

Biochemical markers for prediction of preeclampsia: review of the literature

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Summary

Preeclampsia (PE) is one of the most common diseases worldwide, complicating ~5% of all pregnancies. Although no major progress has been achieved in the treatment of PE, our ability to identify women at high-risk has increased considerably during the past decade.

The early identification of patients with an increased risk for preeclampsia is therefore one of the most important goals in obstetrics. Today, several markers may offer the potential to be used, most likely in a combinatory analysis, as predictors or diagnostic tools. We present here the current knowledge on the biology of preeclampsia and review several biochemical markers which may be used to monitor preeclampsia in a future, that, we hope, is not to distant from today.

Key words: preeclampsia; early diagnosis; hypertension; flt-1; sEng; P-selectin; effDNA; ADAM12; PP-13; PTX3; PAPP-A.

Introduction

Preeclampsia occurs in 2–5% of pregnancies in the Occident, but it complicates up to 10% of pregnancies in the developing countries, where emergency care is often inadequate or lacking. Therefore we are in need of a widely applicable and affordable test that could permit presymptomatic diagnosis in order to identify and monitor the patients at risk and thus provide the best prenatal care for these women and their child. Such a test would also be of benefit to confirm a confounding clinical diag-

nosis and for future studies investigating prophylactic treatments or temporizing therapies.

To be effective a screening test need to be sufficiently sensitive and specific and must provide an adequate positive predictive value. Today, several promising markers have been described, alone or in combination, that might fulfill these criteria. However, these data came often from small case studies with selected populations. Therefore, there is a need for worldwide large scale prospective studies to confirm the sensitivity and specificity of these promising markers and assess their utility in different subtypes of preeclampsia before they could serve in clinically useful screening tests.

Preeclampsia

Preeclampsia is a multi-system disorder of pregnancy, which is characterized by new onset hypertension (systolic and diastolic blood pressure of ≥ 140 and 90 mm Hg, respectively, on two occasions, at least 6 hours apart) and proteinuria (protein excretion of ≥ 300 mg in a 24 h urine collection, or a dipstick of $\geq 2+$), that develop after 20 weeks of gestation in previously normotensive women^{1,2}.

Dependent on the systemic involvement, several other symptoms, such as edema, disturbance of haemostasis, renal or liver failure, and the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet counts) also complicate the clinical picture. Preeclampsia can have an early onset (preeclampsia starting before 34 weeks of gestation) or late onset (preeclampsia starting after 34 weeks of gestation), can show mild or severe symptoms (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg, proteinuria >5 g/24 hours, oliguria, neurological symptoms, other clinical symptoms such as deranged liver function, thrombocytopenia $< 100\,000$ mm³, HELLP syndrome), and can evolve in eclampsia in the most severe cases. In addition, it can manifest as a maternal disorder only, with an appropriate fetal growing, or it can present itself with a growth restricted fetus (intrauterine growth restriction (IUGR)) or sudden fetal distress.

Pathophysiology

The precise origin of preeclampsia remains elusive, but it is believed to be likely multifactorial. A certainty is the central role played by the placenta in its pathology^{3, 4, 5, 6}. A long standing hypothesis has been that preeclampsia develops as a consequence of some kind of immune maladaptation between the mother and the fetus during the very first weeks of pregnancy, leading to a 2-step disorder progression that can be summarized as following:

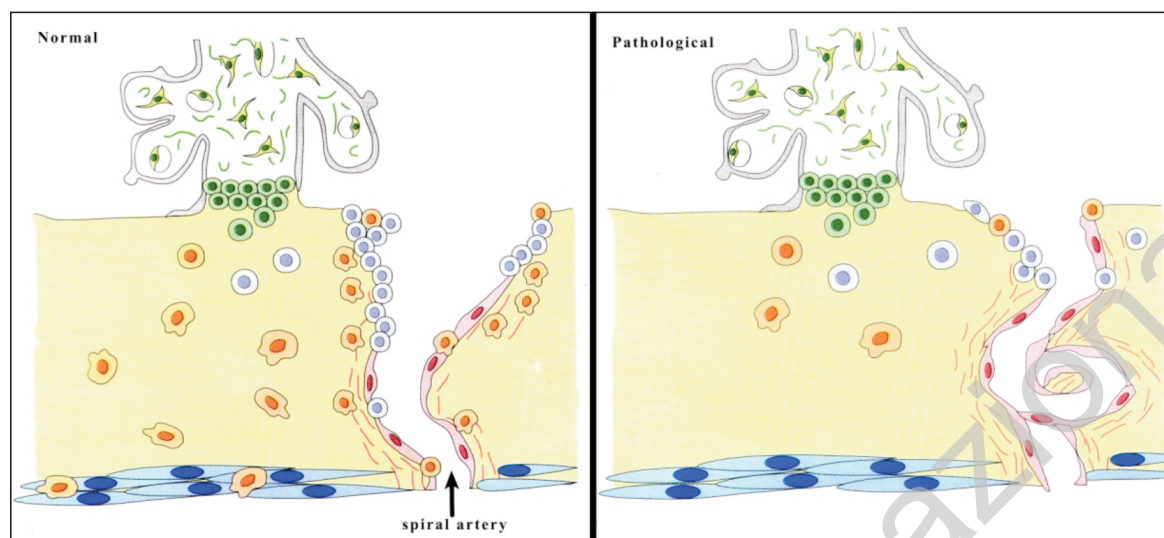


Figure 1

in a first – asymptomatic – step, local aberrant feto-maternal immune interactions within the uterine wall lead to impaired tissue and arterial invasion by trophoblast cells. This results in failed transformation of the uterine spiral arteries and subsequently worsened placental perfusion. Chronic hypoxia or alternate periods of hypoxia/re-oxygenation within the intervillous space is expected to trigger tissue oxidative stress and increase placental apoptosis and necrosis^{7, 8} (Fig. 1).

The clinical disorder arises, in a second step, when the maternal vascular and immune systems cannot handle any longer the increased shedding of placental-produced debris and the aberrant expression of pro-inflammatory, anti-angiogenic and angiogenic factors, leading to a systemic endothelial cell dysfunction and an exaggerated inflammatory response^{1, 9, 10}. Recently, this hypothesis has been challenged¹¹.

It was proposed instead that intrinsic failure in trophoblast differentiation at different time points of ontogeny may lead to either a mild disorder with late-onset appearance, or IUGR complicated or not with the maternal symptoms. However, the origin of preeclampsia might not be restricted to an alteration of trophoblast differentiation, but may also in some cases depend on an underlying maternal constitutional factors such as genetic, obesity, dysfunctional maternal clearance or inflammatory systems¹².

Material and Methods

Since many years, different biophysical and biochemical markers have been investigated, based on pathophysiological observations that have been noted in case of preeclampsia, such as placental dysfunction, a generalized inflammatory response, endothelial dysfunction and activation of the coagulation system.

Using a series of keywords, we reviewed electronic databases (Medline, Elsevier) reporting the performance of biological markers to predict preeclampsia, both single markers and combinations of markers.

Biomarkers

Angiogenic factors

Angiogenesis requires the complex interplay between the pro-angiogenic factors vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) with their cognate receptors VEGF receptor-1 (VEGFR-1, which is alternatively called fms-like tyrosine kinase (flt-1) and VEGFR-2 (for a review on the function of these factors¹³). Interestingly, the placenta is a rich source of these factors^{14, 15}.

In addition to regulating blood vessel homeostasis, VEGF, PlGF and the flt-1 receptor have been shown to be key components in regulating trophoblast cell survival and function^{14, 16}.

Placental cells also secrete a soluble isoform of flt-1, which is generated through alternative splicing of the messenger RNA and acts as an anti-angiogenic factor by interacting with, and thereby neutralizing, PlGF and VEGF¹⁷.

There is strong evidence for the occurrence of higher placental expression of sflt-1 and repeated findings of elevated circulating levels of sflt-1 and reduced free bioactive PlGF and VEGF in preeclamptic patients^{9, 18, 19, 20, 21}.

It was thus suggested that a part of this excess of circulating sflt-1 may stem from the placenta. Maternal blood levels of sflt-1 were shown to correlate with the severity of preeclampsia, whereas, in an opposite manner, the quantities of bioactive VEGF and PlGF were further decreased in patients with severe symptoms compared to normal pregnant women or preeclamptic patients with mild symptoms^{9, 18, 22}. Alterations in sflt-1 and PlGF are also more pronounced in early onset in comparison to late onset preeclampsia^{22, 23}.

However, it was also shown that increased levels of sflt-1 were also associated with IUGR²⁴.

Remarkably, when introduced into pregnant rats, exogenous sflt-1 triggers hypertension and proteinuria, symptoms akin to those in preeclampsia⁹.

According to some studies, the presymptomatic alter-

ations in sflt-1 levels appeared to be specific for preeclampsia as no changes are detected in women who later deliver SGA neonate or whose pregnancies are complicated by IUGR, compared to women with normal pregnancy outcome^{25, 26}.

However, others found that in a selected group of patients with abnormal uterine perfusion, similar alterations in sflt-1 and PIGF levels could be detected during the second trimester in cases with subsequent IUGR²⁷.

Nevertheless, owing to the evolving unbalance of angiogenic factors after 25 weeks of gestation in women with subsequent preeclampsia, the ratio sflt-1/PIGF has been advocated to be a reliable marker of overall preeclampsia risk. As a matter of fact, soluble flt-1 and PIGF have been launched by Roche as a screening test for preeclampsia in the second trimester in Europe and is expected to be submitted to the FDA soon.

It has recently been reported that patients with preeclampsia have lower plasma concentrations of soluble VEGF-R2²⁸.

However, this biomarker may not be specific for preeclampsia as an equivalent decrease was observed in patients with SGA babies in the absence of preeclampsia.

Soluble Endoglin

Endoglin (Eng) is a co-receptor for transforming growth factor (TGF)- 1 and TGF- 3 that is highly expressed on cellular membranes of the vascular endothelium and on the syncytiotrophoblast^{29, 30}.

It functions as a modulator of TGF- signaling and is involved in angiogenesis and the regulation of the vascular tone^{31, 32}.

A circulatory form of endoglin, which consists of the extra cellular part of the molecule that may be produced through the proteocleavage of the placental membrane-bound form, has been identified in normal pregnancy and in preeclampsia.

In vitro, sEng acts as a negative regulator of angiogenesis by competitive interaction with TGF-, thereby impairing capillary formation by endothelial cells. Furthermore, it induces high arterial pressure and vascular permeability in pregnant rats in which the protein was over-expressed. Very interestingly, the combined introduction of sEng and sflt-1 in the pregnant animals induced renal, placental and hepatic changes reminiscent of the HELLP syndrome³³.

Soluble Eng is present in substantial excess in preeclamptic patients compared to normotensive controls, and its concentrations appear to increase with the severity of the symptoms and are the highest in preeclampsia complicated by the HELLP symptom^{24,33,34}. Pregnancies with IUGR without the maternal syndrome may also be characterized by elevated levels of sEng, suggesting that this factor is not specific for preeclampsia, but may be a marker for clinical conditions associated with an underlying placental pathology^{24, 34}.

However, these results remain conflicting as others demonstrated no association between IUGR and the levels of sEng. Moreover, a pilot study has suggested that sEng may prove useful in differentiating preeclampsia from other hypertensive diseases of pregnancy, such as gestational or chronic hypertension³⁵.

Large scale studies will be needed in order to clarify these important issues.

Like sflt-1, sEng concentrations raise during the last 2 months of normal pregnancy. In pregnancies ending with preeclampsia, this increase occurs earlier and is steeper^{26, 36, 37}.

The distinction becomes significant starting 9 - 11 weeks before the clinical symptoms, for both early and late onset preeclampsia, but is more prominent for preterm preeclampsia or in women in whom preeclampsia is complicated with SGA. Altered levels of this factor throughout gestation are also associated with SGA pregnancies without the maternal symptoms^{26, 38}.

Thus, a specific prediction cannot be achieved with this analyses alone.

Several longitudinal case-control studies have therefore evaluated the potential of sEng in combination with the pro- and anti-angiogenic factors PIGF and sflt-1 for the prediction of preeclampsia^{26, 36}.

The studies reported that the pattern of changes in the ratio of different combinations of these factors (PIGF/sEng; (sflt- 1+sEng)/PIGF; etc), collected at 13 weeks and around 20 weeks, was more informative than the individual biomarkers at single time-point screening. One study suggested that a rigorous monitoring of the sequential changes in the profile of these three biomarkers between the first and the second trimesters permits sensitive and specific risk assessment³⁸.

A change in PIGF/sEng ratio that was below the median slope for controls conferred an odds ratio of 7.68 for the development of pre-term preeclampsia, and 2.46 for the development of term preeclampsia, and discriminated SGA pregnancies from preeclampsia. Further studies with large number of patients will be required to confirm these very promising preliminary results and assess the utility of analyzing these biomarkers in the clinical routine.

P-Selectin

P-selectin is a member of the selectin family of cell surface adhesion molecules. It is expressed by platelets and endothelial cells upon activation and plays crucial roles in inflammatory reactions by supporting the recruitment and activation of circulating leucocytes, and in coagulation through the generation of leukocyte-derived "bloodborne" tissue factor^{39, 40}.

P-selectin is rapidly shed from the cellular membrane of activated platelets and this release is suggested to contribute to most of the soluble isoform of the molecule that is found in the plasma⁴¹.

Preeclampsia is associated with extensive platelet activation⁴².

P-selectin-exposing micro particles with procoagulant activity, released from activated platelets, have been detected in the peripheral blood of preeclamptic women^{43, 44}. In addition, soluble P-selectin has been repeatedly, though not constantly, observed in higher amounts in serum or plasma of patients with this disorder^{45, 46}.

Interestingly, it has recently been shown that alterations in the levels of soluble P-selectin before 20 weeks of gestation antedate the symptoms^{47,48}. This early up-regulation of soluble P-selectin has been suggested to reflect the early but still asymptomatic disturbances of the maternal vascular system. In one of these studies, P-selectin was identified as the marker with the highest discriminatory ability among three biomolecules evaluated between gestational weeks 11 to 15⁴⁷.

However, the combination of P-selectin with the two other

markers, namely Activin A and VEGFR, showed a detection rate of only 59% (with a false-positive rate of 5%), which is not sufficient for a possible routine clinical implementation as a screening test.

Cell-free fetal DNA

Since its detection in maternal plasma many approaches have been tested to use cell free fetal DNA for non-invasive diagnostic approaches. These include qualitative analyses like fetal sex analysis⁴⁹, determination of the fetal Rhesus status^{50,51} or the analysis of fetal point mutations⁵² as well as the quantitative analysis as an indicator for several fetal anomalies, e.g. fetal growth restriction⁵³, polyhydramnios⁵⁴, trisomy⁵⁵ or preterm labor⁵⁶.

The value of cffDNA in maternal plasma as an indicator for preeclampsia has first been reported by Lo et al. in a small scale study in the plasma of 20 preeclamptic women and 20 gestational age matched controls in the third trimester, where cffDNA was increased approximately 5-fold in women with preeclampsia⁵⁷. The same effect was observed in the second trimester in a study by Zhong et al. in 10 preeclamptic women and 40 controls⁵⁸. The so far biggest study in that field was conducted by Levine et al. with 120 preeclamptic women and 120 controls: A two- to five-fold increase of cffDNA levels was monitored starting from week 17 until three weeks before the onset of preeclampsia⁵⁹.

As the amount of fetal DNA is routinely determined by quantifying Y-chromosome specific sequences, e.g. *SRY* (sex determining region Y) and *DYS*⁶⁰, alternative approaches have been tested to overcome this limitation: An increase of total cell free DNA was observed in women with preeclampsia at term^{61,62,63} and before the onset of preeclampsia⁶³. Furthermore, approaches to analyze cffDNA independent from fetal sex, using epigenetic differences between maternal and fetal DNA have been developed, e.g. the use of the *maspin* gene, which is hypomethylated in fetal tissue⁶⁴ or the hypermethylated fetal promoter sequence of *RASSF1A*⁶⁵.

Although these approaches are promising, only one study quantifying cffDNA with the *RASSF1A* approach in 10 women with preeclampsia and 20 controls has been published⁶⁶. cffDNA has shown some predictive value for the prediction of preeclampsia between 20–25 weeks of gestation, however, higher sensitivities and specificities can be obtained by combining several markers as has been shown in a nested case-control study for cell free DNA combined with Inhibin A in the second (n = 15 at risk for PE), n = 68 controls) and third trimester (n = 34 preeclampsia, n = 44 controls)⁶⁷.

Currently, several multicenter studies are being performed to confirm the predictive value of cffDNA to predict and monitor preeclampsia in combination with other potential markers, e.g. P-selectin, PAPP-A, PP-13, sIlt-1, sEng, PIGF).

ADAM12

ADAM12 (a disintegrin and metalloprotease 12) is a membrane bound zinc dependant protease and belongs to the ADAM protein family, a group of proteins involved in cell-cell and cell-matrix interactions in fertilization, muscle development and neurogenesis^{68,69}. For this gene, two alternatively spliced transcripts are known, a short secreted form and a long membrane-bound form⁷⁰. The plasma concentration of ADAM12 has been found to be

altered in several pregnancy related disorders. Several studies have demonstrated that the plasma level of ADAM12 is decreased in women carrying a fetus with trisomy 21 and trisomy 18^{71,72,73}. It has also been shown that the ADAM12 concentration is decreased in women with other aneuploidies and in women with low for gestational age birth weights⁷⁴.

The first connection of ADAM12 serum levels to preeclampsia was demonstrated by Laigaard et al. in a study with 160 women with preeclampsia and 324 healthy controls in the first trimester⁷⁵. The serum concentration of ADAM12 was significantly decreased in women that later developed preeclampsia. These results were confirmed by Spencer et al. in a study with two groups (1. n = 64 PE, n = 240 controls, 2. n = 24 cases, n = 144 controls)⁷⁶. However another study failed to confirm these promising results but concluded that measurement of ADAM12 does not provide useful prediction of SGA, preeclampsia, or spontaneous preterm delivery⁷⁷.

PP-13

Placental protein 13 (PP-13, galectin-13) was first isolated in 1983 by Bohn et al.^{78,79}. It is a relatively small protein with 139 amino acids (16,118 kDa) which is highly homologous (69%) to the human eosinophil Charcot-Leyden Crystal protein, a phospholipase that belongs to the beta-galactoside binding S-type animal lectin super family. The homodimer which is linked by disulfide bonds probably has special haemostatic and immunobiological functions at the feto-maternal interface or a developmental role in the placenta⁸⁰. The 600 bp mRNA transcript is only detectable in placental tissue but not in any other fetal or adult tissue^{78,79,81,82}.

The serum levels of PP-13 slowly increase during a normal pregnancy but abnormally low levels of PP-13 were detected in first trimester serum samples of women subsequently developing fetal growth restriction and preeclampsia, in particular cases with early onset^{83,84}. Elevated serum concentrations of PP-13 have been found in the second and third trimester in women with preeclampsia, IUGR and in preterm delivery⁸⁵. For this study 514 controls, 69 cases with preeclampsia, 69 cases with IUGR, 52 cases with preterm delivery and 24 cases with preeclampsia developing before 34 weeks of gestation have been included.

Another study concluded that first-trimester serum levels of PP-13 may serve as a suitable marker for preterm preeclampsia but are weak for the prediction of severe preeclampsia and ineffective for mild preeclampsia at term⁸⁶. Here again the combination of several diagnostic tools results in improved predictive power as was shown by combined measuring of first trimester serum PP-13 levels and median uterine artery pulsatility index by ultrasound. This combination achieved a detection rate for preeclampsia of 90% with a false positive rate of 6%⁸⁷. However, this combination of serum PP-13 levels and uterine artery pulsatility index loses its predictive power when late second trimester (22–24 weeks of gestation) serum is analyzed⁸⁸.

Currently a commercial PP-13 test kit is developed for the first trimester screening for preeclampsia by Diagnostic Technologies, Haifa. The test has already been approved in Europe and approval in the United States is expected in the near future.

Table I - Today the use of biomarkers in combination with uterine artery Doppler screening is promising as a potential screening tool.

Biochemical Marker	Plasma Concentrations			Reported combinations for prediction	Altered levels are also correlated with
	1° trimester	2° trimester	Manifest preeclampsia		
sflt-1	---			- sEng, PIGF, VEGF, - Ultrasound	---
sEng	---			- sflt-1, PIGF, - Ultrasound	- IUGR - HEELP - SGA
PIGF				- sflt-1, sEng - Ultrasound	- IUGR - Preterm delivery - SGA
PP-13					
P-Selectin				Activin A, sflt-1	---
Cell-free fetal DNA				Inhibin A	- IUGR - polyhydramnios - trisomy 21 - preterm labour
Cell free DNA	---	---		---	- trisomy 21 - trisomy 18 - SGA
ADAM12		---	---		
PTX3				---	- IUGR - birthweight - type-2 diabetes m.
PAPP-A					
Visfatin	---				

PTX3

Pentraxin 3 (PTX3, tumor necrosis factor stimulated gene- 14⁸⁹) belongs to the same family as C-reactive protein (CRP) or serum amyloid P component (SAP) and consists of 381 amino acids. The C-terminus is highly homologous to SAP and CRP whereas the N-terminus doesn't show any homology to other proteins. The according gene is organized into three exons⁹⁰ and is extremely evolutionarily conserved from horseshoe crab to human⁹¹.

Responding to proinflammatory stimuli CRP, SAP and PTX3 are produced by various tissues. It is also expressed in tissues undergoing cell death. PTX3 then interacts with several growth factors, extra cellular matrix components and certain pathogens but is also involved in the activation of the complement system⁹² and facilitates pathogen recognition by phagocytes⁹³.

During pregnancy, PTX3 is increasingly expressed in amniotic epithelium, chorionic mesoderm, trophoblast terminal villi, and perivascular stroma of placentae⁹³. Cetin et al. and Rovere-Querini et al. showed that in case of a future preeclampsia and IUGR the PTX3 plasma levels are even more increased in all three trimesters^{94,95}. So far no studies that combine PTX3 with other potential markers have been performed.

PAPP-A

PAPP-A (pregnancy-associated plasma protein A, papalysin 1, insulin-like growth factor binding protein-4 protease, EC 3.4.24.79) is a disulfide bond linked homod-

imeric peptidase of 1628 amino acids and a mass of 400 kDa⁹⁶. It can be detected during pregnancy in maternal circulation mainly as a complex with the proform of the eosinophil major basic protein, an inhibitor of PAPP-A⁹⁷. Although the reaction products are not identified yet, insulin-like growth factor binding proteins are substrates for the hydrolytic activity of PAPP-A⁹⁸. PAPP-A is supposedly involved in local proliferative processes, for example bone remodeling⁹⁹.

In the recent years decreased plasma levels of PAPP-A have been reported in all trimesters in women with preeclampsia^{100, 101, 102, 103}.

Discussion

Regardless of the lack of existing prophylactic and therapeutic means against preeclampsia, the search for noninvasive, blood-borne or urinary biomarkers that could predict the development or assist in the detection of this life-threatening pregnancy disorder is still of utmost importance. The availability of such markers could have decisive impact on the medical management of pregnant women and their child (e.g. refer to a tertiary centre) but also on the health costs associated with this poor medical condition.

So, early identification of pregnant women at risk for preeclampsia is a priority to implement preventive measures.

Conclusion

Despite there exists many different potential markers for preeclampsia, the reliability of these markers in predicting preeclampsia has been inconsistent between different studies. Furthermore, preeclampsia is a multifaceted disorder, certain say it is not one but several diseases. Therefore, there is a need for high quality, large scale multicenter trials which enroll patients with different risks of developing the syndrome and throughout multi-ethnic background, in order to assess the predictive value of different markers and finally propose the best marker combination for a routine use in clinical settings.

Those biomarkers parameters have shown promising predictive performance, but so far there is no clinically validated screening procedure.

Below is a table about summary of potential biochemical markers for the prediction (1° and 2° trimester) or the detection (manifest preeclampsia) of preeclampsia in maternal peripheral blood (Tab. 1).

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