# Current opinions on PlGF and sFlt-1 as reliable markers for preeclampsia

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**Abstract**

*Preeclampsia is an increasingly frequent condition that affects pregnant women and, if undetected in a timely fashion, can be a source of morbidity and mortality for both mother and child. Both PlGF and Flt-1 have been involved in the mechanisms behind this pathology, and are currently being used both as diagnostic markers and possible therapeutic targets.*

***Keywords:*** *PlGF, sFlt-1, preeclampsia, PlGF/sFlt-1 ratio*

**Rezumat**

*Preeclampsia este o condiție patologică ce afectează din ce în ce mai frecvent femeia gravidă și, nedetectată în timp util, poate fi o cauză de morbiditate și mortalitate, atât pentru mamă, cât și pentru făt. Atât PlGF, cât și Flt-1 au fost implicate în mecanismele care generează această patologie și sunt actualmente utilizate ca markeri diagnostici, dar și ca posibile ținte terapeutice.*

***Cuvinte-cheie:*** *PlGF, sFlt-1, preeclampsie, raportul PlGF/sFlt-1*

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## *Opinii actuale despre PlGF şi sFlt-1 ca martori fideli ai preeclampsiei*

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## Introduction

The reduction of both maternal and fetal mortality and morbidity is a long-standing objective in modern medicine. Preeclampsia is an increasingly diagnosed pathology in pregnancy, with an incidence of up to 4.6%, thus explaining why the interest for its early detection and timely treatment has peaked in recent years.

Apart from the classical diagnostic criteria of preeclampsia, the PlGF (placental growth factor), sFlt-1 (soluble fms-like tyrosine kinase receptor-1) and the PlGF/sFlt-1 ratio are gathering momentum as both diagnostic markers and possible options for therapy.

## The structure and function of PlGF and Flt-1

Dimeric glycoproteins, the placental growth factor, the vascular endothelial growth factor (VEGF) and the Fos-induced growth factor (FlGF) are members of a family of structurally related growth factors and share important biochemical and functional features[1].

Abundantly expressed on the endothelial cells, the vascular endothelial growth factor exerts its action through the binding to VEGF receptor-1 (also denoted Flt-1) and VEGF receptor-2 (also denoted Flk-1/KDR). Both the vascular endothelial growth factor and placental growth factor have the ability to stimulate the proliferation of endothelial cells and angiogenesis *in vivo*[2].

In fact, the placental growth factor is observed to be highly expressed in the feto-placental unit in all stages during human gestation, but also in invasive and non-invasive hydatiform moles[3]. Human PlGF gene is encoded by chromosome 14q24 and has four isoforms, but the correlation among diseases located on these chromosomes' regions has not yet been proven[4]. Chromosomal conditions associated with changes in the structure or number of copies of chromosome 14q24 include not only a major susceptibility to a variety of cancers of blood-forming cells (leukemias)[5], cancers of immune system cells (lymphomas)[5], asthma[6], epilepsy[7], but also congenital syndromes (FOXG1 syndrome)[8]. In tumor cells (solid and hematological), PlGF expression correlates positively with cancer severity and there is an inverse relationship between PlGF and survival rate[9]. It was observed that overexpression of PlGF can decrease angiogenesis, VEGF/PlGF heterodimers being less pro-angiogenic than VEGF homodimers[10], and blocking PlGF can improve the quality of tumor vascularization, as shown in another study[11]. Data from a phase-1 study in patients with recurrent glioblastoma did not show improved survival after the treatment with an anti-PlGF monoclonal antibody in combination with anti-VEGF antibody bevacizumab[12].

The presence of PlGF was detected and researched through immunohistochemistry studies in the vasculosyncytial membrane and in the media of large vessels of the placenta villi[13]. Another analysis, *in situ* hybridization, shows that PlGF is found in the villous trophoblast, while VEGF is present in cells of mesenchymal origin within the chorionic plate[14]. Northern blot analysis detected low levels of PlGF messenger ribonucleic acid in human heart, lung, thyroid, brain and skeletal muscle[3]. The same PlGF messenger ribonucleic acid was observed in choriocarcinoma, umbilical vein endothelial cells and hepatoma[2]. PlGF was detected

in the endometrial tissue during the secretory phase of the human menstrual cycle, but the role in embryo implantation was not closely characterized[15].

It is known that placental growth factor expression undergoes transcriptional and post-transcriptional regulation and the major stimuli of PlGF mRNA are the hypoxic conditions[2]. Another research showed that PlGF stimulates the growth of certain types of endothelial cells, which have derived from specific tissues, and the main target for the angiogenic effects of PlGF is the endothelium of post-capillary venules, which is more prone to respond to mitogenic stimuli in comparison with the umbilical cord vein endothelium[16].

The PlGF homodimer binds and induces autophosphorylation of VEGF receptor-1 (also known as Flt-1), through the immunoglobulin-like domain 2 of the Flt-1[16,17]. PlGF 1 and 3 are diffusible isoforms and probably affect targets in a paracrine manner, whereas PlGF 2 and 4 have heparin binding domains and act in an autocrine manner. The activation of the VEGF receptor-1 intracellular signaling pathway leads to mitogenic and chemotactic effects of PlGF on endothelial cells[2]. Placental growth factor is pro-angiogenic, as it enhances the activity of vascular endothelial cells, by competitively binding to the VEGFR-1 receptor, allowing VEGF to bind to VEGFR-2 which has stronger tyrosine kinase activity. PlGF amplifies VEGFR-2 response to VEGF binding through intermolecular transphosphorylation and forms a heterodimer with VEGF, which may have either pro-angiogenic, or anti-angiogenic effects[9].

Among the members of tyrosine kinases receptor-type family, the vascular endothelial growth factor 1 (VEGFR-1) was first isolated from the human placenta and mentioned as Flt-1 or Fms-like tyrosine kinase, due to its structural similarity to members of the Fms family[18]. Flt-1 binds the VEGF-related growth factor PlGF with high affinity and stimulates angiogenic activities. PlGF is expressed in placental tissues, but the amino-acid sequence of PlGF is 53% identical to that of VEGF[2].

The Ftl-1 gene is described on human chromosome 13q12-13[19] and has specific functions under conditions of embryonal and pathological angiogenesis[20]. In relation to chromosome 13, women carrying trisomy 13 fetuses have an increased risk of preeclampsia and high circulating concentrations of sFlt-1 (soluble Flt-1)[21]. During the early stages of the embryonal development, Flt-1 is expressed in the primitive endothelial cells outside the blood islands at 8.5 days and in the vascular endothelial cells of the embryo[22]. Flt-1 mRNA is strongly expressed under hypoxic conditions in endothelial cells[23]. sFlt-1 is thought to be responsible for the maternal dysfunctional hypoxic placenta (Figure 1), causing peripheral vasoconstriction in the attempt to raise maternal blood pressure. The reason for this mechanism is the tendency to increase the oxygenated maternal blood flow through the intervillous space and can lead to systemic vascular findings with preeclampsia[24]. Flt-1 captures and inhibits pro-angiogenic factors involved in the proliferation, survival and fenestration of endothelial cells, such as placental growth factor and vascular endothelial growth factor[25]. It was observed that once an individual threshold of angiogenic imbalance is reached, the first clinical manifestations of preeclampsia appear. The sFlt-1/PlGF imbalance can become evident as early as one month before preeclampsia develops clinically, especially in the most severe and early-onset forms[26].

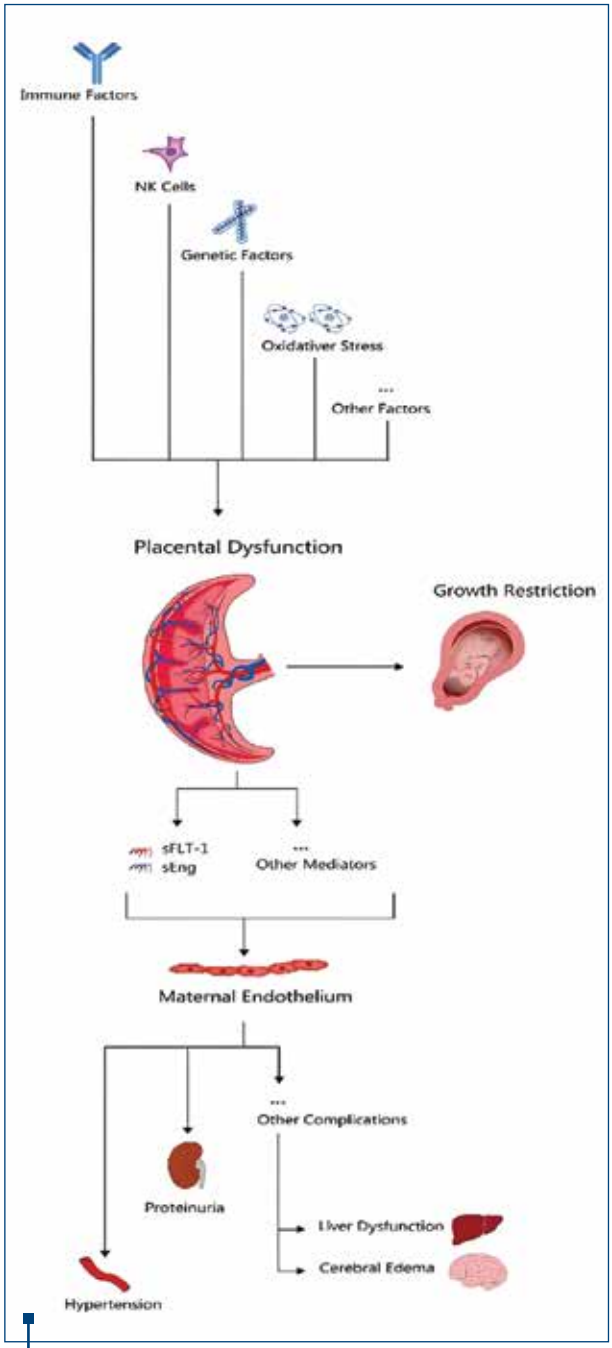


***Figure 1.*** *Implication of sFlt-1 in the dysfunctional placenta (sEng: soluble endoglin; sFlt-1: soluble fms-like tyrosine kinase receptor-1)*

## The importance of sFlt-1/PlGF ratio

The concentrations of PlGF depend on gestational age, with a low level in the first trimester of normal pregnancies, and they have the tendency to increase from week 11 to week 12, with a peak value at week 30, after which they decrease. This is in contrast with sFlt-1, which increases towards the completion of pregnancy[27].

At the time of diagnosis with preeclampsia, as well as in advance of syndrome onset, both urinary and serum PlGF are low, due to a combination of decreased expression and sFlt-1 binding[26]. In early pregnancy, the PlGF expression in the placenta is decreased in women who develop preeclampsia, the level of serum PlGF being lower than in normal pregnancies, while the sFlt-1 level is unchanged. Later in pregnancy, there is a reciprocal relationship between sFlt-1 and PlGF, with a rising levels of total sFlt-1 and lower PlGF levels, which is bound by sFlt-1[28]. An important observation is that both women with clinical suspicion of preeclampsia and women without preeclampsia, who give birth to small-for-gestational-age babies, have also low PlGF early in pregnancy[29].

Based on the previously mentioned mechanisms and useful for establishing the real risk of developing preeclampsia among pacients, the sFlt-1/PlGF ratio has been studied enthusiastically. The PROGNOSIS study shows that the sFlt-1/PlGF ratio and none of the markers taken individually are predictive of the short-term absence or presence of preeclampsia in women between 24 and 36+6 weeks of pregnancy[30]. Another research, the PELICAN study, showed that the PlGF test alone had a very high accuracy for predicting preeclampsia requiring delivery within 14 days for women presented with suspicion of preeclampsia between 20 and 34 weeks of gestation[31] (Table 1). Verlohren et al., in 2014, observed that the sFlt-1/PlGF ratio could be useful for establishing an accurate diagnosis of preeclampsia at different cutoffs, depending on the gestational age, the early or late onset of preeclampsia[32] (Table 1). The same research team raised the hypothesis that the sFlt-1/PlGF ratio can also be useful to differentiate the various hypertensive disorders of pregnancy, especially before 34 weeks[33]. The studies aforementioned have the disadvantage of being conducted on selected population singleton pregnancies without congenital anomalies, but insufficiently studied in situations such as structural and chromosomal abnormalities or multiple pregnancies. On the other hand, a combination of maternal characteristics, mean arterial pressure, uterine artery pulsatility index, PAPP-A and PlGF, currently widely used as a predictive algorithm at 11-13 weeks gestation by the Fetal Medicine Foundation, detects 95% and 46% of women with early and late preeclampsia, respectively with a false-positive rate of 10%[34].

Abnormal sFlt-1/PlGF ratio offers limited information regarding similar antiangiogenic states, such as HELLP (Hemolysis, Elevated Liver enzymes, Low Platelets) syndrome, acute renal failure, refractory hypertension, pulmonary edema or fetal well-being impairment, and therefore requires further research and other detailed maternal and fetal evaluations to complete the diagnosis and future outcome[35].

**Table 1** sFlt-1/PlGF ratio research data

| Study | Gestational age | Results | |
|---|---|---|---|
| PROGNOSIS study[30] | 24-36$^{+6}$ weeks of gestation | sFlt-1/PlGf ratio≤38<br>**NPV 99.3% – first week**<br>(95% CI; 97.9-99.9)<br>NPV 97.9% – 2$^{nd}$ week<br>NPV 95.7% – 3$^{rd}$ week<br>NPV 94.3% – 4$^{th}$ week | sFlt-1/PlGf ratio>38<br>**PPV 36.7%**<br>(95% CI; 28.4-45.7)<br>**Sp 83.1%**<br>(95% CI; 54-77)<br>**Sn 66.2%**<br>(95% CI; 79.4-86.3) |
| PELICAN study[31] | 20-34 weeks of gestation | PlGF<100 pg/Ml<br>**Sp 56%** (95% CI; 89-99)<br>**Sn 96%** (95% CI; 49-63)<br>**PPV 44%** (95% CI; 36-52)<br>**NPV 98%** (95% CI; 93-100) | |
| Verlohren et al., 2014[32] | early-onset preeclampsia | sFlt-1/PlGf ratio>85<br>**Sp 99.5%**<br>(95% CI; 97.7-100) | |
| | late-onset preeclampsia | sFlt-1/PlGf ratio>100<br>**Sp 95.5%**<br>(95% CI; 92.9-100) | |

**NPV: negative predictive value; PPV: positive predictive value; Sp: specificity; Sn: sensitivity*

## Potential therapeutic targets

Despite its important predictive value, the sFlt-1/PlGF ratio and both biomarkers alone were studied as a potential target for therapies. In a pilot study, Thadhani et al. confirmed that removing sFlt-1 with dextran sulfate apheresis, from the excessive placental level secreted in the maternal circulation, was safe to use during pregnancy and the gestational age of the treated patients was prolonged[36,37].

Therefore, further randomized controlled trials must confirm this hypothesis. Brownfoot et al. conducted an experimental treatment for preeclampsia, with pravastatin, which reduced sFlt-1 level in the maternal circulation and decreased endothelial dysfunction in cell culture experiments[38].

The previous group published a pilot data suggesting that pravastatin can positively interfere with clinical and biochemical features of preterm preeclampsia, but studies to confirm the importance of pravastatin in randomized controlled trials are still under careful observation[39].

Different PlGF treatment reports, on rodent models, evaluated the possibility of new means of treating preeclampsia, using exogenous PlGF, by continuous infusion *via* an intraperitoneal osmotic pump of recombinant human PlGF (rhPlGF)[40] or by transfection of mice with adenovirus[41]. The first method is shown to reduce blood pressure, proteinuria and improve glomerular filtration rate in addition to reducing markers of oxidative stress, while the second one reduced hypertension but not proteinuria. Therefore, the treatment of preeclampsia with PlGF needs to be further studied; it seems promising, but many uncertainties remain. ■

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## References

1. Bussolino F, Mantovani A, Persico G. Molecular mechanisms of blood vessel formation. *TIBS*. 1997; (22):251-6.
2. Persico M, Vincenti V, DiPalma T. Structure, expression and receptor-binding properties of placenta growth factor (PlGF). In: Current topics in microbiology and immunology. Berlin, Springer; 1999:31-41.
3. Maglione D, Guerriero V, Viglietto G, et al. Two alternative mRNAs coding for the angiogenic factor, placenta growth factor (PlGF), are transcribed from a single gene of chromosome 14. *Oncogene*. 1993; (8):925-31.
4. DiPalma T, Tucci M, Russo G, et al. The placenta growth factor gene of the mouse. *Mammalian Genome*. 1996; (7):6-12.
5. Dessen P, Huret J. Chromosomal band 14q24. [Online].; 2012 [cited 2019 May 21. Available from: http://atlasgeneticsoncology.org/Bands/14q24.html
6. Hakonarson H, Bjornsdottir U, Halapi E, et al. A Major Susceptibility Gene for Asthma Maps to Chromosome 14q24. *Am J Hum Genet*. 2002;b(71):483-91.
7. Giovanni S, Marangio L, Fusco C, et al. Epilepsy in ring 14 syndrome: a clinical and EEG study of 22 patients. *Epilepsia*. 2013; (54): 2204-13.
8. Kortum F, Das S, Flindt M, et al. The core FOXG1 syndrome phenotype consists of postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and corpus callosum hypogenesis. *J Med Genet*. 2011; (48):396-406.
9. Chau K, Hennessy A, Markis A. Placental growth factor and preeclampsia. *Journal of Human Hypertension*. 2017; (31):782-6.
10. Xu L, Cochran D, Tong R, et al. Placenta growth factor overexpression inhibits tumor growth, angiogenesis, and metastasis by depleting vascular endothelial growth factor homodimers in orthotopic mouse models. *Cancer Res*. 2006; 66(8):3971-77.
11. Van de Veire S, Stalmans I, Heindryckx F, et al. Further pharmacological and genetic evidence for the efficacy of PlGF inhibition in cancer and eye disease. *Cell*. 2010;(141):178-90.
12. Lassen U, Chinot O, McBain C, et al. Phase 1 dose-escalation study of the antiplacental growth factor monoclonal antibody RO5323441 combined with bevacizumab in patients with recurrent glioblastoma. *Neuro Oncol*. 2015; (17(7)):1007-15.
13. Khaliq A, Li X, Shams M. Localization of placenta growth factor (PlGF) in human term placenta. *Growth Factors*. 1996; (13):243-50.
14. Vuorela P, Hatva E, Lymboussaki A, et al. Expression of vascular endothelial growth factor and placenta growth factor in human placenta. *Biol Reprod*. 1997; (56):489-94.
15. Binder N, Evans J, Salamonsen L, et al. Placental growth factor is secreted by the human endometrium and has potential important functions during embryo development and implantation. *PLoS ONE*. 2016;11(10):e0163096.
16. Sawano A, Takahashi T, Yamaguchi S, et al. Fit-I but not KDR/Flk-I tyrosine kinase is a receptor for placenta growth factor, which is releated to vascular endothelial growth factor. *Cell Growth Differ*. 1996; (7):213-21.
17. Davis-Smyth T, Chen H, Park J, et al. The second immunoglohulin-like domain of the VEGF tyrosine kinase receptor Fit-I determines ligand hinding and may initiate a signal transduction cascadc. *EMBO J*. 1996; (15):4919-27.
18. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *CELL*. 1990; (61):203-12.
19. Satoh H, Yoshida M, Matsushime H, Shibuya M, et al. Regional localization of the human c-ros on 6q22 and flt on 13q12. *Jpn J Cancer Res*. 1987; (78):772-5.
20. Wernert N, Raes M, Lassalle P, et al. C-ets 1 proto-oncogene is a transcription factor expressed in endothelial cells during tumor vascularization and other forms of angiogenesis in humans. *Am J Pathol*. 1992; (14):119-27.
21. Bdolah Y, Palomaki G, Yaron Y, et al. Circulating angiogenic proteins in trisomy 13. *Am J Obstet Gynecol*. 2006; (194):239-45.
22. Fong G, Rossant J, Gertsentein M, et al. Role of the Flt-1 receptor tyrosine kinase is regulating the assembly of vascular endothelium. *Nature*. 1995; (376):66-70.
23. Shweiki D, Itin A, Soffer D, et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*. 1992; (359):843-5.
24. Roberts J. Pathophysiology of ischemic placental disease. *Semin Perinatol*. 2014; (38):139-45.
25. Hagmann H, Thadhani R, Benzing T, et al. The promise of angiogenic markers for early diagnosis and prediction of preeclampsia. *Clin Chem*. 2012; (58):837-45.
26. Levine R, Maynard S, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*. 2004; (350): 672-83.
27. Saffer C, Olson G, Boggess K, et al. Determination of placental growth factor (PlGF) levels in healthy pregnant women without signs or symptoms of preeclampsia. *Pregnancy Hypertens*. 2013; 3(2):124-32.
28. Maynard S, Min J, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003; 111(5):649-58.
29. Poon L, Zaragoza E, Akolekar R, et al. Maternal serum placental growth factor (PlGF) in small for gestational age pregnancy at 11(+0) to 13(+6) weeks of gestation. *Prenat Diagn*. 2008; 28(12):1110-15.
30. Zeisler H, Llurba E, Chantraine F, et al. Predictive value of the sFlt-1/PlGf ratio in women with suspected preeclampsia. *N Engl J Med*. 2016; (374):13-22.
31. Chappell L, Duckworth S, Seed P, et al. Diagnostic accuracy pf placental growth factor in women with suspected preeclmapsia: a prospective multicenter study. *Circulation*. 2013; (128):2121-31.
32. Verlohren S, Herraiz I, Lapaire O, et al. New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. *Hypertension*. 2014; (63):346-52.
33. Verlohren S, Herraiz I, Lapaire O, et al. The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am J Obstet Gynecol*. 2012; (206:58): e1-e8.
34. Poon L, Syngelaki A, Akolekar R, et al. Combined screening for preeclampsia and small for gestational age at 11-13 weeks. *Fetal Diagn Ther*. 2013; 33(1):16-27.
35. Gomez-Arriga P, Herraiz I, Lopez-Jimenez E, et al. Uterine artery Doppler and sFlt-1/PlGF ratio: prognostic value in early-onset pre-eclampsia. *Ultrasound Obstet Gynecol*. 2014; (43):525-32.
36. Thadhani R, Kisner T, Hagmann H, et al. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. *Circulation*. 2011; (124):940-50.
37. Thadhani R, Hagmann H, Schaarschmidt W, et al. Removal of soluble fms-like tyrosine kinase-1 by dextran sulfate apheresis in preeclampsia. *J Am Soc Nephrol*. 2016; (27): 903-13.
38. Brownfoot F, Tong S, Hannan N, et al. Effects of pravastatin on human placenta, endothelium, and women with severe preeclampsia. *Hypertension*. 2015; (66):687-97.
39. Constatine M, Cleary K. Pravastatin for the prevention of preeclampsia in high-risk pregnant women. *Obstet Gynecol*. 2013; (121):349-53.
40. Spradley F, Tan A, Joo W, et al. Placental growth factor administration abolishes placental ischemia-induced hypertension. *Hypertension*. 2016; 67(4):740-7.
41. Suzuki H, Ohkuchi A, Matsubara S, et al. Effect of recombinant placental growth factor 2 on hypertension induced by full-length mouse soluble fms-like tyrosine kinase 1 adenoviral vector in pregnant mice. *Hypertension*. 2009; 54(5):1129-35.