Novel Angiogenic Factors for Predicting Preeclampsia: sFlt-1, PlGF, and Soluble Endoglin

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Abstract: Preeclampsia is a devastating multisystem syndrome and is a major cause of maternal, fetal and neonatal morbidity and mortality. Angiogenic factors contribute to the molecular mechanisms of preeclampsia and its main phenotypes such as hypertension and proteinuria. Very recently, novel anti-angiogenic proteins including soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), and one pro-angiogenic protein placental growth factor (PlGF) have been found to be significantly abnormal several weeks preceding the onset of clinical signs and symptoms. Automatic immunoassays are being developed for sFlt-1 and PlGF. This article will summarize our current knowledge of these markers and their roles on predicting preeclampsia.

Key Words: Preeclampsia, sFlt-1, PlGF, and soluble endoglin.

INTRODUCTION

Preeclampsia is a heterogeneous syndrome of pregnancy that is characterized by hypertension and proteinuria in mother and/or growth restriction of fetus developing at late pregnancy. Preeclampsia and other hypertensive disorders of preganancy such as chronic hypertension and gestational hypertension remain leading causes of maternal and perinatal morbidity and mortality. It has been estimated that preeclampsia occurs in 5-8 % of all pregnancies worldwide resulting in a very large health burden [1-3]. According to the guideline of National Institutes of Health publication No. 00-3029 [4], preeclampsia is defined as (1) maternal systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg on two occasions, separated by 6 hours, and (2) proteinuria > 300 mg in a 24 hours period after 20 weeks of gestation. The criteria for severe preeclampsia include anyone or more of the following: blood pressure $\geq$ 160/110 mmHg, proteinuria > 2+ by dipstick or > 2 g/24 h, visual disturbances, pulmonary edema, epigastric or right upper quadrant pain, impaired liver function, thrombocytopenia, fetal growth restriction, and the most severe form of preeclampsia is the so-called HELLP syndrome (hemolysis, elevated liver enzymes and low platelets count) [5, 6].

Although preeclampsia has been extensively studied for several decades, its etiology is still unclear. Preeclampisa is mostly considered as a two-stage disorder [7, 8]. In preclinical stage 1, the endothelialization of cytotrophoblasts is impaired and the invasion of spiral arteries into myometrium is inadequate, remaining small calibre resistance vessels. Poor placentation results in possible placenta ischemia and hypoxia. Stage 2 occurs in late pregnancy. The oxidatively stressed placenta releases anti-angiogenic proteins such as soluble fms-like tyrosine kinase-1 (sFlt-1), prostaglandins and cytokines into the maternal circulation. Meanwhile, the hypoxic placenta reduces the production of pro-angiogenic factors including placental growth factor (PlGF) and vascular endothelial growth factor (VEGF). These changes cause the systemic endothelial dysfunction and an inflammatory response that leads to elevated systemic vascular resistance, vasoconstriction, activation of the coagulation cascade, and eventually clinical manifestations such as hypertension, porteinuria, hepatic dysfunction, neurological disturbances, hematological disturbances, and fetal growth restriction. To support this hypothesis, Makris *et al*. induced uteroplacental ischemia (UPI) by uterine artery ligation in a pregnant non-human primate model and found that UPI resulted in the development of a clinical entity analogous to human preeclampsia and significant elevation of circulating sFlt-1 [9]. However, an alternative molecular mechanism has been proposed that angiogenesis imbalance (increase of sFlt-1 and decrease of PlGF and VEGF) by genetic, immunological and other unknown factors, may be the cause of placental hypoxia which in turn results in more sFlt-1 production, thus leading to a vicious cycle and eventually causing preeclampsia [10]. In brief, sFlt-1 rise and PlGF/VEGF is down regulated. This imbalance results in abnormal placentation and placenta hypoxia that in turn results in further angiogenesis imbalance, maternal endothelial dysfunction and preeclampsia. The work of Thadhani *et al*. showed that the reduction of serum PlGF occured as early as in the first trimester in patients destined to develop preeclampsia and suggested that imbalance of pro- and anti-angiogenic factors may be the primary cause of preeclampsia [11]. The cause or effect of angiogenesis imbalance and placenta hypoxia is basically a chicken and egg issue, however, the precedence of angiogenesis imbalance over clinical signs and symptoms has shred off lights on the prediction of the onset of preeclampsia.

The aim of this mini-review is to provide clinical laboratory professionals and clinicians with our current under-

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standing of novel preeclampsia-related pro- and anti-angiogenic factors (sFlt-1, PlGF, and soluble Endoglin) and recent advances in the utilities of these markers for predicting preeclampsia.

sFlt-1

sFlt-1, also known as soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), is the soluble receptor for VEGF and PlGF (Table **1**). In the human VEGF family system, there are two transmembrane receptors involved in angiogenesis, i.e., VEGFR-1 (or Flt-1) and VEGFR-2 (or fetal liver kinase-1/kinase insert domain-containing receptor). These receptors contain seven extracellular immunoglobulin (Ig)-like domains (including three ligand binding domains and a dimerization domain), a membrane-spanning region, and a split intracellular tyrosine kinase domain [12-15]. While VEGFR-1 is specific for the angiogenic factors VEGF-A, VEGF-B, and PlGF, VEGFR-2 is responsible for ligands including VEGF-A, VEGF-C, VEGF-D, and VEGF-E (viral) [14]. Unlike *VEGFR-2*, *VEGFR-1* gene expresses two types of mRNA of 3.0 and 2.4 kb, corresponding to the full-length VEGFR-1 of 180 kDa and the soluble receptor of sFlt-1 (100 kDa) respectively [12, 16]. The truncated sFlt-1 consists of only the first six extracellular Ig-like domains [16].

VEGFR-1 has a about 10-time weaker kinase activity than that of VEGFR-2 and it is a dual regulator for angiogenesis. VEGFR-1 plays a negative role in angiogenesis at embryogenesis [17, 18], however, the tyrosine kinase domain of VEGFR-1 does promote the functions of monocytes/macrophages and play an angiogenic role [19, 20]. The binding affinity of VEGFR-1 as well as sFlt-1 for VEGF-A is about one order of magnitude higher than that of VEGFR-2, which may explain that excess circulating sFlt-1 antagonizes normal VEGF and PlGF signals by binding and preventing their interaction with VEGFR-1 or VEGFR-2 [14, 15]. Furthermore, sFlt-1 may form inactive heterodimers with VEGFR-2 through dimerization domains that are located in the Ig-like loops [16].

VEGFR-1 was originally discovered by screening of a human placental cDNA library [12]. The production of sFlt-1 is mostly from placenta, with only small amounts are generated by endothelial cells and monocytes [19, 21, 22]. sFlt-1 may play an important role in the pathogenesis of preeclampsia and may actually be the cause of the syndrome. Karumanchi and Epstein [23] had summarized 10 lines of accumulating evidence from their own as well as other investigators' findings, e.g., sFlt-1 antagonizes VEGF signalling; administration of sFlt-1, anti-VEGF antibodies or inhibitors to animals or cancer patients leads to preeclampsia like symptoms; and the endothelial dysfunction induced by preeclampsia can be reversed by removal of sFlt-1 or by addition of excess VEGF [24-29].

In normal pregnancy, the serum concentration of sFlt-1 decreases from 8-12 weeks to 16-20 weeks, gradually increases at 26-30 weeks, rapidly elevates at 35-39 weeks of gestation, and it is back to normal level after delivery [30, 31]. However, sFlt-1 level in preeclamptic pregnancy is significantly higher than that of normal pregnancy. Levine *et al*. have shown that sFlt-1 level increased beginning approximately five weeks before the onset of preeclampsia [31]. The mean serum concentration of sFlt-1 in the women with the onset of clinical disease was highest and about 3 times of that of normal pregnancies of similar gestational age and its levels correlated with the severity of the disease [31, 32]. In a systematic review, all five available studies consistently demonstrated the increase of sFlt-1 level after gestational week 25 in preeclampsia, especially in severe preeclampsia, compared with the control group [33]. The receiver operator characteristic (ROC) curves of sFlt-1 for prediction of preeclampsia with onset prior to 34 weeks' gestation was constructed using serum specimens obtained 22-26 weeks' gestation and the area under curve was 0.9 [34]. sFlt-1 has also been demonstrated diagnostic utilities to differentiate preeclampsia from normal pregnancy, gestational hypertension and chronic hypertension with sensitivities of 79-90%, specificities of 88-95%, positive likelihood ratio (LR) of 6.7-16, negative LR of 0.1-0.2, and area under the ROC curve of 0.88-0.94 [35].

PlGF

PlGF is a member of the VEGF family that shares 42% amino acid (aa) sequence identity with VEGF, and they share significant structural similarity [36]. PlGF has four alternatively spliced forms: PlGF-1, PlGF-2, PlGF-3, and PlGF-4 [36, 37]. PlGF is a small protein (~30 kDa) and is filtered into urine even in the absence of renal damage [6]. Unlike VEGF-A, PlGF only binds to VEGFR-1 but not VEGFR-2. It has been suggested that PlGF synergistically enhances VEGF-induced angiogenesis by binding to VEGFR-1 to displace VEGF, and the latter will be freed to activate VEGFR-2 which has much potent kinase activity [38]. Interestingly, PlGF may also regulate inter- and intramolecular cross talk between the VEGFR-1 and VEGFR-2, e.g., activation of VEGFR-1 by PlGF results in intermolecular transphosphorylation of VEGFR-2 hence VEGF-

Table 1. Summary of sFlt-1, PlGF, and sEng

	sFlt-1	PlGF	sEng
Molecular size	100 kD	~30 kD	~65 kD
Characteristics and biological function	Truncated form of VEGFR-1 Antagonizes VEGF and PlGF signalling Anti-angiogenic	A member of VEGF family Binds to VEGFR-1 Pro-angiogenic	Truncated form of Endoglin Impairs TGF-β1 signalling Anti-angiogenic
Changes in preeclampsia	Increase	Decrease	Increase

driven angiogenesis through VEGFR-2 is amplified [39]. The interaction of PlGF and its receptor can be inhibited by sFlt-1 and thereby leads to endothelial dysfunction [40].

As the name suggested, PlGF was first identified in human placenta [41]. It is expressed mainly in the villous cytotrophoblasts and syncytiotrophoblasts which may indicate a role for placenta formation [42]. In addition, PlGF may be expressed in endothelial cells, natural killer cells, bone marrow cells, and keratinocytes [42]. In normal pregnancy, the serum concentration of PlGF increases from 8-12 weeks, reaches to peak at 29-32 weeks, and then decreases at 33-40 weeks of gestation [30, 31]. PlGF levels in women who later had preeclampsia were significant lower than the controls from 13-16 weeks of gestation till delivery and the PlGF was lowest in women with clinical preeclampsia at similar gestational age [31]. In a systematic review of 10 available studies, all reports consistently demonstrated a decrease of PlGF level at the second and the third trimester of preeclampsia compared with normal pregnancy and the decrease of PlGF was shown to correlate with the severity of the disease in all those studies investigated on severe form of preeclampsia [33]. The ROC area under curve of PlGF for prediction of preeclampsia with onset prior to 34 weeks' gestation using serum specimens obtained 22-26 weeks' gestation was 0.97 [34]. Because the change of PlGF happens earlier than that of sFlt-1, it may be considered as a better marker for predicting preeclampsia. Serum sFlt-1 to PlGF ratio is significantly increased in preeclamptic pregnancy compared with the control and it has been shown a fairly good power for the prediction as well with an area under the ROC curve as 0.94 [34, 43].

Another important aspect of PlGF is its small molecular size and because it is generated predominantly from placenta during preganacy, urinary PlGF may be used as a convenient preeclampsia marker. In the Calcium for Preeclampsia Prevention (CPEP) trial, Levine *et al.* found that during normal pregnancy urinary PlGF demonstrated a same pattern as serum PlGF, which increased during the first 2 trimesters, peaked at 29-32 weeks then decreased thereafter [6]. Similarly, urinary PlGF (from both random and first morning urine) increased at preeclamptic pregnancy but was significant lower compared with the controls. Furthermore, urinary PlGF and PlGF normalized for creatinine both correlated with the severity of disease. Interestingly, neither urinary PlGF nor PlGF normalized for creatinine were significantly decreased in gestational hypertension or normotensive pregnancy with small for gestational age infants [6].

SOLUBLE ENDOGLIN

Endoglin, also known as CD105, is a transmembrane glycoprotein first identified by Letarte's group [44, 45]. There are two Endoglin splice variants (S and L) identified, i.e., Endoglin-L consisting of 633 aa with a 47 aa cytoplasmic tail and Endoglin-S consisting of 600 aa with a 14 aa cytoplasmic tail [46]. Endoglin is a 180 kDa homodimeric co-receptor for members of the transforming growth factor (TGF)-β superfamily including TGF-β1, TGF-β3, bone morphogenic proteins, and activin A [47]. The protein regulates TGF-β signalling by interacting with TGF-β type I and type II receptors and their ligands [47, 48]. Endoglin is highly expressed on the cell membranes of syncytiotrophoblasts, vascular endothelial cells, and it is also expressed on other cells such as monocytes and hematopoietic stem cells [49-52]. Given that, Endoglin is suggested to involve in angiogenesis and hematopoiesis and play a role in cancer and cardiovascular development [53, 54]. In human, mutations in the Endoglin gene cause hereditary hemorrhagic telangiectasia or *Osler Rendu Weber Syndrome* type 1 (HHT1), an autosomal-dominant disorder characterized by arteriovenous malformations in multiple organ systems and bleeding telangiectases of mucous membranes [54].

Soluble Endoglin (sEng) is the ~65 kDa truncated form of Endoglin [55]. Evidence indicated that sEng is an anti-angiogenesis factor and it contributes to the pathogenesis of preeclampsia [48]. It has been shown to impair the binding of TGF-β1 to its receptors and downstream signalling such as activation of eNOS and vasodilation [56]. Adenoviral-mediated overexpression of sEng in pregnant rats led to hypertension, and coadministration of sEng and sFlt-1 resulted in severe preeclampsia including the HELLP syndrome [56]. In addition, sEng correlates tightly with sFlt-1, PlGF, and sFlt-1/PlGF ratio, as well as disease severity in preeclampsia [57]. The serum level of sEng of normal pregnancy is quite stable till slightly increase at 33-42 weeks of gestation [43]. However, circulating sEng concentrations are significantly elevated in preeclamptic pregnancy and intrauterine growth restriction [43, 58]. Very interestingly, sEng levels increased significantly before the onset of disease in women with preterm preeclampsia (< 37 weeks) by 11-9 weeks, and in term preeclampsia (at ≥ 37 weeks) by 14-12 weeks respectively [43]. The second trimester sEng has been suggested as a marker for predicting preterm and severe preeclampsia [59, 60]. Soluble Endoglin has also been demonstrated fairly good diagnostic utilities to differentiate preeclampsia from normal pregnancy, gestational hypertension and chronic hypertension with sensitivities of 84-90%, specificities of 79-95%, positive LR of 4-17.9, negative LR of 0.1-0.2, and area under the ROC curve of 0.75-0.93 [35]. The predictive abilities of sFlt-1 and PlGF for preeclampsia were excellently reviewed by Widmer *et al.* in a systematic review [33]. The test utility for sENG is summarized from 4 most recent studies [35, 43, 61, 62] as shown in Table **2**.

CONCLUDING REMARKS

As discussed above, circulating sFlt-1, PlGF, and sEng all have specific alterations in preeclamptic pregnancy and because these changes usually happen before the onset of clinical presentation of the disorder, these proteins are believed to be useful for predicting preeclampsia. Serum sFlt-1/PlGF ratio and urinary PlGF have also been demonstrated utilities and convenience for the prediction. These angiogenic factors may be used as markers for the third or second trimester screening test, but before we adopt these strategies, there are several challenges need to overcome.

There is large variation in the published values of these markers within the same gestational age window which may reflect the heterogeneity in sample handling, processing and laboratory procedures, and this variation induces difficulties to compare the diagnostic values across different studies. In addition, there is no adequate information on a cut-off for clinical practice to predict preeclampsia. So far numerous studies provided the associations between alterations of an-

Table 2. Predictive Ability of Soluble Endoglin for Preeclampsia

Study	Gestational Week	Sample Size (PE/Control)	Cut-off (ng/mL)	Sensitivity (%)	Specificity (%)	Odds Ratio (95% CI)	ROC Area	LR (+)	LR (-)
Levine 2006 [43]	13-20	Preterm PE 23/27	7.9			2.2 (1.1-4.6)			
		Term PE 25/27	7.9			1.1 (0.6-2.3)			
		PE + SGA 13/27	7.9			1.7 (0.7-4.1)			
	21-32	Preterm PE 43/27	7.2			9.4 (4.3-20.7)			
		Term PE 45/27	7.2			2.6 (1.4-4.8)			
		PE + SGA 25/27	7.2			8.7 (3.4-21.9)			
	33-42	Term PE 62/22	13.6			7.0 (3.4-14.4)			
		PE + SGA 14/22	13.6			40.7 (6.7-246.1)			
Salahuddin 2007 [35]	34.6±3.3	PE/Ctrl 19/20	24.8	90	95		0.93	17.9	0.1
		PE/ GHTH 19/17	33	84	88		0.87	7.2	0.2
		PE/CHTH 19/19	31.5	79	99		0.87	4	0.2
Baumann 2008 [61]	11-13	46/92	5	63	57		0.628		
Stepan 2008 [62]	19-25	14/45	4.14	80	43.2	2.67 (0.65-10.9)		2.09	

PE, preeclampsia; CI, confidence interval; ROC, receiver operating characteristic; LR, likehood ratio; SGA, small-for-gestational-age infants; Ctrl, control; GHTH, gestational hypertension; CHTH, chronic hypertension.

giogenic factors and subsequent preeclampsia, however most were retrospective studies with limited patients numbers. The evidence is insufficient to recommend these factors to be used for screening tests at present [33]. Prospective data with rigorous study design and enough patient participants are needed to further evaluate the clinical utility of these tests.

Currently the only methodology for the measurement of sFlt-1, PlGF, and sEng is the manual enzyme-liked immunosorbent assay kit from the R&D Systems. This kit is not validated for diagnostic procedures. The methods for sFlt-1 and PlGF are under development at Beckman Coulter for its Access® Immunoassay System. The preliminary data of their assays showed that areas under the ROC curves were 0.993 for sFlt-1 and 0.986 for PlGF (2008 American Association for Clinical Chemistry (AACC) annual meeting, abstract A-107). The Ortho Clinical Diagnostics is working on new sFlt-1 and PlGF assays for the Vitros® ECiQ immunodiagnostic system (personal communication). The Abbott Diagnostics and Dade Behring GmbH also joined the arena and are developing methods for sFlt-1 and PlGF for their automatic immunoassay platforms (2008 AACC annual meeting, abstracts D-108 and B-95).

In conclusion, sFlt-1, PlGF, and sEng are important angiogenic (pro or anti) factors involved in the pathogenesis of preeclampsia. These proteins may be promising markers for the disease prediction. With the early detection of preeclampsia, appropriate treatment, timely delivery and continued intensive postpartum monitoring, most cases of severe preeclampsia, eclampsia and seizure can be prevented. Although there is no curative treatment yet, these angiogenic factors may provide potential target for future therapy and animal experiments are underway.

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ABBREVIATIONS

aa = Amino acid

Flt-1 = Fms-like tyrosine kinase-1

Ig = Immunoglobulin

LR = Likelihood ratio

PlGF = Placental growth factor

ROC = Receiver operator characteristic

sEng = Soluble endoglin

sFlt-1 = Soluble fms-like tyrosine kinase-1

TGF = Transforming growth factor

VEGF = Vascular endothelial growth factor

VEGFR = Vascular endothelial growth factor receptor

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