CEOG Clinical and Experimental Obstetrics & Gynecology

Review Articles

The importance of angiogenic and antiangiogenic biomarkers in pathogenesis and early diagnosis of preeclampsia

R. Einikytė[1], A. Dapkeviciute[1], V. Dzenkeviciute[2,3], S. Kasnauskiene[3], V. Sapoka[2,3], D. Ramasauskaite[3,4]

[1]Faculty of Medicine, Vilnius University, Vilnius
[2]Faculty of Medicine, Clinic of internal medicine, oncology and family medicine;Vilnius University, Vilnius
[3]Vilnius University Hospital Santariskiu Clinics, Vilnius
[4]Faculty of Medicine, clinic of obstetric and gynaecology, Vilnius University, Vilnius (Lithuania)

Summary

The early diagnosis of preeclampsia (PE) remains one of the great medical problems worldwide. PE is a multisystemic disorder and the etiology is still unclear. The equilibrium between anti-angionenic and angiogenic factors is essential in the PE pathogenesis. In this review, the authors highlight the role of key circulating anti-angionenic and antiangiogenic factors as pathogenic biomarkers and as well as early diagnostic biomarkers for PE. They analyzed the main anti-angiogenic factors: soluble FMS–like tyrosine kinase (sFlt-1), soluble endoglin (sEng), and the angiogenic factors – vascular endothelial growth factor (VEGF) and placental growth factor (P1GF). An accurate algorithm for diagnosing PE using only biomarkers is still absent.

Key words: Preeclampsia; Anti-angiogenic factors; Angiogenic factors; Biomarkers.

Introduction

Preeclampsia (PE) is one of the most important maternal and neonatal morbidity and mortality reasons and probably accounts for more than 50, 000 maternal deaths worldwide each year [1-3]. Up to 20% of premature births are associated with PE, which could cause greater risk of fetal death, low birth weight, hypoxia-related neurologic injury, perinatal death, and long term cardiovascular morbidity associated with low birthweight [4]. PE can also contribute to the increased risk of maternal cardiovascular morbidity later in life [4, 5].

It is clear that despite high quality medical care in most countries, the prevalence of PE is still high. Moreover, despite the lack of complete understanding of the pathogenesis, there are many hypotheses explaining the onset of PE. However, at this time, there is no single, reliable explanation of PE mechanism, which would help to diagnose and to treat this condition prior the onset of the symptoms.

An attempt has been made to predict PE by maternal history and risk factors; however, mechanisms through which these factors cause PE are still not entirely clear. It has been hypothesized that: a) maternal mean arterial pressure, b) mean uterine artery Doppler ultrasound indices, c) markers of placenta (PAPP-A, plasma protein 13), and d) angiogenic factors (PIGF, VEGF, sFlt-1, sEng) can predict the onset of PE.

In the last decade, biochemical markers (angiogenic and antiangiogenic) gained much importance as possible PE predictors. Biomarkers alone or in combination could accurately predict the onset of PE and could help to detect pathological changes before the appearance of the first maternal symptoms of PE. Although biomarkers are a promising step towards the diagnosis of PE, an exact algorithm or standardized diagnostic criteria that would help to identify high-risk women and adequately organise medical care, are still lacking.

Risk factors

PE is associated with various risk factors, such as: age – 35 years and above, medical history of chronic hypertension, kidney disease, diabetes, obesity, birthplace in Africa, and pregnancy characteristics, such as twin or molar pregnancy, previous PE, or fetal congenital abnormality. High altitude has also been shown to increase the incidence of PE, and is attributed to greater placental hypoxia, smaller uterine artery diameter, and lower uterine artery blood flow [6-8] (Table 1).

It is thought that healthy pregnancy itself is a state of systemic inflammation, at least in the third trimester [3]. Based on this concept, PE is not a separate condition, but simply a form of maternal systemic inflammatory responses

Revised manuscript accepted for publication January 9, 2017

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLV, n. 4, 2018
doi: 10.12891/ceog4046.2018

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Table 1. — *Major risk factors for PE.*

Risk factor	Relative risk (95% Cl)
Antiphospholipid antibodies	9.7 (4.3–21.7)
Renal disease	7.8 (2.2–28.2)
Prior PE	7.2 (5.8–8.8)
Systemic lupus erythematosus	5.7 (2.0–16.2)
Nulliparity	5.4 (2.8–10.3)
Chronic hypertension	3.8 (3.4–4.3)
Diabetes mellitus	3.6 (2.5–5.0)
High altitude	3.6 (1.1–11.9)
Multiple gestations	3.5 (3.0–4.2)
Strong family history of CV diseases	3.2 (1.4–7.7)
Obesity	2.5 (1.7–3.7)
Family history of PE in first-degree relative	2.3–2.6 (1.8–3.6)
Advanced maternal age (> 40 years)	1.68 (1.23–2.29) – for nulliparas
	1.96 (1.34–2.87) – for multiparas

caused by the pregnancy itself, therefore, any factor that would enhance this response would predispose to preeclampsia [3].

It is important to note, that PE affects 5–7% of nulliparas and only 1–3% of multiparas with no history of previous PE; therefore PE is considered to be a disease of the first pregnancy (Table 1) [9]. However, if a woman had PE during the first pregnancy, then the risk to have PE during the second pregnancy rises to 14.7% and to 31.9% after two pregnancies affected by PE, respectively [10]. It is also noticed that among women, with a prior pregnancy that had chronic hypertension, the risk for PE rises by seven- to eight- fold [7]. Important risk factor is obesity: the higher the body mass index (BMI), the higher the risk for PE to develop during pregnancy. Therefore, it is thought that increased prevalence of obesity can be associated with increased incidence of PE [3].

It is important to note that the presence of at least one pre-existing condition (probable or definite hypertension, pre-existing diabetes, multiple gestation or obesity) is associated with 22.3% of all PE cases in nulliparous women [7]. Moreover, the more risk factors a women has, the higher the risk is for PE to occur [6, 7, 10].

PE pathogenesis

PE is a multisystemic disorder and despite intensive research, the etiology is still unclear. It has been thought that PE results from reduction in urethroplacental perfusion, which leads to urethroplacental ischemia. The ischemic placenta is thought to secrete soluble factors during the third trimester that induces systemic endothelial dysfunction [6]. Therefore, recent studies have led to the hypothesis that clinical manifestations of PE result, in part, due to an imbalance between circulating pro-angiogenic and anti-angiogenic factors in the maternal circulation [6]

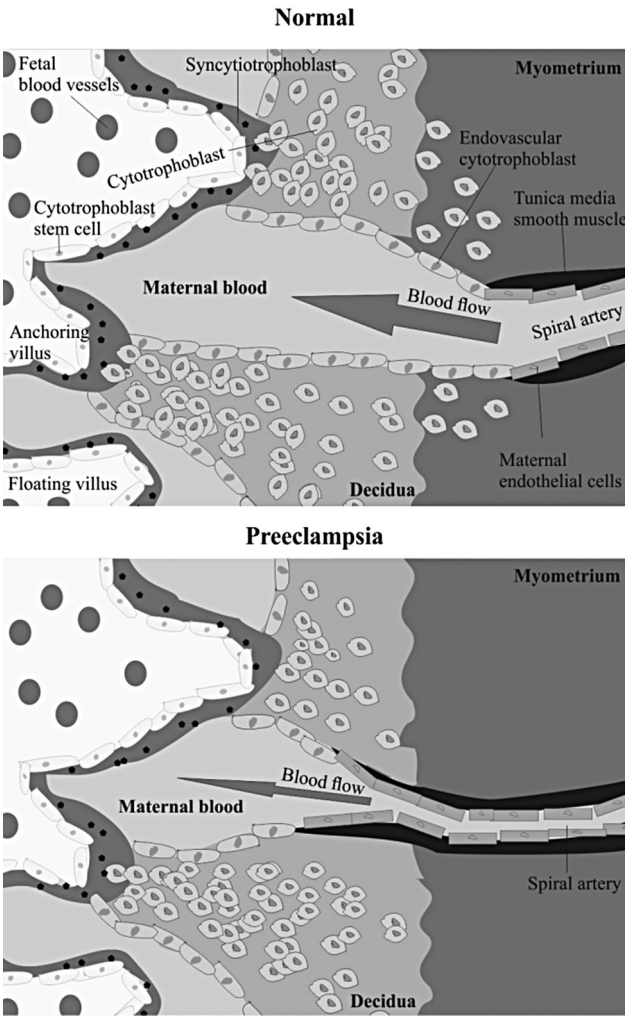

Figure 1. — Mechanism of PE pathogenesis.

During normal placental development, invasive cytotrophoblasts of fetal origin invade the maternal spiral arteries, transforming them from small-caliber resistance vessels to high-caliber capacitance vessels capable of providing placental perfusion adequate to sustain growing fetus. During the process of vascular invasion, the cytotrophoblasts differentiate from epithelial phenotype to an endothelial phenotype, a process referred to as "pseudovasculogenesis" or "vascular mimicry" (upper picture).
PE:
During PE, cytotrophoblasts fail to adapt an invasive endothelial phenotype. Instead, invasion of the spiral arteries is shallow, and they remain small calibre, resistance vessels (bottom picture) Adopted from Wang A., Rana S., Karumanchi S.A.

It has been proposed that PE is a two stage disorder: *asymptomatic stage* is marked as abnormal placentation, followed by the secretion of certain soluble factors that enter maternal circulation and cause subsequent generalised

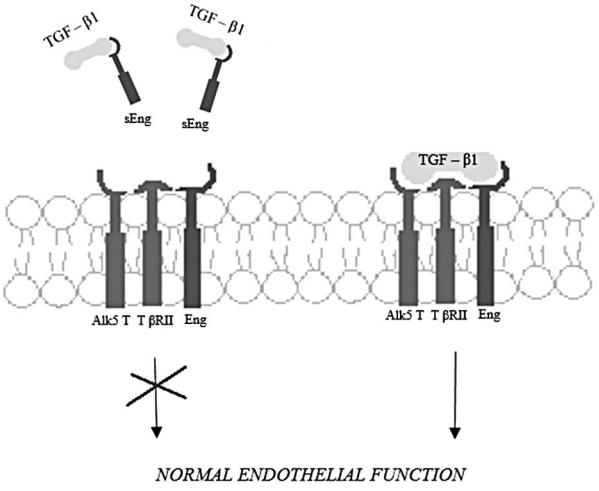

Figure 2. —Schematic mechanism of sEng role in PE pathogenesis. *TGF-β1 are maintained. During PE excess secretion of sEng inhibits TGF-β1 signaling and therefore disrupts normal endothelial formation.*

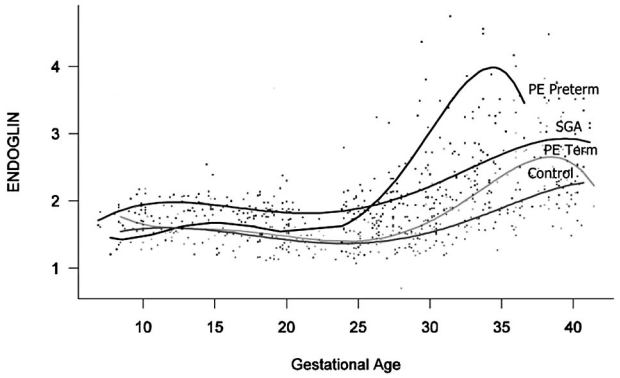

Figure 3. —sEng concentration in normal pregnancy. *Maternal plasma concentration of s-Eng in patients with normal pregnancies and those with pregnancy complications. Patients who delivered a small for gestational age (SGA) neonate had a significantly higher plasma concentration of s-Eng from ten weeks of gestation onwards than controls ($p < 0.0001$). The increment in plasma s-Eng in patients destined to develop preterm PE surpassed that of patients with normal pregnancies at 13 weeks and became significant at 23 weeks. In patients destined to develop PE at term, the maternal plasma concentration of s-Eng became significantly higher than that of normal patients at 30 weeks. Adopted from Romero R., Nien J.K., Espinoza J., et al.*

endothelial dysfunction [11]. During normal pregnancy cytotrophoblasts invade the endometrium reaching tunica media of the spiral arteries (Figure 1). At the cellular level, cytotrophoblasts undergo pseudovasculogenesis by switching their adhesion molecules to mimic those of vascular cells, and therefore the remodelling of vascular walls occurs: high resistance low diameter vessels become low resistance high diameter vessels (Figure 1). Remodelling is very important for adequate blood flow to growing placenta and if this process does not occur appropriately, invasion into spiral arteries is incomplete, and leads to reduced placental perfusion and later – ischemia, causing serious maternal and fetal outcomes [6]. *Symptomatic (maternal) stage is* characterized primarily by hypertension and proteinuria [11]. This syndrome usually occurs after 20 gestational weeks and is caused by secretion of soluble factors by the ischemic placenta [12]. These factors in turn induce systemic endothelial dysfunction and are in part, responsible for the clinical manifestation of PE. Oxidative stress has been emphasized as key mechanism that causes increased production of soluble humoral factors (cytokines, chemokines, anti-angiogenic factors, etc.) by the poorly perfused placenta [13]. These humoral factors trigger multiple organ injuries responsible for the clinical manifestations of PE. As the disease progresses, angiospasms in the brain and brain edema may lead to eclampsia.

Many of secreted factors are pro-inflammatory and some contribute to the angiogenic balance as well as stimulating vascular inflammation [14]. Many factors are involved that are both proangiogenic and anti-angiogenic [14]. The two factors that have received the greatest attention are vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) [6]. Syncytiotrophoblasts secrete P1GF and receptor for VEGF and PlGF (soluble VEGF receptor–1 or sFlt 1), which inhibits their activity (VEGF and PlGF signalling is disrupted) [14]. Thus, sFlt-1 is considered to be a major antiangiogenic factor along with other factors (for instance, soluble endoglin: sEng). sEng is antiangiogenic protein that is thought to act by disrupting transforming growth factor beta signalling in the vasculature [6]. Therefore, PE is characterised by excessive release of sFlt-1 and sEng and reduced circulating P1GF levels. In PE sEng and sFlt-1 expression is increased and reflects the degree of placental ischemia[6].

In normal pregnancy sFlt1 remains fairly constant until 30-32 weeks, then it begins to rise. In contrast, circulating PlGF increases steadily, peaks at about 30 weeks, and then decreases, therefore, it is clear that in normal pregnancy, syncytiotrophoblasts become increasingly stressed for the last 8–10 weeks of pregnancy [14]. Patients, who later developed PE had low levels of PlGF from 13–16 weeks up until the delivery, while sFlt-1 and sEng – increased later in pregnancy, suggesting that syncytiotrophoblasts of preeclamptic women are stressed earlier in pregnancy [14-16].

It is important to note, that anti-angiogenic factors sEng and sFlt-1 are found to rise in the second trimester, while angiogenic (PlGF and VEGF) – are early onset PE markers.

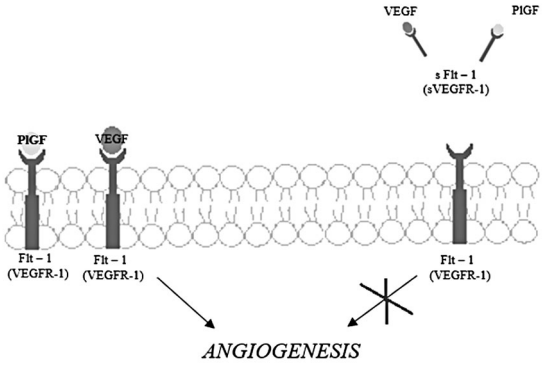

Figure 4. —Schematic mechanism of sFlt-1 role in PE pathogenesis.
In normal placental formation (angiogenesis), a proper interaction between Flt-1 and VEGF, as well as PlGF is essential. In PE, when excess of sFlt-1 is formed, circulating level of VEGF is decreased and therefore its signalling is disrupted, causing defective development of placenta.

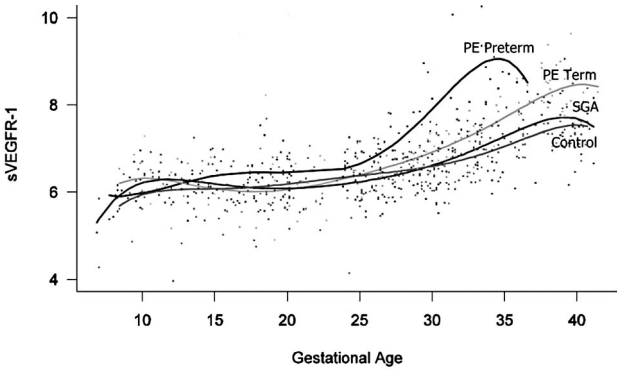

Figure 5. —sFlt-1 concentration in PE.
Maternal plasma concentration of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) in patients with normal pregnancies and those with pregnancy complications. Patients destined to develop preterm term and term PE had a significantly higher plasma concentrations of sVEGFR-1 at 26 and 29 weeks of gestation, respectively, than controls. However, there was no difference in the increment of sVEGFR-1 between control patients and those who delivered an SGA neonate. Adopted from Romero R., Nien J.K., Espinoza J., et al.

That is why the latter is more favourable in clinical situations as it would allow for early intervention. Each marker will be separately discussed below.

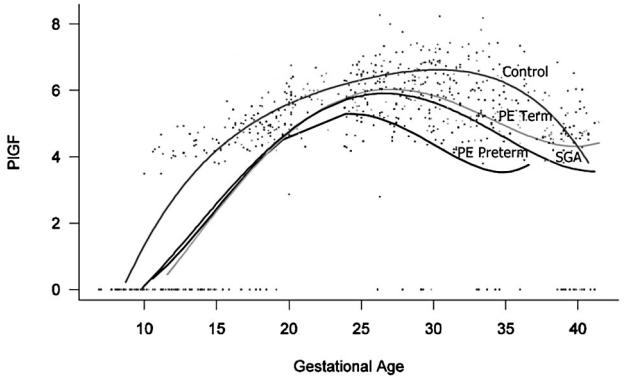

Figure 6. —P1GF concentration in maternal blood during normal pregnancy and during pregnancy complications
Maternal plasma concentration of PlGF in patients with normal pregnancies and those with pregnancy complications. Patients destined to develop PE (term or preterm) and those who delivered an SGA neonate had lower plasma concentration of PlGF throughout gestation than controls. These differences were statistically significant at ten weeks of gestation for SGA and term PE and at 11 weeks for preterm PE.

Normal placental development

In normal placental development, invasive cytotrophoblasts of fetal origin invade the maternal spiral arteries, transforming them from small-caliber resistance vessels to high-caliber capacitance vessels capable of providing placental perfusion adequate to sustain growing fetus. During the process of vascular invasion, the cytotrophoblasts differentiate from epithelial phenotype to an endothelial phenotype, a process referred to as "pseudovasculogenesis" or "vascular mimicry" [12].

The equilibrium between anti-angiogenic (sEng, sFTL-1) and angiogenic (P1GF, VEGF) factors in PE pathogenesis is of a critical importance. It is also important that angiogenic and anti-angiogenic factors can be detected in the maternal blood prior the onset of the symptoms. Cytotrophoblast invasion occurs between 8 and 16 gestational weeks, therefore, it should be possible to assess the risk of the PE in the first trimester. The main anti-angiogenic factors sFtl-1 and sEng are both elevated in those with PE compared with healthy women [2, 15, 17].

sEng is a monodimeric transmembrane glycoprotein. It is a cell-surface co-receptor for transforming growth factor TGF-β1 and TGF-β3 isoforms, is highly expressed in endothelial cells and syncytiothrophoblasts, modulates actions of TGF-β1 and TGF-β3, and thus affects transcriptional responses in the vasculature [18, 19].

It is thought that sEng plays a role in PE pathogenesis by regulating the synthesis of nitric oxide synthase (sNO) in

the endothelium and thus affecting blood pressure. sEng inhibits TGF-β1 adhesion to endothelium cells and therefore the activation of sNO and thus sNO – dependant vasodilatation is stopped [19] (Figure 2).

Although it is suggested that the placenta is a major source of sEng during pregnancy, other sources such as maternal vasculature cannot be ruled out [19]. sEng is mainly expressed in endothelial cells, macrophages, syncitiothrophoblast cells, activated monocytes, and stroma cells. The role of sEng in PE pathogenesis is defined by four findings: (1) sEng inhibits endothelium formation *in vitro*, (2) sEng causes hypertension *in vivo,* (3) higher sEng mRNR gene expression in the placenta of women with PE, and (4) elevated level sEng in maternal sera [19]. Moreover, it was found that not only sEng level rise in PE, but also that it correlates with disease severity: concentrations of sEng were three-, five-, and ten-fold higher in individuals with mild PE, severe PE, and HELLP syndrome, respectively, compared to gestational age-matched preterm controls [19]. However circulating concentrations increase also by two- and three-fold in a normal pregnancy at preterm and term, respectively, as compared with non-pregnant state[18].

Romero *et al.* have shown that in normal pregnancies sEng rises with gestational age (Figure 3*)* [15]. In women, with PE, sEng begins to rise from 13 weeks and becomes significant at 23 weeks (Figure 3) [15]. These sEng fluctuations in complicated pregnancy allow to predict the onset of PE (Figure 3). The prediction rate of early onset of PE in the first trimester was found to be 63% (a 10% false positive rate) [20]. Used in combination with Doppler ultrasound (PI) and PIGF, the precision rate for early onset PE was 77.8%. (a 5% false positive rate) [2].

Although sEng seems to be important in PE diagnostics, it is still unclear when it should be tested. Some studies state that sEng level becomes significant at 11-14 weeks [2, 20], while others argue that sEng is important only in the second trimester [15, 21-23]. Therefore, it is unclear whether sEng may serve as diagnostic or prognostic marker for the disease in the first trimester. If the former is true, and the significant difference in sEng levels appears from the second trimester onwards, then sEng testing would be unfavourable in clinical situation, because it would not allow for early intervention [24].

According to the most recent studies, the predictive value of only sEng has been shown to be inconsistent and sEng alone is considered to be a poor screening test [24]. It has been suggested to use sEng in combination with other markers or factors that affect pregnant women. Maternal circulating biomarkers, clinical signs or mean arterial pressure with sEng values might give a better approach for an accurate prediction of early-onset PE [6].

Soluble FMS-like tyrosine kinase (sFlt-1)

sFlt-1 is also known as sVEGFR1 and is composed of seven extracellular domains, one transmembrane, and one intracellular tyrosine kinase domain. sFlt-1 causes endothelium dysfunction by binding to VEGF and PlGF. VEGF and PIGF binding to sFlt-1 are no longer available to their innate receptors on endothelial cells, and VEGF signalling is disrupted; therefore, in PE, sFlt-1 is increased, whereas free concentrations of PlGF and VEGF are decreased [25]. This angiogenic imbalance correlates with severity of signs and symptoms of PE [15, 25] (Figure 4)

sFtl-1 as a prognostic marker is particularly well-studied, discussed, and argued. Some studies state that sFtl-1 levels significantly rise in the second trimester. In normal pregnancy, sFlt-1 plasma concentration between first and second semester remains the same, in the third trimester it slightly rises, whereas in PE it increases significantly well before the onset of clinical signs and symptoms [15, 21, 22, 25, 26] (Figure 5). Noori *et al.* evaluated sFlt-1 concentrations longitudinally throughout gestation (between 10 to 40 weeks) in women with normal pregnancies and those with PE and stated that sFlt-1 concentrations in PE women seemed to be increased throughout gestation and significantly increased at 5-6 weeks before the onset of the symptoms [27].

Recent studies have demonstrated that sFlt-1 measurement alone gives diagnostic specificity and sensitivity up to 80% and 70%, respectively, for the prediction of PE during the second trimester. sFlt-1 and PlGF ratio has been proven to be even more valuable (discussed in section "s-Flt-1/P1GF ratio"). Although sFlt-1 can be used as a prognostic factor, as a possible predictor sFtl-1 alone is less useful than other biomarkers, because it is argued that it rises later in pregnancy, just few a weeks before the onset of PE and in general it has been shown that testing for placental diseases (including PE) before the second trimester is of limited use; however, because PlGF alterations occur early in the first trimester, PIGF has been tested alone and in combination with other biomarkers as a potential predictive test [25, 28].

Angiogenic factors

VEGF is responsible for stabilising endothelial cells in matured blood vessels. This factor is especially important in fenestrated or sinusoidal endothelial cells which are found in kidney glomerulus, brain and liver – these organs are affected the most in case of PE [29]. VEGF and sFlt-1 are produced fundamentally by the placenta but these molecules and their receptors are also synthesized and secreted by endothelial cells and peripheral blood mononuclear cells (PBMCs) [30-33]. VEGF receptors are also found in glomerular endothelial cells. Anti-VEGF therapy for grown up animals cause glomerular endothelial dysfunction with proteinuria [34]. This explains why the lack of VEGF caused by higher concentration of sFlt-1 may cause kidney dysfunction characteristic to PE [35]. VEGF gene family

comprises several members including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and P1GF [36]. VEGF-A is one of the most important endothelial growth factors, others structurally resemble VEGF-A [37].

There are two main VEGF and P1GF receptors – VEGFR-1 (also known as Flt-1 or FMS-like tyrosine kinase-1) and VEGFR-2 (also known as flk-1 – fetal liver kinase) [38]. VEGFR-1 has a very strong affinity to VEGF, which explains why the majority of VEGF is connected to receptors, and free VEGF concentration in blood serum or plasma is very low [39]. This is the reason for technical difficulties in measuring free VEGF concentration with commercially available ELISA [25].

Studies showed that free VEGF concentration in women who later developed PE and control group women were statistically insignificant [16, 40]. This could have occurred due to VEGF-A concentration being lower than commercially available tests sensitivity in 80% of PE cases [40]. All VEGF-A concentration (free VEGF-A and VEGF combined with receptors) in plasma was statistically significantly different in PE patients and IUGR patients[41].

Although free VEGF concentration did not give significant results, other studies were designed to measure VEGF effect on PE patients. Cardenas-Mondragon *et al.* assessed the relationship between PE and anti-angiogenic and angiogenic factors produced by peripheral blood mononuclear cells (PBMCs) and their serum levels. Data showed that VEGF produced by stimulated PBMCs was lower than in healthy pregnant women and VEGF levels produced by stimulated PBMCs were even lower in severe PE [42].

P1GF belongs to VEGF gene family and 42% of P1GF amino acids matches VEGF amino acids, with structural similarities seen as well [43]. This factor is mainly expressed by cytotrophoblast and syncytiotrophoblast, but it can also be expressed by endothelial cells, natural killer cells (NK), bone marrow cells, and keratinocytes [44]. P1GF stimulates angiogenesis during episodes of ischemia, inflammation, and wound healing. It can also have an influence in development of atherosclerosis [45]. Blocked VEGF and P1GF action caused PE-like symptoms in experiments with pregnant rats, showing that a lower concentration of P1GF is important in sFlt-1 caused endothelial dysfunction [46].

During normal pregnancy, P1GF concentration begins to elevate at around 8-12 weeks of pregnancy, reaches its peak at 29-32 week of pregnancy, and begins to decrease at 33-40 weeks of pregnancy [47]. However, patients who later developed PE had lower P1GF concentration from 13-16 weeks of pregnancy until delivery [15, 40]. The lowest P1GF concentration was seen in patients who already had clinically manifested PE at 13-16 weeks of pregnancy *(*Figure 6*)* [16]. Lowered concentration of P1GF also correlates with severity of PE [48]. P1GF concentration changes earlier than sFlt-1 – that is why it is considered a better prognostic marker [48]. Also, due to low molecular weight P1GF is easily filtrated through kidneys which allows measuring P1GF concentration in urine [49]. P1GF urine concentration was analysed and proved to correlate positively with serum P1GF concentrations [50]; it also rises during the first two pregnancy trimesters, reaches its peak at 29-32 weeks of pregnancy, and begins to decrease thereafter. Urine P1GF also correlates with the severity of PE. Neither urine P1GF nor serum P1GF concentration changed during cases with gestational hypertension or normotensive pregnancy with SGA newborns [49].

P1GF improved the positive predictive value of an abnormal ultrasound array Doppler velocimetry (UADV). Abnormal UADV and maternal plasma PlGF of < 280 pg/mL conferred a much higher risk for the development of early onset and/or severe PE (89% of the women with abnormal UADV results developed early onset PE and 84% of the women developed severe PE) had a plasma PlGF concentration of < 280 pg/mL [51]. In addition, another study showed that P1GF can improve algorithm based on mean blood pressure and uterine artery Doppler [40].

s-Flt-1/P1GF ratio

Various studies confirm that P1GF concentration changes are clinically important not only alone, but also combined with sFlt-1. sFlt-1 and P1GF ratio has shown to be useful in PE onset prediction. The time when sFlt1/P1GF ratio increases among women in whom PE subsequently develops varies from 15-17 weeks for preterm PE and from 20-21 weeks for term PE [15, 52]. Backward analysis indicated that patients who developed preterm and term PE had a significantly higher s-Flt-1/P1GF plasma ratio at 20 and 14 weeks before the clinical diagnosis, respectively [15]. Furthermore, sFlt-1/P1GF ratio has shown to be a reliable tool to discriminate between different types of pregnancy-related hypertensive disorders. In the window before 34 weeks, neither patients with gestational hypertension (GH), nor chronic hypertension patients (chrHTN) tend to have increased sFlt-1/P1GF ratio, compared with controls, and this ratio is decreased when compared to PE/HELLP patients. Although after 34 weeks there were statistically significant differences between GH and chrHTN patients and control group, significantly lower concentration was still seen when compared to PE/HELLP patients [53]. These results match other studies [52]. It is also important that this ratio might be useful to predict imminent delivery. A study showed that PE/HELLP patients who delivered within seven days had a significantly higher sFlt-1/PlGF ratio than patients who delivered later than seven days. These findings could be crucial to maternal and fetal morbidity and mortality because the timely referral to a perinatal care center alone is able to reduce them by 20% [53]. In addition, sFlt-1/P1GF ratio has effect on maternal and fetal manifestations. sFlt-1/PlGF ratio correlated positively with proteinuria and length of maternal hospital stay, and correlated

negatively with birth weight. The sFlt-1/PlGF ratio correlated positively with length of newborn stay in the neonatal ICU [50].

Conclusions

Establishing PE diagnosis by measuring blood pressure and proteinuria is not providing any information regarding the severity of the disease or its clinical course. There is no doubt that angiogenic and anti-angiogenic factors are of critical importance in the pathogenesis of PE. Therefore, in PE, sEng and sFlt-1 are increased, whereas free concentrations of PlGF and VEGF are decreased. This angiogenic imbalance correlates with severity of signs and symptoms of PE. Unfortunately, heterogeneity between different studies makes it difficult to compare these biomarkers. It is clear that this field necessitates large-scale prospective multicentric studies. Newly found anti-angiogenic and angiogenic biomarkers should not take over the former ones; on the contrary, they should be seen as another piece of the puzzle which would help to create a reliable way to forewarn the development of PE.The best predictive marker(s) can be identified in the future and can improve the management of women destined to develop PE.

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Corresponding Author:
V. DŽENKEVIČIŪTĖ, M.D.
Fiziku 29
Vilnius NA 09441 (Lithuania)
e-mail: vilma.dzenkeviciute@santa.lt