Angiogenic factors in preeclampsia: so complex, so simple?

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Introduction

Preeclampsia is a common cause of fetal and maternal morbidity and mortality, that affects ~2–7% of all healthy nulliparous women [1]. Until recently, the pathophysiology of preeclampsia was not well understood. Successive hypotheses have been proposed, each being challenged by subsequent publications. The current most plausible hypothesis involves abnormal placental development leading to placenta ischaemia [2]. Recently, ischaemic trophoblast cells [3] were shown to synthesize anti-angiogenic factors, notably the soluble form of fms-like tyrosine kinase-1 (sFlt-1), which is a receptor for vascular endothelial growth factor (VEGF). In addition, sFlt-1 mRNA is highly expressed in the placenta from preeclamptic patients, and administration of sFlt-1 induces a preeclampsia-like syndrome in pregnant rats [4]. Therefore, sFlt-1 could be the link between implantation disorders and maternal symptoms of preeclampsia.
We also showed that between 25 and 28 weeks of gestation, plasma sFlt-1 levels above 957 ng/l had 100% specificity and 80% sensitivity for predicting preeclampsia [11]; however, this was an initial study using frozen samples and this value needs to be refined for larger populations and with fresh serum samples rather than thawed frozen samples. Furthermore, although the sFlt-1 levels that we reported in normal and preeclamptic pregnancies were in the same range as those found in two prospective studies, the issue of sFlt-1 stability remains to be examined. Regardless, all studies to date have shown that sFlt-1 levels are elevated during preeclampsia. This elevation of SFlt-1 may not be specific to preeclampsia because one (isolated) report showed an increase in sFlt-1 levels during intrauterine growth retardation without preeclampsia [14]. However, this point remains debated [12].

Because PI GF is a small molecule, it is filtered by the glomerulus and excreted in the urine. In 2005, Levine et al. [15] reported that, like sFlt-1, urinary PI GF levels could be used to predict preeclampsia. Specifically, preeclamptic women with high plasma sFlt-1 values had lower PI GF concentrations in plasma and urine, and a decrease in urinary PI GF at mid-gestation was found to be strongly associated with the early development of preeclampsia. The authors therefore recommended measuring the urinary PI GF concentration in all pregnant women at mid-gestation and the sFlt-1 levels in the serum of women with low urinary PI GF levels [15].

Interestingly, two karyotypic anomalies, trisomy 13 and hydatidiform mole, are known to be associated with a risk for preeclampsia. The association between trisomy 13 and preeclampsia was first suggested in 1987 by Redman et al. [16] and was confirmed by a larger retrospective study published in 1992 by Tuohy and James [17]. The authors predicted that a fetal factor was involved in the pathogenesis of preeclampsia in this setting, and they suspected that proteins encoded by genes located on chromosome 13 play a role. Indeed, the sFlt-1 gene is localized on chromosome 13, and a recent study comparing 17 pregnancies with trisomy 13 and 80 normal pregnancies showed that the plasma sFlt-1/PI GF ratio is significantly higher in those with trisomy 13 [18]. In retrospect, because there are 69 chromosomes including three copies of chromosome 13 in molar pregnancies, the association with preeclampsia is not surprising.

Viral infections can also lead to the overexpression of sFlt-1 [19]. A transient preeclampsia syndrome lasting about a month was recently described during a pregnancy with maternal parvovirus seroconversion and fetal anaemia (fetal ascites and hydropic placenta) [19]. This was successfully treated by intravascular transfusion of the fetus. The maternal plasma sFlt-1 level was greatly increased during the preeclamptic phase and fell as the clinical manifestations of preeclampsia subsided. Interestingly, this case indicates that preeclampsia may be reversible during pregnancy. The concomitant fall in the sFlt-1 level and the clinical...
improvement (i.e. resolution of hypertension and proteinuria) further supports the direct pathophysiological role of sFlt-1 in the maternal syndrome in preeclampsia. All these reports argue that sFlt-1 is involved in the pathophysiology of preeclampsia.

Impaired cytotrophoblast invasion of the maternal spiral arteries is the main characteristic of severe and/or early preeclampsia and it begins very early in pregnancy, long before the appearance of maternal symptoms [2]. All published studies on the kinetics of plasma sFlt-1 show that the rise in sFlt-1 levels occurs during the second part of pregnancy and is therefore not the primary mediator of classical preeclampsia. Rather, it is currently thought that the impaired cytotrophoblast invasion leads to deficient nutrient and oxygen supply to the fetoplacental unit leading to local hypoxia and increased apoptosis. Studies performed in human placenta explants suggest that it is the hypoxia that is responsible for abnormal sFlt-1 synthesis [3]. They showed that cytotrophoblasts up-regulate sFlt-1 expression in vitro under conditions of low oxygen.

In addition to these classical severe forms of preeclampsia, there are mild forms of term preeclampsia that lack clear signs of impaired cytotrophoblast invasion. These later and more moderate forms could be due to simple exaggeration of physiological placental apoptosis and inflammatory status [2]. Indeed, a physiological increase in sFlt-1 expression occurs towards the end of pregnancy. This increase was recently shown to positively correlate with the physiological micro-albuminuria observed at the end of normal pregnancy and to negatively correlate with the maternal serum VEGF concentration [20]. In this context, it is interesting that some degree of glomerular endotheliosis is observed in renal biopsy samples obtained from healthy women at the end of normal pregnancies [21]. Although this study raised ethical issues, it showed that 5 out of 12 women with normal pregnancies had mild signs of glomerular endotheliosis, which had not been previously described except in preeclampsia. Overall, it appears that the renal symptoms observed during preeclampsia are due to an exacerbation of a normal process, (i.e. glomerular endotheliosis) due to a deficiency in angiogenic factors.

Using DNA chip analysis, Venkatesha et al. [22] recently found that placental expression of the soluble form of endoglin is increased in preeclampsia. Endoglin is a co-receptor for types 1 and 3 transforming growth factor- (TGF-β) highly expressed by angiogenic endothelial cells. Overexpression of the soluble form of endoglin is thought to blunt the angiogenic effects of TGF-β and to oppose the physiological NO-dependent vasodilation elicited by this growth factor. This study [22] also showed that co-administration of sFlt-1 and soluble endoglin to pregnant rats induces severe preeclampsia along with a HELLP (haemolysis, elevated liver enzymes, and low platelet count)-like syndrome. Also, using samples from the CPEP study, they found that soluble endoglin levels are significantly increased in preeclamptic women. Therefore, there appears to be a strong link between the HELLP syndrome and preeclampsia.

These advances in the understanding of preeclampsia suggest the following pathophysiological mechanism. In classical preeclampsia, trophoblast invasion of the maternal spiral arteries proceeds abnormally for unknown reasons, with endothelial and smooth muscle cells persisting in the wall of the decidual and myometrial vessels. The presence of these persistent smooth muscle cells can be visualized by uterine artery Doppler screening as the presence of persistent bilateral notches during the second part of gestation. Failure of trophoblast invasion and spiral artery transformation leads to insufficient oxygen and nutrient supply to the fetoplacental unit. The resulting placent al hypoxia leads to overexpression of sFlt-1, which absorbs free VEGF and PIGF in the maternal circulation, preventing these two growth factors from binding to their respective membrane receptors. This in turn provokes a relative systemic vasoconstriction (hypertension) and glomerular endotheliosis (proteinuria). At the same time, the increase in placental apoptosis leads to the release of trophoblast debris into the maternal circulation [2]. This debris contributes to the pro-inflammatory state associated with preeclampsia and participates in the maternal endothelial insult that favours a prothrombotic state and systemic vasoconstriction [2].

If, as expected, large prospective studies confirm that there is a significant increase in maternal sFlt-1 levels 5 or more weeks before the clinical onset of preeclampsia, sFlt-1 could be useful as a biomarker for identifying women who require closer monitoring. The enzyme-linked immunosorbent assay for sFlt-1 is expensive but relatively simple; however, before it can be used routinely, the measurement procedures must be standardized. Specifically, the cut-off should take into account the low prevalence of preeclampsia in the general population to avoid a high false positive rate. Second, though closer monitoring is known to improve pregnancy outcome, no specific monitoring guidelines have been defined to date. These assays could be used to monitor pregnant women with chronic hypertension or renal disorders because it is often very difficult to distinguish an aggravation of the underlying chronic disease from superimposed preeclampsia. Again, appropriate cut-off values must be determined in these specific populations. Moreover, although sFlt-1 may be useful for identifying women at risk for preeclampsia, this marker increases too late in pregnancy to be used to direct preventive treatment, for example, with aspirin.

Finally, low free circulating VEGF levels are responsible for the maternal symptoms and certain fetal signs in preeclampsia. Therefore, administration of VEGF to the mother might be beneficial, but lowering maternal blood pressure and decreasing uterine arterial blood flow could be hazardous to the fetus. Moreover, through its angiogenic action (and possibly other effects), VEGF has been shown to promote the proliferation of a wide range of normal
and tumour cells, including breast tumour cells, and increased endogenous levels of VEGF are associated with a poor response to hormone antagonist therapy in breast cancer patients. This possible pro-tumorigenic side-effect of VEGF should be considered before advocating its widespread therapeutic use.

**Conclusion**

Recent advances in the understanding of preeclampsia have opened up new possibilities for the identification of women at risk for developing this disease and intrauterine growth restriction. The use of sFlt-1 and PI GF as biomarkers should be compared with traditional screening such as uterine artery Doppler imaging in women at low and high risk for preeclampsia. Although low maternal plasma VEGF levels supports the therapeutic use of VEGF infusion in preeclamptic women, its cancer-promoting effects and side-effect of VEGF should be considered before advocating its widespread therapeutic use.

**Conflict of interest statement.** None declared.

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