertension and proteinuria. This again emphasizes the interaction of fetal and maternal factors, and the likelihood that a more appropriate interpretation is the predominance of maternal or preponderance of placental features in preeclampsia, rather than dichotomy of these factors in the genesis of preeclampsia.

The hypothesis that PlGF will discriminate a placental or a maternal predominance in the genesis of preeclampsia was supported by a recent study of the relationship of PlGF across pregnancy to preeclampsia.¹⁵⁶ Fifty women with preeclampsia could be clearly subdivided into 2 groups. Approximately half of the preeclampsia cases had consistently low PlGF from the start of the second trimester, whereas the other half had normal or high PlGF until delivery, similar to that of normal pregnant women. Those with persistent low PlGF had higher blood pressure in early pregnancy and after preeclampsia diagnosis, earlier gestational age at delivery, and more preterm birth compared with preeclamptic women with normal or high PlGF. These findings represent further evidence in favor of our proposal of using PlGF in a novel redefinition of preeclampsia.¹⁵⁶

Limitations to Definitions Based on Circulating PlGF: Challenges of Measurement

In Cellular Sources and Regulation of Circulating PlGF in Nonpregnancy and Pregnancy section, the complex dynamic equilibria that may affect measurements of PlGF, and certainly its interpretation, in the circulation or tissues, are described. We describe here additional factors that need to be taken into account when using PlGF as a placental-derived biomarker to redefine preeclampsia. Also, a role of other potential alternative biomarkers is briefly reviewed.

Different PlGF Assay Platforms

There are several commercial PlGF assay platforms (using different antibodies and reagents) being used in preeclampsia research today.^{37,157,158} Different assays may differentially detect the 4 different isoforms of the PlGF molecule present in human pregnancy. As described in Cellular Sources and Regulation of Circulating PlGF in Nonpregnancy and Pregnancy section, there is a complex interaction between free and bound PlGF molecules and other angiogenic regulators, and at present it is not clear whether one analytic platform has an advantage over others in measuring the most clinically relevant PlGF concentration, or even whether a platform more specifically measures the PlGF form released by the placenta. We are not aware of any preeclampsia prediction studies comparing different platforms. However, there may be differences in the diagnostic performance between assays in diagnosing early-onset preeclampsia.¹⁵⁷

Time and Costs for PlGF Analyses

Most angiogenic factor assays are designed for experimental laboratory use. In daily clinical practice, it would be preferable to have access to a simple and rapid assay, based on a noninvasive procedure such as urine testing, to facilitate clinical decision-making. Testing of urinary PlGF is feasible, because the small PlGF molecule passes the glomerular filtration barrier, unlike the larger sFLT1 molecule. The interactions with other cellular and tissue components are much less of a problem but, to date, urinary concentrations have been less promising than circulating levels of PlGF for diagnosing preeclampsia.^{149,159}

Other Potential Biomarkers for Redefinition of Preeclampsia

In this review, we have proposed a redefinition of preeclampsia on the basis of placental contributions to the disease, using low-circulating PlGF as a biomarker for poor placental health. This does not exclude other relevant placentally associated biomarkers, which we have previously reviewed.¹⁴⁹ Among the most relevant biomarkers are the other angiogenic proteins that have been extensively investigated in relation to preeclampsia, namely sFLT1 and sENG.

It is also possible that markers of maternal sensitivity might further divide preeclampsia into pathophysiological relevant subtypes. For example, the Renin–Angiotensin System is dysregulated in preeclampsia, both in the circulation and in uteroplacental tissues.¹⁶⁰ In preeclampsia, circulating angiotensin II is not increased, but Angiotensin II Type I receptor–mediated signaling pathways are activated,¹⁶⁰ of which agonistic autoimmune antibody against angiotensin II Type I receptor seems to be an important mediator. These autoantibodies¹⁶¹ or other markers of maternal sensitivity (eg, greatly increased inflammatory activation) could indicate a subset of women in whom specific preventive or palliative therapy would be beneficial. This could be the next step in subdividing in preeclampsia in a pathophysiological relevant manner.

Future Research

To validate this proposal, we have begun work with The Global Pregnancy CoLaboration (CoLab). This was established in 2011 to facilitate cooperation among researchers who have data and sample collections for pregnancy-associated diseases (<http://pre-empt.cfri.ca/Members/ListofMembers/CoLaboratory.aspx>). The CoLab Angiogenic Factor Study is a substudy within this collaboration. We are undertaking a meta-analysis of individual patient measurements of angiogenic factor concentrations that have been published from different laboratories, on different analytic platforms. We will test whether normal maternal blood PlGF concentrations (based on gestational-age–specific normograms, and normalized to allow comparison between various platforms) will be more characteristic of the maternal preeclampsia phenotype without a placental component, and whether these cases will show more constitutional susceptibility factors (such as preexisting diabetes mellitus, chronic hypertension, or obesity). We will also be able to analyze in more detail the differences in the distribution of PlGF concentrations in early- and late-onset disease. The concepts which we have presented here, with

PIGF as an example, will also be applied to other trophoblast-derived factors, such as sFLT1 and sENG. It is our expectation that a combination of biomarkers reflecting both the placental (such as PIGF) and maternal (such as the recently investigated MR-proANP, midregional proatrial natriuretic peptide)¹⁶² pathophysiology of preeclampsia may improve diagnostic accuracy and clinical management. Such combinations of placental- and nonplacental-derived biomarkers may also prove helpful in identifying pregnancies with FGR.

Advantages and Disadvantages of the Proposed Definition

An improved classification of preeclampsia, based on pathologically relevant biomarkers such as PIGF, would be expected to improve the reliability and reproducibility of outcome assessment in studies of the prediction, diagnosis, or prevention of preeclampsia. This, in turn, would improve diagnostic clarity, risk stratification, and allocation of care, thus reducing unnecessary health expenditure. For example, given that preeclampsia is associated with long-term cardiovascular disease, as described in Heterogeneity of Preeclampsia, a biomechanistic classification of its components could improve prediction of short- and long-term cardiovascular consequences for mother and child, thus identifying target groups with the highest risk. These benefits remain to be proved. If, indeed, presentations that fit the classical definition of preeclampsia without evidence for placental dysfunction are compatible with the diagnosis of maternal preeclampsia, this would clarify many issues for research and clinical uses.

A major limitation is that application of the principle that we propose would probably address the definition only of a subset of the preeclampsia, namely that with a prominent placental component. Hence, other biomarkers would need to be sought to identify better the subsets that are not covered by this methodology. A potential flaw would be if the maternal and placental factors overlapped too much to discriminate the disease subtypes. Based on preliminary data, we believe that, at least, the inclusion of PIGF in the definition of preeclampsia would discriminate the 2 tails of the distribution, which would still be useful. Nonetheless, we await the results of the CoLab Angiogenic Factor Study metanalysis to resolve this concern. A disadvantage in the specific PIGF-based redefinition of preeclampsia would be that this blood-based tool would not be immediately accessible in the developing countries.

We look forward to a time when all laboratory and clinical studies of preeclampsia are conducted using objective outcomes; ones that are not defined solely by tertiary maternal features, but rather by whether or not preeclampsia is secondary to maternal, placental, or mixed (maternal and placental) inputs.

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