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# Clinical, Biochemical, and Biophysical Markers of Angiogenesis in Preeclampsia

*Osredkar Joško and Kumer Kristina*

## Abstract

Preeclampsia/eclampsia is described as a pregnancy-specific systemic disorder of unknown etiology and is a potentially life-threatening disease with symptoms related to a general vascular endothelial cell activation and dysfunction. Preeclampsia can be defined as a new onset of hypertension ( $>140/90$  mmHg) after gestational week 20 together with significant proteinuria (300 mg/24 h). Preeclampsia has a complex pathophysiology, the primary cause likely being abnormal placentation. Angiogenic factors and biophysical markers may be combined for predicting preeclampsia. Various high-throughput techniques have evolved, thus allowing us simultaneous examination of thousands of genes (genomics), gene transcripts (transcriptomics), proteins (proteomics), metabolites (metabolomics), protein interaction (interactomics), and chromatin modifications (epigenomics) in single experiments, and the results suggest that the use of transcriptomic, proteomic, and metabolomic profiles may be predictive for preeclampsia.

**Keywords:** preeclampsia, biomarkers, sFlt-1, PlGF, sEndoglin

## 1. Classification and epidemiology of hypertension during pregnancy

Hypertension is the second most prevalent maternal complication worldwide after anemia in pregnancy, and it is associated with a significant morbidity and mortality of the mother and fetus. The American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy has modified the older classification of hypertension during pregnancy in only four categories: (1) preeclampsia-eclampsia, (2) chronic hypertension (of any cause), (3) chronic hypertension with superimposed preeclampsia, and (4) gestational hypertension (**Figure 1**). It has been suggested that an older category, “unclassified,” be reintroduced or replaced by “suspected” or “presumptive” preeclampsia [1].

In 2017, the American College of Cardiology and American Heart Association (ACC/AHA) issued a clinical practice guideline on hypertension that reclassified the previous category of prehypertension into elevated BP (systolic BP 120–129 mmHg) and stage 1 hypertension (systolic BP 130–139 mmHg or diastolic BP 80–89 mmHg) [2]. However a rise of diastolic blood pressure over prepregnant levels (delta hypertension) rather than a rise above absolute value is also a significant predictive marker.

Severe features of preeclampsia (any of these findings):

A. Systolic blood pressure of 160 mmHg or higher or diastolic blood pressure of 110 mmHg or higher on two occasions at least 4 h apart

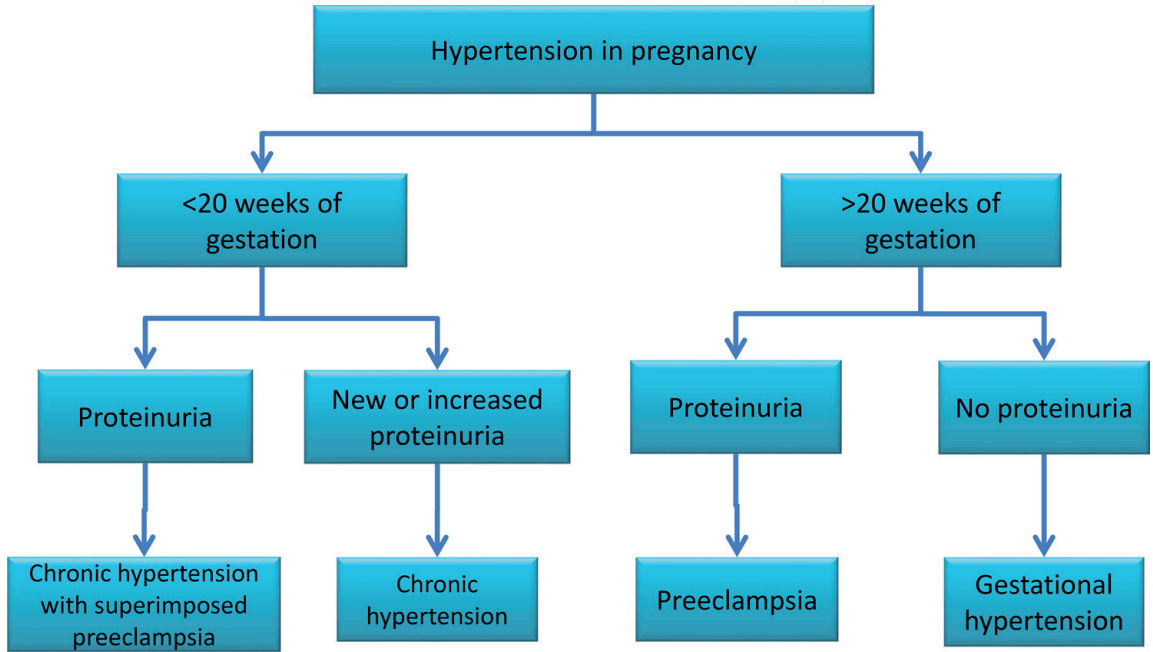
B. Thrombocytopenia

C. Impaired liver function as indicated by abnormally elevated liver enzymes

D. Progressive renal insufficiency

E. Pulmonary edema

F. New-onset cerebral or visual disturbances



**Figure 1.**  
*Classification of hypertensive disorders in pregnancy.*

## 2. Definition of preeclampsia

Preeclampsia/eclampsia is described as a pregnancy-specific systemic disorder of unknown etiology and is a potentially serious disease with symptoms related to a generalized vascular endothelial activation. The placenta seems to be a crucial component in the pathophysiology of the disease. Preeclampsia is a multisystemic disease characterized by the development of hypertension after 20 weeks of gestation, with the presence of proteinuria or, in its absence, of signs or symptoms indicative of target organ injury [3, 4].

Preeclampsia can be defined as a new onset of hypertension ( $>140/90$  mmHg) after gestational week 20 together with significant proteinuria (300 mg/24 h) [5, 6]. Hypertension is considered mild until diastolic or systolic levels reach or exceed 110 and 160 mmHg, respectively. It is recommended that a diagnosis of hypertension requires at least two determinations at least 4 h apart. Proteinuria is diagnosed when 24-h excretion equals or exceeds 300 mg in 24 h or the ratio of measured protein to creatinine in a single-voided urine measures or exceeds 0.3 (each measured as mg/dL), termed the urinary protein/creatinine ratio [1]. The definitive treatment of preeclampsia is delivery to prevent development of

maternal or fetal complications from disease progression. Timing of delivery is based upon gestational age, the severity of preeclampsia, and maternal and fetal condition.

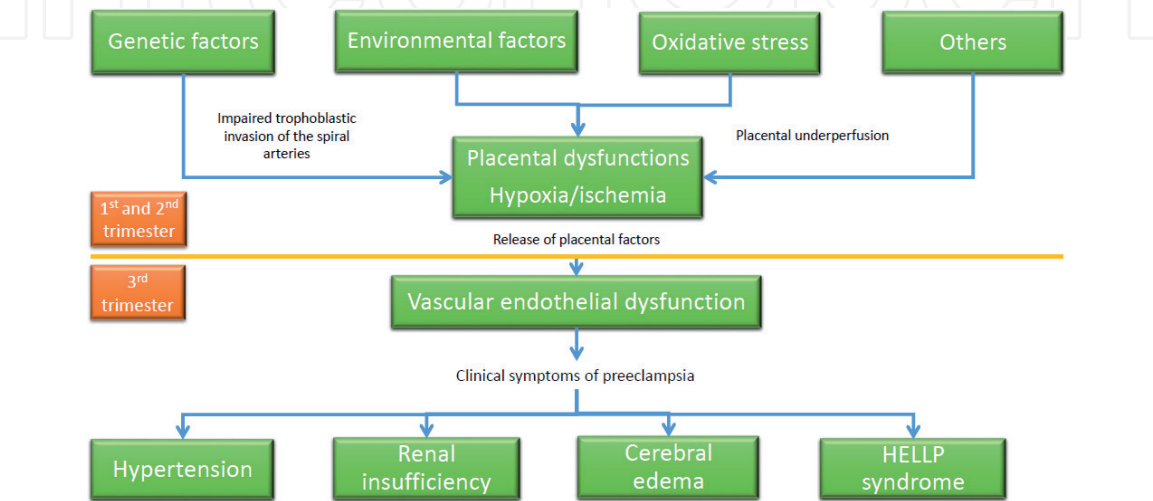
### 3. Key elements of the pathophysiology

Precise causes of preeclampsia are still unknown, but contributors are impaired angiogenesis [7], systemic endothelial dysfunction [8], and decreased vascular compliance resulting in impaired accommodation of the volume expansion required for healthy gestation [9].

During normal pregnancy, the villous cytotrophoblast invades into the inner third of the myometrium, and spiral arteries are remodeled. The remodeling contains four steps: decidua-associated remodeling, the intraluminal appearance of migratory endovascular trophoblasts, their intramural incorporation and trophoblast-associated remodeling, and maternal reendothelialization.

Preeclampsia has a complex pathophysiology, the primary cause likely being abnormal placentation. Defective invasion of the spiral arteries by cytotrophoblast cells is observed during preeclampsia. Recent studies have shown that cytotrophoblast invasion of the uterus is actually a unique differentiation pathway in which the fetal cells adopt certain attributes of the maternal endothelium they normally replace. In preeclampsia, this differentiation process is defective [10].

In normal pregnancy the uterine arteries are resilient and elastic, and they lose their sensitivity to vasoconstrictors. In a preeclamptic pregnancy there is increased uterine arterial resistance and higher sensitivity to vasoconstrictors and thus chronic placental ischemia and oxidative stress. This chronic placental ischemia causes fetal complications, including fetal growth restriction (FGR) and intrauterine death. In parallel, oxidative stress induces release into the maternal circulation of substances such as free radicals, oxidized lipids, cytokines, and serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1/sFlt-1). These abnormalities are responsible for endothelial dysfunction [8] with vascular hyperpermeability, thrombophilia, and hypertension, so as to compensate for the decreased blood flow in the uterine arteries due to peripheral vasoconstriction. Endothelial dysfunction is responsible for the clinical signs observed in the mother, i.e., impairment of the hepatic endothelium contributing to onset of the HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome, impairment of the cerebral endothelium inducing cerebral edema or posterior



**Figure 2.**  
*Pathogenesis of preeclampsia.*

reversible encephalopathy syndrome (PRES), refractory neurological disorders, or even eclampsia. In kidney, the depletion of vascular endothelial growth factor (VEGF) in the podocytes leads to endotheliosis, and these block the slit diaphragms in the basement membrane, exacerbating the already decreased glomerular filtration and causing proteinuria. Finally, endothelial dysfunction promotes microangiopathic hemolytic anemia, and vascular hyperpermeability associated with low serum albumin causes edema, particularly in the lower limbs or lungs. The crucial issue to understand is that the prime mover of preeclampsia is abnormal placentation [11] [Figure 2].

#### 4. Symptoms

Preeclampsia sometimes develops without any symptoms. High blood pressure may develop slowly, or it may have a sudden onset. Monitoring of blood pressure is an important part of prenatal care because the first sign of preeclampsia is commonly a rise in blood pressure. Blood pressure that exceeds 140/90 mmHg—documented on two occasions, at least 4 h apart—is considered abnormal.

Other signs and symptoms of preeclampsia may include:

- Excess protein in urine (proteinuria)
- Severe headaches
- Vision changes include sensations of flashing lights, light sensitivity, blurry vision, or spots
- Upper abdominal pain, usually under ribs on the right side
- Nausea or vomiting
- Decreased urine output
- Decreased levels of platelets in the blood (thrombocytopenia)
- Impaired liver function
- Shortness of breath and anxiety
- Sudden weight gain
- Swelling (edema)

Many of these symptoms also occur in normal pregnancies, so they are not considered reliable signs of preeclampsia though they will alert the obstetrician.

The International Society of Study of Hypertension in Pregnancy (ISSHP) recently suggested that a clinical diagnosis is made even in the absence of proteinuria if organ-specific signs or symptoms are present with new onset of hypertension [12].

Hemolysis, abnormal elevation of liver enzymes levels, and low platelet count occur together as the HELLP syndrome [13]. HELLP syndrome is a severe variant of preeclampsia that occurs in 5% of cases and can progress rapidly to a life-threatening condition [14]. The presence of seizures in preeclampsia is eclampsia and is another complication during pregnancy and at delivery. In **Table 1**, diagnostic criteria are summarized.



<b>Blood pressure</b>
• Greater than or equal to 140 mm Hg systolic or greater than or equal to 90 mm Hg diastolic on two occasions at least 4 h apart after 20 weeks of gestation in a woman with a previously normal blood pressure
• Greater than or equal to 160 mm Hg systolic or greater than or equal to 110 mm Hg diastolic; hypertension can be confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy
and
<b>Proteinuria</b>
• Greater than or equal to 300 mg per 24 h urine collection (or this amount extrapolated from a timed collection)
or
• Protein/creatinine ratio greater than or equal to 0.3 <sup>*</sup>
• Dipstick reading of 1+ (used only if other quantitative methods not available)
Or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following:
<b>Thrombocytopenia</b>
• Platelet count less than 100,000/ $\mu$ l
<b>Renal insufficiency</b>
• Serum creatinine concentrations greater than 1.1 mg/dL or a doubling of the serum creatinine concentration (normal levels in pregnancy are 0.8 mg/dL) in the absence of other renal disease
<b>Impaired liver function</b>
• Elevated blood concentrations of liver transaminases to twice normal concentration
<b>Pulmonary edema</b>
Cerebral or visual symptoms
<sup>*</sup> Each measured as mg/dL.

**Table 1.**  
*Diagnostic criteria for preeclampsia by ACOG.*

5. Risk factors of preeclampsia

Risk factors include health conditions, lifestyle, and family history that can increase the risk for high blood pressure.

Some of the risk factors for high blood pressure cannot be controlled, such as age or family history. But we can take steps to lower our risk by changing the factors we can control.

Some medical conditions can raise the risk for high blood pressure. If one of these risks is present, the pregnant women can take steps to control it and lower the risk. However preeclampsia cannot be prevented, but the complications of pre-eclampsia can be prevented.

ACOG recommendation, any risk factor [1]	NICE guidelines, one high risk or two moderate risk factors [15]
<b>Risk factors</b>	<b>High risk factors</b>
Nulliparity	Hypertensive disease in previous pregnancy
Age > 40 years	Chronic kidney disease
BMI > 30 kg/m <sup>2</sup>	Diabetes mellitus
Family history of PE	Chronic hypertension
History of previous pregnancy with PE	Autoimmune disease

ACOG recommendation, any risk factor [1]	NICE guidelines, one high risk or two moderate risk factors [15]
Conception by in vitro fertilization	
Chronic hypertension	Moderate risk factors
Chronic renal disease	Nulliparity
Diabetes mellitus	Age > 40 years
Systemic lupus erythematosus	Interpregnancy interval > 10 years
Thrombophilia	BMI at first visit > 35 kg/m <sup>2</sup>
	Chronic hypertension
ACOG, American College of Obstetricians and Gynecologists; NICE, National Institute of Clinical Excellence; PE, preeclampsia	

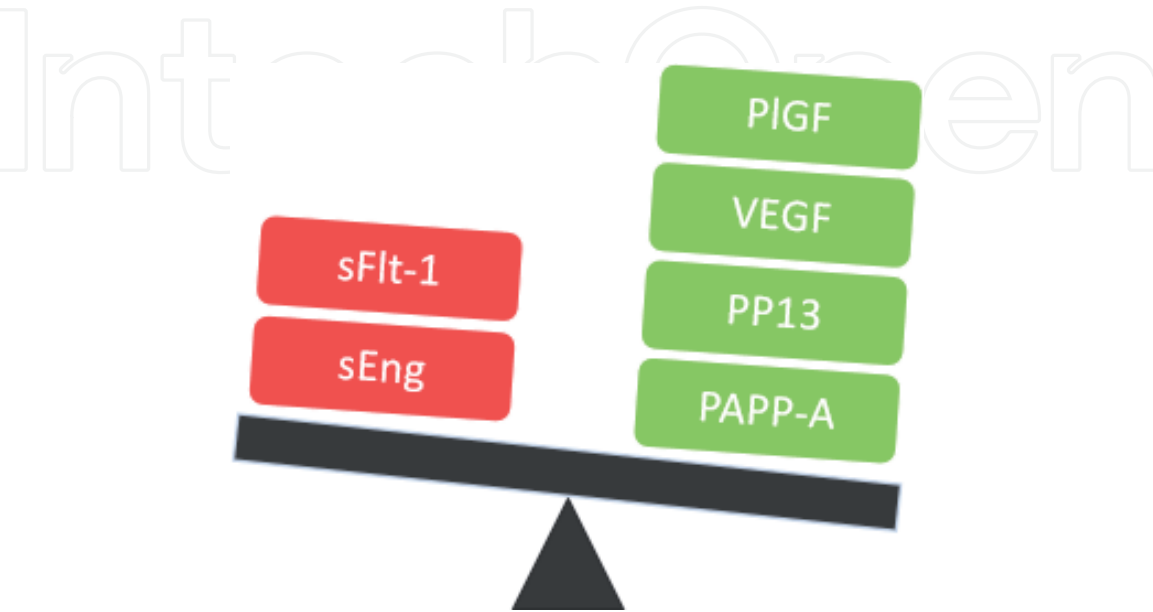
**Table 2.**  
Risk factors for preeclampsia by ACOG and NICE recommendations.

Preeclampsia develops only as a complication of pregnancy. Risk factors are presented in **Table 2** together with data of increased risk for some items [15].

The National Institute for Health and Care Excellence (NICE) recommends that women with high and more than one of the moderate risk factors for preeclampsia should be advised to take aspirin from 12 weeks gestation [16].

6. Biochemical markers

The role of biomarkers in preeclampsia diagnosis is becoming increasingly important. A literature review gives us a range of biomarkers that have proved to be sufficiently specific and sensitive to be classified as potential biomarkers (**Figure 3**). The most researched with data on specificity and sensitivity are given in **Table 3**. A good biomarker would be one, which may have the potential of identifying women earlier in their disease course. There have been also many studies investigating multiple-marker algorithms for predicting preeclampsia.



**Figure 3.**  
Biochemical markers in preeclampsia. sFlt-1, soluble fms-like tyrosine kinase 1; PlGF, placental growth factor; sEng, soluble endoglin; PP13, placental protein 13; PAPP-A, pregnancy-associated plasma protein A.

Biomarker	Sensitivity	Specificity
sFlt-1	26–73.1%	88.5–100%
PlGF	64.1%	89.5%
sFlt-1/ PlGF	78%	84%
sEng	18–85%	69–84.6%
PP13	79–100%	80–90%
PAPP-A 1st trimester	49.7–69.7%	68.6–85.7%
NGAL	97.89%	93.55%
Insulin resistance	73%	85%
SHBG	85%	37.7%
Inhibin A and activin A	87%	80%
Copeptin 1st trimester	88%	81%
Uterine artery Doppler	Positive likelihood ratio 9:1	
Podocytes	38–100%	70–100%

*sFlt-1*, soluble *fms*-like tyrosine kinase 1; *NGAL*, neutrophil gelatinase-associated lipocalin; *PAPP*, pregnancy-associated plasma protein; *PP*, placental protein; *sEng*, soluble endoglin; *SHBG*, sex hormone-binding globulin

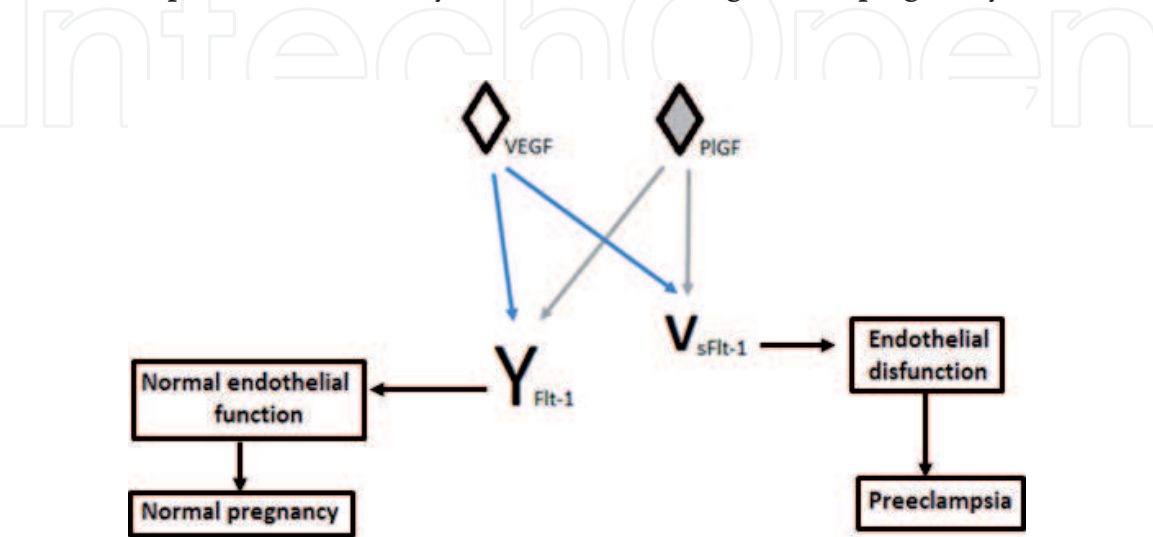
**Table 3.**  
Biomarker test characteristics for prediction.

6.1 Soluble *fms*-like tyrosine kinase 1 (sFlt-1)

Soluble Flt-1 is an anti-angiogenic form of VEGF receptor 1. sFlt-1 acts as a potent scavenger of VEGF and PlGF (**Figure 4**), thus preventing their interaction with endothelial receptors on the cell surface, and subsequently induces endothelial dysfunction. Elevated concentration of sFlt-1 has been as early as 5 weeks before the diagnosis of preeclampsia and correlates with severity of disease [17, 18]. Some other studies also support this sFlt-1 role in the pathogenesis of preeclampsia [19–21].

6.2 Placental growth factor

Placental growth factor (PlGF) is a prominent angiogenic factor in the development of the placental vascular system [22, 23]. During normal pregnancy, PlGF can



**Figure 4.**  
Circulating sFlt-1 in the maternal blood leads to a net decrease in PlGF and VEGF in the vasculature, which are necessary for normal placental angiogenesis. In PE angiogenic balance is disturbed and may result in endothelial dysfunction.



be detected in the maternal circulation from 8 weeks gestation, reaching a maximal concentration toward the end of second trimester and declining thereafter until delivery [24]. In line with its pro-angiogenic function, reduced levels of PlGF were found in preeclampsia [18, 25, 26].

The commercial kits available for determination of PlGF are mostly using sandwich enzyme-linked immunosorbent assay (ELISA) (Roche Diagnostics International, Thermo Fisher Scientific, IBL International, Abcam) or fluoroimmunoassay (PerkinElmer). In a multicenter, prospective study PROGNOSIS the Elecsys (Roche) sFlt-1/PlGF ratio proved to be a helpful tool in enabling clinicians to rule out the occurrence of preeclampsia for 1 week at cutoff of 38 or lower in women in whom the syndrome is suspected clinically. A ratio more than 38 indicates an increased risk of developing preeclampsia in the next 4 weeks [27].

### **6.3 sEndoglin**

Endoglin (Eng) is a type I membrane glycoprotein localized to the cell membrane where it constitutes the transmembrane co-receptor for TGF beta receptor complex (TGF- $\beta$ 1 and TGF- $\beta$ 3) [28]. Circulating sEng was found to be high in pre-eclamptic women even prior to the disease manifestations correlating with disease severity and falls after delivery [17, 29], making it a reliable predictor of patients destined to develop severe early-onset preeclampsia [30].

Research has shown that near the time of delivery there is a rise in anti-angiogenic factors including [31, 32] soluble endoglin (sEng) [33], a drop in the pro-angiogenic placental growth factor (PlGF) [17], and slight changes in the vascular endothelial growth factor (VEGF) [34]. These have been associated with increases in the anti-angiogenic sFlt-1/PlGF ratio [35] and a decrease in the pro-angiogenic PlGF/(sFlt-1 + sEng) ratio [36, 37].

Other studies have reported increases in inhibin A [38] and placental protein 13 (PP13) [39] near delivery. The elevated tumor necrosis factor alpha (TNF $\alpha$ ) has been detected in preterm delivery [40] and also in FGR [41].

### **6.4 Placental protein 13 (PP13)**

PP13 is a member of the galectin family, predominantly expressed by the syncytiotrophoblast, during placental vascular development [42, 43]. Serum concentrations of PP13 are significantly lower in women who later develop pre-eclampsia, FGR, and preterm birth [39, 44]. Combining first trimester PP13 with other parameters may further improve predictive performance.

### **6.5 Pregnancy-associated plasma protein A (PAPP-A)**

PAPP-A is a peptidase produced by syncytiotrophoblast with hydrolytic activity for insulin-like growth factor-binding proteins [45, 46]. Decreased levels of PAPP-A in the first trimester have been associated with increased risk of preeclampsia [47], not a good predictor of late-onset preeclampsia [48].

### **6.6 Free fetal nucleic acids**

The examination of fetal cells, specifically erythroblasts, and of cell-free fetal DNA from the blood of pregnant women is a subject of intense research, with the aim of developing new risk-free methods for prenatal diagnosis [49, 50]. In preeclamptic pregnancies [51], cell-free fetal DNA is elevated long before the

Biomarker	The characteristics for prediction (95% CI)
PAPP-A	Sensitivity 0.30 (0.29–0.32) Specificity 0.92 (0.92–0.92)
Inhibin A	Sensitivity 0.32 (0.25–0.39) Specificity 0.90 (0.89–0.91)
PP13	Sensitivity 0.37 (0.33–0.41) Specificity 0.88 (0.87–0.89)
PlGF	Sensitivity 0.65 (0.63–0.67) Specificity 0.89 (0.89–0.89)

**Table 4.**  
*The pooled sensitivity and specificity of the separate meta-analyses for some biomarkers.*

clinical onset of the disease [52, 53]. Total free DNA has also been used and has been reported to be increased in women who subsequently develop preeclampsia [54].

New methods based on immunodiagnostics that measure the level of biomarkers as well as sonographic devices that measure the uterine artery blood flow have emerged as promising avenues that can lead to more accurate differential diagnoses.

### 6.7 Biophysical markers

Biophysical markers have also been developed to evaluate blood flow through the uterine arteries to the placenta. In the case of preeclampsia, an abnormal placenta-tion results in decreased penetration of maternal spiral arteries in the junctional zone myometrium by cytotrophoblast cells. The consequence is that high blood flow and low-resistance vessels do not occur. Doppler ultrasonography has been evaluated as a potential predictive test for preeclampsia. Parameters such as the resistance index to the flow (RI), the average pulsatility index (PI), and the peak systolic flow (PSF) have been identified [55–58, 76].

### 6.8 Combination of tests

Angiogenic factors and biophysical markers may be combined for predicting preeclampsia. The combinations which give us best results are biochemical markers sFlt-1 and PlGF with Doppler [59, 60] and additional sEng [36] or PP13 [36, 61–63] and PAPP-A [63–66]. The pooled sensitivity of all single biomarkers for PE was 0.40 (95% CI 0.39–0.41) at a false-positive rate of 10%. The area under the summary of receiver operating characteristic curve (SROC) was 0.786. The pooled sensitivity and specificity of the separate meta-analyses for some biomarkers are shown in **Table 4**. Wu et al. in their study got a pooled sensitivity of 0.91 (95% CI: 0.90–0.91) and SROC of 0.893 for a combination of clinical characteristics, biomarkers, and Doppler pulsatility indexes [67].

## 7. Novel methods of diagnosis

Nowadays, various high-throughput techniques have evolved, thus allowing us simultaneous examination of thousands of genes (genomics), gene transcripts (transcriptomics), proteins (proteomics), metabolites (metabolomics), protein interaction (interactomics), and chromatin modifications (epigenomics) in single experiments.

mRNA-circulating placenta-specific mRNA in serum from preeclamptic women might be useful for the prediction of preeclampsia. In this study inhibin A,

p-selectin, and VEGF receptor mRNA values were higher in preeclampsia, whereas human placental lactogen, KISS-1, and plasminogen activator type 1 were lower, both compared to normotensive controls [68]. Similar results were reported from some other studies also [69, 70], where circulating cells of fetal/placental origin were a source of mRNA. mRNAs were increased in women with preeclampsia, and there was a direct correlation between expression levels and the severity of the disease.

Protein, a functional product of gene expression can be measured. A set of differently expressed proteins which are involved in lipid metabolism, coagulation, complement regulation, extracellular matrix remodeling, protease inhibitor activity, and acute phase responses can be measured. A different pattern of proteins between the group of women who subsequently developed preeclampsia on one side and without preeclampsia on the other side [71] was reported. It is also reported that women with severe preeclampsia have a unique urine proteomic pattern [72] and that this proteomic profile appeared more than 10 weeks before the clinical manifestations, and this distinguished preeclampsia from other hypertensive or proteinuric disorders in pregnancy [73].

Some studies revealed that metabolomic strategies might be appropriate for investigating the metabolic function of trophoblast or placental tissue, and it was found that preeclamptic pregnancies have a different metabolomic profile when compared to normal pregnancies [74, 75].

These novel technologies in preeclampsia appear quite promising. The number of studies is growing, and the results suggest that the use of transcriptomic, proteomic, and metabolomic profiles may be predictive for preeclampsia. These techniques open new possibilities to find a new set of biomarkers for preeclampsia. Future studies are needed, with the collaborative efforts of bioinformatics, biostatistics, researchers, and clinicians.

Key points

- A. Preeclampsia is a pregnancy-specific hypertensive disorder with or without proteinuria that occurs after 20 weeks of gestation in a previously normotensive woman. In the absence of proteinuria, the diagnosis can still be made if new-onset hypertension is accompanied by signs or symptoms of significant end-organ dysfunction.
- B. Major risk factors for development of preeclampsia include past history of preeclampsia, pregestational diabetes, chronic hypertension, and autoimmune disease.
- C. The pathologic changes are present long before clinical manifestations.
- D. Endothelial dysfunction and disturbed angiogenic balance are one of the key features of the disease.
- E. Preeclampsia serum levels of VEGF, PLGF, PP13, and inhibin A are decreased, and sFlt-1 and sEng are increased.

8. Conclusions

Many studies demonstrate the importance of optimal management of blood pressure in pregnancy hypertension. The use of angiogenic biomarkers gives us promising results for the prediction and diagnosis of preeclampsia, but there is still a lack of specific and reliable biomarkers to predict preeclampsia, particularly in the first trimester of pregnancy. New methods to isolate and characterize markers outside the protein field (lipids, nucleic acids, etc.) from serum/plasma/urine/saliva are useful.

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