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Markers for the prediction of preeclampsia and their relevance in the first trimester of pregnancy

Predikční markery preeklampsie a jejich výpovědní hodnota v prvním trimestru gravidity

Bachelor's thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstract

Preelampsia is a clinical syndrome found uniquely in a pregnant patient with an incidence 2 – 8 % of pregnancies worldwide. It is defined as the new onset of hypertension and proteinuria after 20 weeks of gestation, resolving with delivery or soon thereafter. Its worst consequences are eclampsia with tonic and clonic seizures and possibly coma. Also pulmonary edema, CNS hemorrhage, anaemia, hepatorenal failure, circulation failure and other complications can occur. In its most severe form, it affects nearly every organ. Preeclampsia remains a major cause of premature delivery and both maternal and neonatal mortality and morbidity. The origin and the cause of the disease remain unknown and therefore the medical treatment focuses only on clinical manifestations. Timely prediction of preeclampsia would enable accurate therapeutic treatment and a decrease of the threat to maternal and fetal health. In this study, the most important predictive biomarkers of PE and their relevance in the first trimester of gestation are presented. Furthermore, a first trimester screening with the best prediction rates is described.

Key words: preeclampsia, early markers, gestational hypertension, biochemical markers, immunological markers, antenatal care, first trimester screening

Abstrakt

Preeklampsie je komplexní syndrom, který se může objevit pouze u těhotných pacientek. Vyskytuje se asi ve 2 – 8 % těhotenství v celosvětovém měřítku. Preeklampsie je definována jako proteinurie spolu s novým výskytem hypertenze u jinak normotenzní pacientky. Nejhoršími důsledky preeklampsie jsou eklampsie spojená s tonickými a klonickými křečemi, a eventuálně koma. Mohou nastat i jiné komplikace jako plicní edém, CNS krvácení, anémie, hepatorenální selhání, oběhové selhání a další. Původ preeklampsie není znám stejně jako její příčina, současná zdravotnická péče se tedy soustředí pouze na klinické projevy onemocnění. Včasná predikce preeklampsie by umožnila adekvátní terapeutický zásah a snížilo by se tak riziko ohrožení zdraví matky i plodu. V této studii jsou uvedeny nejdůležitější predikční markery preeklampsie a jejich výpovědní hodnota v prvním trimestru gravidity. Představen je také nejvhodnější prvotrimestrální screening s co nejlepší predikční hodnotou.

Klíčová slova: preeklampsie, časné markery, gestační hypertenze, biochemické markery, imunologické markery, antenatální péče, prvotrimestrální screening

Abbreviations

ACLA	anti-cardiolipin antibodies
ADAM12	a disintegrin and metalloprotease 12
AFP, α-FP	alpha fetoprotein
Ang II	angiotensin II
BMI	body mass index
BP	blood pressure
cffDNA	cell-free fetal deoxyribonucleic acid
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
Eng, sEng	endoglin, soluble endoglin
FasL	Fas ligand
GH	gestational hypertension
hCG, β-hCG	(beta) human chorionic gonadotropin
HELLP syndrome	Hemolysis, Elevated Liver enzymes and Low Platelets syndrome
HIF	hypoxia-inducible factor (HIF-1α, HIF-2α)
HLA	human leukocyte antigen
Ig	immunoglobulin
IGF, IGFBP	insulin-like growth factor (-binding protein)
IL	interleukin
INF-γ	interferon gamma
IUGR	intrauterine growth restriction
IVF	*in vitro* fertilization
KDR, sKDR	(soluble) kinase domain region protein (see also sFlk1)
KIR	killer immunoglobulin receptor
L-NAME rats	N-nitro-L-arginine methyl ester developed rats
MFCT	maternal-fetal cell trafficking
MHC	major histocompatibility complex
NK cells, dNK cells	(decidual) natural killer cells
NO	nitric oxide
NOS, eNOS	(endothelial) nitric oxide synthase (also Cav-1, Hsp90)
NT	nuchal translucency
PAPP-A	pregnancy-associated protein A
PE	preeclampsia/preeclamptic
PP13	placental protein 13
PTX3	pentraxin-3
sFlk-1	soluble vascular endothelial growth factor receptor-2, also VEGFR-2
sFlt-1	soluble fms-like tyrosine kinase 1, also VEGFR-1
SGA	small for gestational age infant
SHBG	sex hormone-binding globulin
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
T-reg	regulatory T-cells
UtA-PI	uterine artery pulsatility index
VEGF	vascular endothelial growth factor
VEGFR-1	vascular endothelial growth factor receptor-1, also sFlt-1

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1. Introduction

Preeclampsia (PE) is one of the severe conditions that may occur during human pregnancy. It affects around 2 – 8% pregnancies worldwide – the exact incidence is difficult to determine since it varies by many factors e. g. ethnicity, pre-existing diabetes and hypertension, previous pregnancy complicated by PE, and others. Therefore, the highest prevalence of PE is in multicultural countries such as the USA. The incidence in the Czech Republic is much lower and one of the lowest worldwide. It was 0,83% in 2013 and has been under 0,91% in the recent years (ÚZIS 2006-2013). However, preeclampsia remains a major cause of premature delivery and both maternal and neonatal mortality and morbidity. This is especially true in the case of early onset PE. The cause of maternal death is often not PE itself as it is often related to other pregnancy complications such as HELLP syndrome, liver rupture, stroke, pulmonary edema or the most serious consequence of PE – eclampsia. Fetuses are frequently affected by developmental defects, intrauterine growth restriction (IUGR), small for gestational age infants (SGA), or various malformations (reviewed in Steegers et al. 2010; Lin et al. 2014).

The syndrome of preeclampsia is a pathophysiological clinical state which arises only during the pregnancy or shortly after the delivery. It is characterized by a new onset of hypertension (systolic blood pressure ≥ 140 mm Hg, diastolic BP ≥ 90 mm Hg; measured two times with at least 4 hours between them) and proteinuria (≥ 300 mg in 24 hours) at or after 20 weeks of gestation (reviewed in Steegers et al. 2010; Anderson et al. 2012; Lin et al. 2014).

Current medical treatment focuses only on clinical manifestations of the disorder as its origin and cause have not yet been fully confirmed. Remarkably, the first description of eclampsia, the end state of preeclampsia associated with severe tonic and clonic seizures, was given 2,000 years ago already as seizures during pregnancy resolving with delivery (Chesley 1978; Roberts 2000). Roberts also commented that only at the end of the 19th century an association with blood pressure and proteinuria led to the conclusion that this gestation abnormality was more than just a seizure disorder.

Contradictorily, according to Anderson et al. the condition of what might have been preeclampsia was described more than 3,000 years ago already by ancient Egyptians in the Kahun papyrus, the most ancient gynaecology document known to exist, describing various gynaecology cases and medical practices of the time (Stevens 1975; Anderson et al. 2012).

Regardless of the precise time of the first reference, it is still evident that these disorders do not appear in our society only in the present day and that they have in fact been around since time immemorial. Still, the risk of PE is increasingly high nowadays - one of the most alarming statistics points out that in comparison to 1980, women delivering in 2003 were claimed to be at 6.7-fold increased risk of severe form of PE. However, the actual risk rate may be different as the authors of this USA study admit that different period and cohort effects may both contribute to

this trend, as well as some of the secular changes, mostly increasing obesity and the declining smoking prevalence trend. Also, the diagnostic criteria of PE changed in recent decades and an earlier identification of preeclamptic symptoms is now possible (Ananth, Keyes, and Wapner 2013).

Yet, many years of investigation of PE have not provided a compelling elucidation of the cause and etiopathogenesis of the disease process, although the pathophysiology has been established deeply. Nevertheless, PE is still called „the disease of theories" (Pipkin & Rubin 1994; Roberts & Cooper 2001; Pennington et al. 2012). It is believed that the origin depends on more than one factor – the combination of risk factors plays a big role. Age, parity, time between pregnancies, previous PE and various pre-existing medical conditions including body mass index (BMI), and blood pressure or diabetes are of significance during the antenatal care already (Duckitt & Harrington 2005; Yu et al. 2005; Poon & Nicolaides 2014).

Timely prediction of PE would enable accurate therapeutic treatment and lowering of the threat to maternal and fetal health both in the perinatal period and later in life. Moreover, the disease might be more preventable.

Here, the biomarkers that are specified during the screening of gravidity are evaluated, with particular focus on the ones involved in the first-trimester screening. Initially, the physiology of the disease is explored in the next chapter. Subsequently, the description of the markers of PE follows, with explanations about their roles and mechanisms of action and predictive values if they are known. This is the main and also the largest part of the thesis. An immunological aspect of PE then follows with the description of immunological markers. The evaluation of the current and possible novel first trimester screening is then presented. And finally, several Czech scientists of great importance are introduced.

The aim of this thesis is to present the most important predictors of PE and refer to their prediction rate in the first trimester of pregnancy, to be able to conclude the best possible model of prediction of PE in the first trimester screening.

2. Pathophysiological viewpoint of preeclampsia

2.1 The origin of the disease

Preeclampsia manifests as a complex syndrome characterized by hypertension, proteinuria and edema in pregnancy (although edema is often observed in physiological pregnancies as well). Both origin and cause of the disease are still being discussed. Here, we present the most referred theories.

Normally, in the first stage of pregnancy, the trophoblast proliferates and differenciates into two-layer mass, cytotrophoblast (inner side) and syncytiotrophoblast (outer side). The syncytiotrophoblast invades myometrium and maternal spiral arteries to establish an adequate

uteroplacental blood flow into the fetus. The endovascular invasion destroys the endothelial and muscular layer of the maternal vessels and transforms them into high-capacitance vessels capable of providing placental perfusion huge enough to supply the growing fetus (L Myatt 2002; Powe, Levine, and Karumanchi 2011; Rockwell, Vargas, and Moore 2003). This physiological process leaves the arteries distended and funnel-shaped right in the myometrial and decidual part (Figure 1).

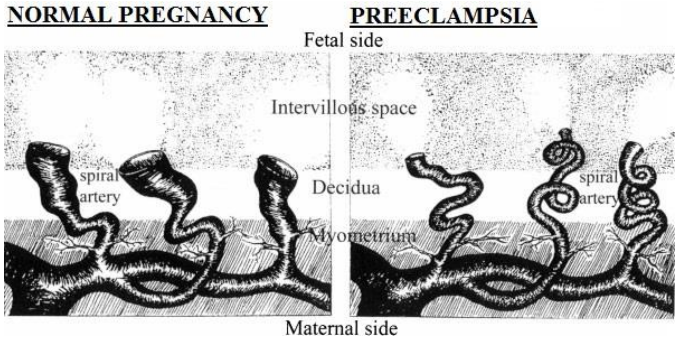


Figure 1. The maternal spiral arteries in normal pregnancy and during preeclampsia (adapted from Rockwell et al. 2003).

In a physiological pregnancy, endothelium no longer lines spiral arteries – instead, there are trophoblast cells that express endothelial antigens (Y. Zhou, Fisher, and Janatpour 1997). This process is called „pseudovasculogenesis". However, an abnormal invasion of the cytotrophoblast and a failure to adopt an endothelial adhesion phenotype correlates with PE. The pathophysiological process of PE is characterized by shallow invasion of the cytotrophoblast cells when the changes made in the uterine spiral arteries are less dramatic and are created more distally from the maternal side (Figure 1). This leads to a decreased blood flow into the fetus and results in an oxidative stress in the fetal-placental unit (Rockwell, Vargas, and Moore 2003). This can have severe consequences for the fetus therefore compensatory mechanisms must be activated. The release of the placental factors that enter the maternal circulation cause endothelial dysfunction and result in hypertension and proteinuria (Levine et al. 2004).

On the other hand, it has been discovered that there is no blood flow of maternal blood into the intervillous space of the placenta during the first trimester of pregnancy. The trophoblast cells blocking the lumen of the spiral arteries are being disrupted only at around 11 to 12 weeks of gestation and thus the flow of maternal blood to the placenta is established then (Jauniaux et al. 2000). However, some of the biomarkers of PE show significant alterations as early as at 7 weeks of gestation. This leads to the conclusion that PE certainly stems from a failure arising already during the period of placentation and implantation, but there may be lots of various failures in the trophoblast development that can lead to PE onset (Huppertz 2008).

The failed differentiation of the trophoblast tissue resulting in placental hypoxia and ischemia is also considered to be the cause of intrauterine growth restriction (IUGR). If there is a failure during the very early differentiation steps (Figure 2), it may result in the combination of early onset PE and IUGR. The early onset PE associated with IUGR is the most life-threatening case for both mother and child (Huppertz 2008).

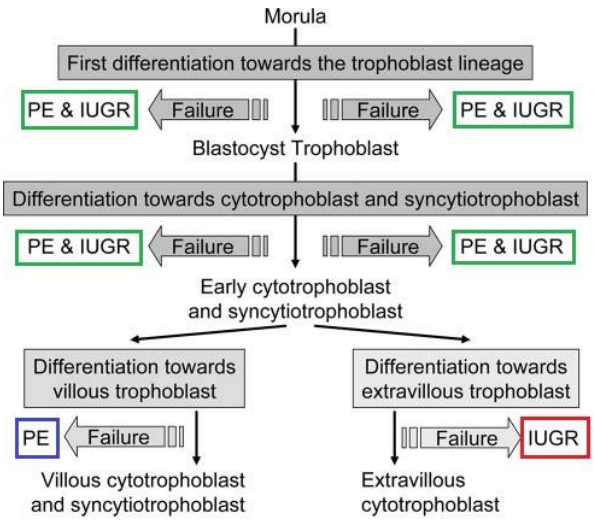


Figure 2. The early development of the trophoblast tissue and its possible failures (adapted from Huppertz 2008).

It is widely accepted that it is the presence of the trophoblast tissue (or placenta) in a mother's body that is crucial for the onset of PE, not the presence of the fetus - PE can also evolve in the case of gestational trophoblastic disease, mola hydatidosa (Rockwell, Vargas, and Moore 2003). Moreover, the presence of the placenta can cause post-partum eclampsia (the final stadium of PE) and only the removal of the placenta abolishes the disease (Matsuo et al. 2007). Nevertheless, what is actually so puzzling about this disease is the fact that placental and trophoblast cells in maternal circulation are present in all pregnancies so that these two tissues cannot be the actual cause of PE. However, the placental hypoxia/ischemia is the key factor for the onset of PE (Granger et al. 2001).

2.2 The pathophysiology of the disease

As stated in Introduction, the syndrome of preeclampsia is characterized by hypertension and proteinuria in a pregnant woman who has been normotensive before. Proteinuria is defined as a presence of more than 300 mg of protein in urine within 24 hours. Blood pressure is considered as hypertension when 140/90 or higher. This clinical state may also be associated with a lot of other symptoms such as edema, headache, epigastric pain, and visual disturbance. ("Diagnosis and Management of Preeclampsia and Eclampsia" 2002).

Although the cause of PE remains unclear, the following consequences are known to be induced by fetal and placental factors in maternal circulation (Levine et al. 2004). However, the maternal-fetal cell trafficking (MFCT) is prevalent in all pregnancies. It results in fetal microchimerism in mothers, and maternal microchimerism in fetuses and ultimately, the fact that we can detect fetal cell-free DNA in maternal circulation is commonly used in clinical practice to detect chromosomal abnormalities of the fetus by scanning maternal blood during gestation (reviewed in Nijagal & MacKenzie 2013).

On the other hand, it was proved nearly 20 years ago that there are significantly more fetal particles in maternal circulation in preeclamptic pregnancies compared to physiological control ones (Cockell et al. 1997; Holzgreve, Ghezzi, and Di Naro 1998). Also, as explained earlier, during PE the fetus reacts to hypoxia in the placenta by counteracting actions, for instance enhancing the production of erythroblasts, and producing vascular endothelial growth factors (VEGF) and

placental growth factor (PlGF) to enhance vasculogenesis and angiogenesis (reviewed in Lin et al. 2014). PlGF is an analogue of VEGF produced in placenta, endothelial cells and smooth muscle (Pan et al. 2010).

Thus, in PE, there are significantly more fetal-placental factors and fetal cells in maternal circulation in comparison with physiological gestation.

Those fetal and placental factors (VEGF, PlGF, and sFlt1 – their function is further described below) are released to the maternal circulation because of hypoxia in placenta, they lead to dysfunctional endothelium and consequently cause the outset of PE. The endothelium has a key role in the onset of hypertension and proteinuria. The role of functioning endothelium is to buffer the response to circulating dilatational or contractive substances, to prevent activation of platelets, to activate circulating anticoagulants and to maintain fluids in the intravascular space (reviewed in Roberts 1998; Roberts 2000). Thus, because all of these functions are disturbed in PE, it is implicated that the endothelium is the target of the disease.

Dysfunctional endothelium subsequently increases the formation of vasoconstrictors such as endothelin and thromboxane, increases vascular sensitivity to angiotenzin II and decreases the formation of vasodilators, nitric oxide (NO) and prostacyklin. This leads to hypertension due to the impairment of the renal pressure natriuresis and the increase of total peripheral resistance (Granger et al. 2001). So the hypertension of preeclampsia stems from peripheral vasoconstriction and arterial incongruity.

However, it has to be emphasized that some changes in blood pressure during gestation are normal and totally physiological. The borderline between the pathological changes and normality is therefore not easy to define. But generally speaking, any BP above 130/80 is yet conceived to be abnormal anytime during gestation (Pridjian & Puschett 2002). Eventually, BP above 140/90 is always considered as hypertension even if only one of the measured blood pressures (diastolic or systolic) exceeded the set border.

Nevertheless, there are other important symptoms apart from hypertension characteristic for preeclamptic patients, such as proteinuria, which originates by the activity of the same factors that cause hypertension. Proteinuria of PE is associated with renal lesion, a specific thrombotic microangiopathy called glomerular endotheliosis. It is characterized by glomerular endothelial swelling, loss of endothelial fenestrae and occlusion of the capillary lumens (Stillman & Karumanchi 2007). The capability of glomerular filtration is therefore reduced. It was confirmed by Maynard et al. 2003, that the key role has a circulating antiangiogenic factor released by placenta, soluble fms-like tyrosine kinase 1 (sFlt1, also known as soluble VEGF receptor 1, VEGFR-1). It is an antagonist to both VEGF and PlGF. VEGF is a promotor of angiogenesis; it also induces NO and vasodilatory prostacyclins in endothelial cells. The presence of VEGF and PlGF therefore results in a decrease of the blood pressure and the vascular

tone. The study of Maynard et al. shows that the increased level of circulating sFlt1 correlates with the decreased circulating level of VEGF and PlGF, which in the end results in endothelial dysfunction (described above), hypertension and proteinuria – typical symptoms of preeclampsia (Maynard et al. 2003). The hypothesis that sFlt1 might have a key role in the pathogenesis of PE, due to the fact that sFlt1 is a soluble receptor for both VEGF and PlGF, was further endorsed by a nested case-control study of Levine et al. 2004.

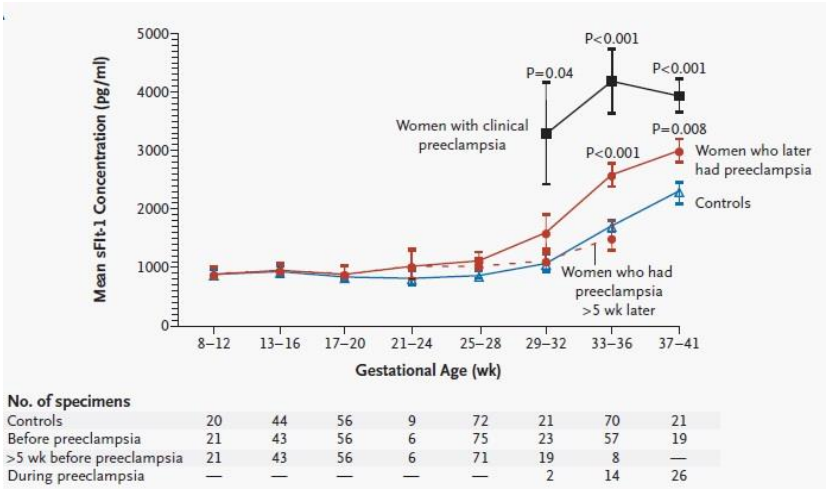
3. Biochemical and other specific clinical markers of PE

Three of the substantial markers and their role in the pathology of PE were already mentioned– VEGF, PlGF and sFlt1. Soluble fms-like tyrosine kinase 1 (sFlt1) binds to and inactivates the proangiogenic factors VEGF and PlGF. Diagnostically important is the fact that high concentration of sFlt1 in maternal circulation along with a decreased concentration of VEGF and PlGF are seen before the onset of preeclamptic symptoms already (Venkatesha et al. 2006).

3.1 Early increase of sFlt1 indicate the later onset of PE

In a clinical study released in 2004, the data shows that in women who later developed PE, the concentration of sFlt1 began to increase at 21 to 24 weeks of gestation, whereas the concentration of sFlt1 in controls remained constant until 33 to 36 weeks of gestation and then increased approximately 145 pg/ml per week until delivery. In the preeclamptic patients, the concentration further increased steeply at 29 to 32 weeks – see Figure 3 (Levine et al. 2004).

In women who later developed PE, the concentrations of sFlt1 were increased beginning 11 to 9 weeks before the onset of clinical symptoms (Figure 4). In the following weeks, the concentration went still higher and 1 week before the onset of clinical signs, the concentration was virtually equal to the women with manifested disease (Levine et al. 2004).



No. of specimens								
Controls	20	44	56	9	72	21	70	21
Before preeclampsia	21	43	56	6	75	23	57	19
>5 wk before preeclampsia	21	43	56	6	71	19	8	—
During preeclampsia	—	—	—	—	—	2	14	26

Figure 3.
The concentration of sFlt1 in women with clinical PE, in women who later developed PE, and in control ones according to gestational age in weeks (adapted from Levine et al. 2004).

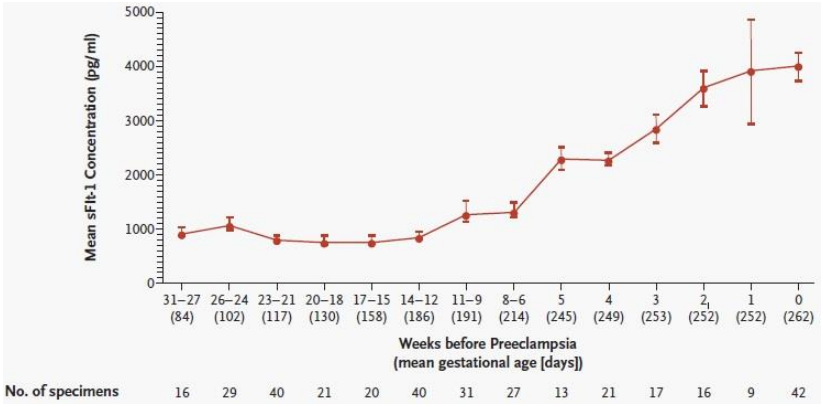


Figure 4.
The concentration of sFlt1 in weeks before the onset of PE (adapted from Levine et al. 2004).

Obviously, the level of sFlt1 is particularly important for the onset of PE. Injected sFlt1 (VEGFR-1) alone was sufficient to create several clinical symptoms of PE in rats, e. g. hypertension and glomerular endotheliosis, while exogenously administrated sFlk1 (VEGFR-2) did not produce the phenotype of PE in pregnant rats. The pathological changes after injecting sFlt1 happened both in pregnant and non-pregnant mice models (Maynard, J. Y. Min, et al. 2003).

Anyhow, the level of sFlt1 is the same for healthy pregnancies and those later developing PE during the first trimester (1st to 13th week of gestation), therefore the first trimester screening of sFlt1 concentration can absolutely not provide satisfactory predictive rates.

3.2 The decrease of PlGF and VEGF five weeks before the onset of PE

In the control pregnancies, the concentrations of PlGF increased in the first two trimesters already with the peak at 29 to 32 weeks, and decreased afterwards, as shown in Figure 5. Likewise, in women with PE, the concentrations of PlGF had similar trend throughout the gestation but the values were significantly lower from 13 to 16 weeks onward.

Remarkably, the concentrations of PlGF began to decrease at 11 to 9 weeks before the onset of PE (Figure 6). This data therefore supported the affirmed mechanism of activity of PlGF with sFlt1, as sFlt1 increased at the same time, 9 to 11 weeks to the onset of PE.

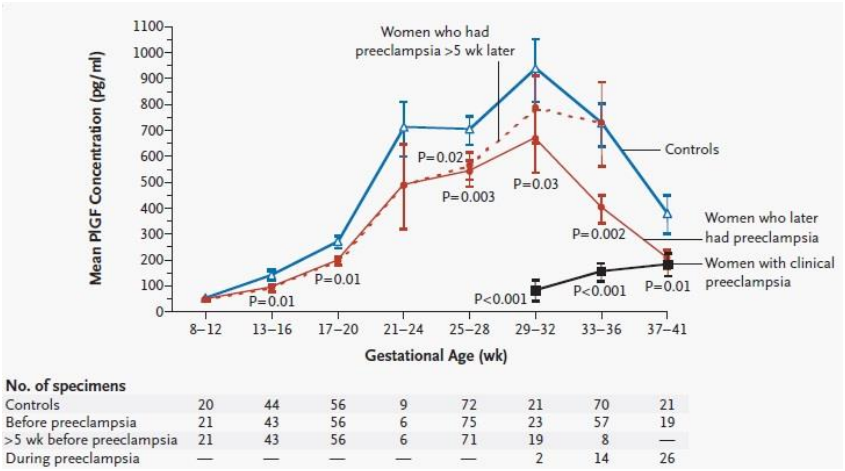


Figure 5.
The concentration of PlGF in controls, women who developed PE later and in women with clinical PE according to gestational age (adapted from Levine et al. 2004).

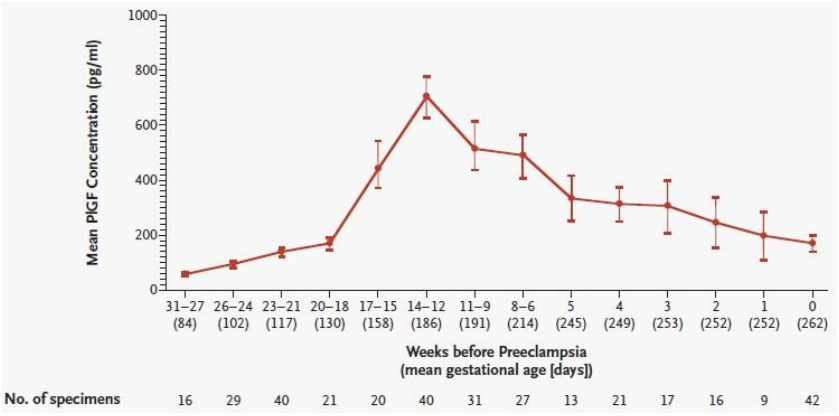


Figure 6.
The concentration of PlGF in weeks before the onset of PE (adapted from Levine et al. 2004).

Figures 7 and 8 clearly show the increase of the concentration difference between PlGF and sFlt1 at 21 to 32 weeks and 33 to 41 weeks of gestation. Similar concentrations of sFlt1 and PlGF were measured by Masuyama et al. 2007, as shown in Figure 9.

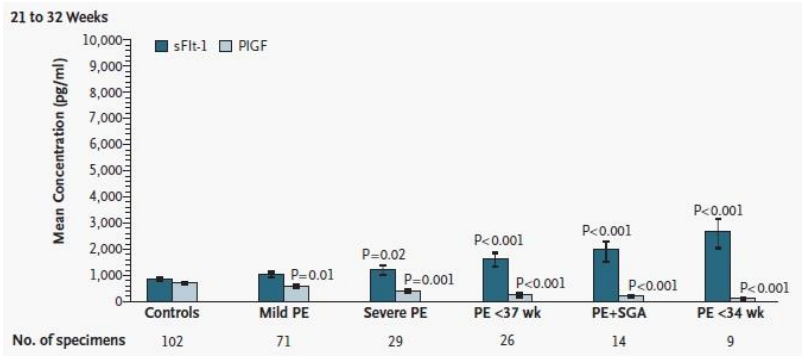


Figure 7.
The concentration difference between PlGF and sFlt1 at 21 to 32 weeks of gestation in women with mild PE, severe PE, the onset of PE before 37 weeks of gestation, both PE and small for gestational age infant (SGA) and the onset of PE before 34 weeks of gestation (adapted from Levine et al. 2004).

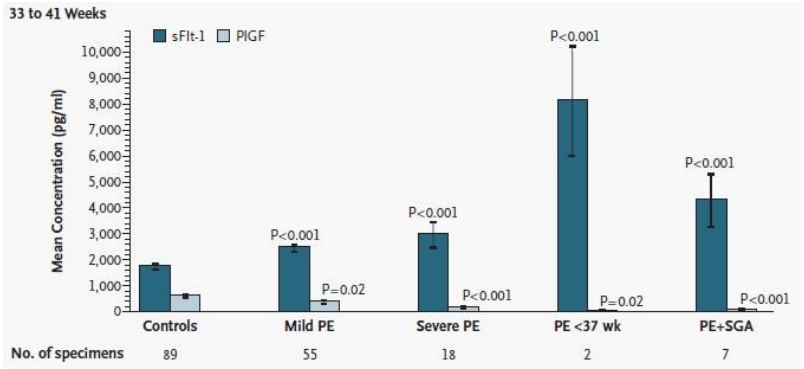


Figure 8.
The concentration difference between PlGF and sFlt1 at 33 to 41 weeks of gestation women with mild PE, severe PE, the onset of PE before 37 weeks of gestation and both PE and small for gestational age infant (adapted from Levine et al. 2004)

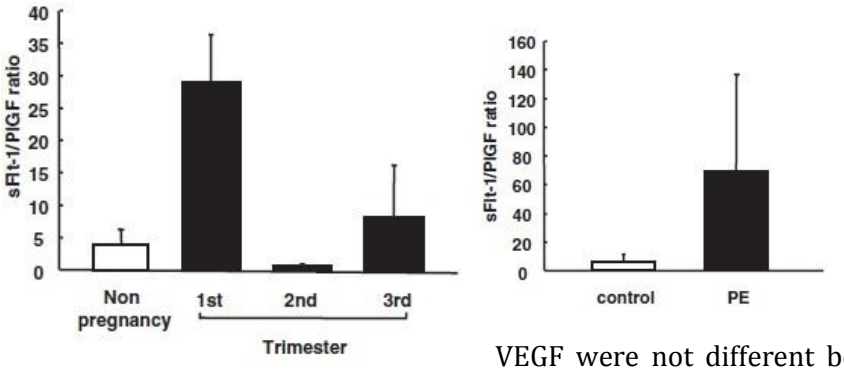


Figure 9. Left: The sFlt1/PlGF concentration ratio in non-pregnant women compared to the 1st, 2nd and 3rd trimester of healthy pregnancy. Right: The sFlt1/PlGF concentration ratio in controls compared to women with PE (adapted from Masuyama et al. 2007).

The concentrations of VEGF were not different between controls and women who later had PE, and were low throughout the gestation with two exceptions - the concentrations of VEGF at 37 to 41 weeks of gestation were lower in preeclamptic women, and

at 21 to 32 weeks the VEGF levels were lower only if the specimen was obtained within 5 weeks before the onset of PE (Levine et al. 2004).

The invariable concentration of VEGF is explained by the presence of KDR receptor (kinase domain region protein, also known as VEGF receptor 2, VEGFR-2 and Flk-1) on vascular endothelial cells (the soluble form of VEGFR-2 is sFlk-1). KDR receptor binds VEGF with high affinity, whereas it does not bind PlGF. Therefore, while VEGF binds to both sFlt1 and KDR receptors, PlGF binds solely to sFlt1. However, PlGF itself has no effect on vascular permeability, although it potentiates the actions of VEGF in cultured endothelial cells and in an in vivo vascular permeability model (Park et al. 1994). For the onset of PE, the concentration difference between PlGF and VEGF is needed. It was proved that VEGF alone is insufficient in producing PE during pregnancy due to the fact that high levels of PlGF is released in placenta. On the contrary, in nonpregnant state, there is no PlGF, and VEGF is therefore sufficient to cause the onset of PE by disrupting the balance of pro- and anti-angiogenic factors (Maynard et al. 2003).

To summarize, the substantial increase of sFlt1 about 5 weeks before the clinical signs of PE and equally the decrease of free PlGF and free VEGF at the same time may have considerable diagnostic potential during the second and third trimesters of pregnancy. Nonetheless, further investigation has shown that these markers have only limited role for the prediction of late PE and mild PE, although they are rather predictive of early onset PE and severe PE and have high predictive values during second trimester screening (P. H. Andraweera, G. A. Dekker 2012). VEGF, PlGF and sFlt1 were also investigated for their possible predictive values of the risk of future cardiovascular disease. However, it was only discovered that none of these markers can contribute to the prediction of this risk in women who previously developed PE, as no difference in these angiogenic factors was found 10 years later between women that had suffered from severe early onset PE and women with uncomplicated pregnancies (Gaugler-Senden et al. 2012).

But apparently, the predictive values of VEGF and PlGF in the first trimester of pregnancy are of no use as they show no significant concentration difference then, just like sFlt1.

3.3 The level of sEng binding TGF-β1 and TGF-β3 correlates with the severity of PE

Another angiogenic factors contributing to the severity of PE are endoglin (Eng) and the transforming growth factor family TGF-β.

TGF-β is one of the most potent inhibitors of cell growth. It has been localized on the syncytiotrophoblast in both the first trimester and the term placenta, which leads to the conclusion that the local production and the activation of this growth factor helps the syncytiotrophoblast to maintain in the non-proliferative and functional states throughout the gestation, as it inhibits its invasive capacity (DUNGY, SIDDIQI, and KHAN 1991; GRAHAM, LYSIAK, and MCCRAE 1992).

Endoglin interactes specifically and with high affinity with two isoforms of transforming growth factor - TGF-β1 and TGF-β3. There is another receptor for TFG-β family, betaglycan. Conversely, betaglycan binds all three isoforms of TGF-β and is widely distributed on most cell types (St-Jacques et al. 1994). Endoglin (Eng, also known as CD105) is a dimeric integral membrane glycoprotein present at high levels mainly on human vascular endothelial cells and placental syncytiotrophoblasts, but it can also be found on the cell surface of macrophages, pre-erythroblasts, lymphoid and myeloid leukemic cells (Cheifetz et al. 1992).

Eng is a component of the TGF-β receptor, and it is up-regulated along the villous pathway of differentiation and at the onset of migration of cytotrophoblast into maternal tissues. Eng is therefore thought to be required for the initiation of the migration process and the regulation of the effects of TGF-β, mainly the stimulation of the synthesis of extracellular proteins and integrins (St-Jacques et al. 1994).

Eng was detected in caveolae of endothelial cells where it associates with endothelial nitric oxide synthase (eNOS) and its regulators, Cav-1 and Hsp90. Endothelial cells lacking endoglin simultaneously lose their capacity to produce NO in response to specific eNOS activation. It was proposed that Eng acts as a scaffolding protein for cytoplasmic Hsp90 and caveolar eNOS which enables the eNOS activity (Toporsian, Gros, and Kabir 2005). And indeed, NO is considered to be the ultimate factor that seems to be crucial in the pathogenesis of hypertension and possibly preeclampsia, as it is a central factor for both VEGF and TGF-β signaling. It was observed that both TGF-β and VEGF have additive effect on NOS-dependent vasodilatation. On the other hand, this effect is not demonstrated in preeclamptic patients due to the distinctively high concentrations of sEng and sFlt1. Eng is therefore involved not only in cardiovascular development but also in vascular homeostasis, as it modulates the actions of TGF-β1 and TGF-β3 (Venkatesha et al. 2006).

The placenta-derived and soluble form of endoglin, sEng, is present in the sera in all pregnant women and it is significantly elevated in women with PE (Masuyama et al. 2007). The concentration of sEng correlates with the severity of the disease. It induces vascular permeability and hypertension and amplifies the endothelial dysfunction caused by sFlt1 and hence induces clinical signs of severe PE including other affiliated complications such as HELLP syndrom (Hemolysis, Elevated Liver enzymes, Low Platelets) and restriction of fetal growth (Venkatesha et al. 2006).

As described above, by binding TGF-β1, Eng induces vasorelaxation through activation of eNOS, which is the mechanism of endothelial-dependent vasoregulation. Conversely, present sEng competes with other TGF-β receptor binding and downstream signaling and thus decreases the eNOS activation in endothelial cells. It is considered that sEng is produced by the placenta as a compensatory mechanism to balance the effects of membrane Eng. The excessive production

of membrane Eng leads to increased sEng in maternal circulation in preeclamptic state and that may be responsible for the manifestation of clinical signs of PE (Venkatesha et al. 2006). The alteration of sEng concentrations during physiological pregnancy compared to non-pregnant women and compared to preeclamptic state are shown in Figure 9.

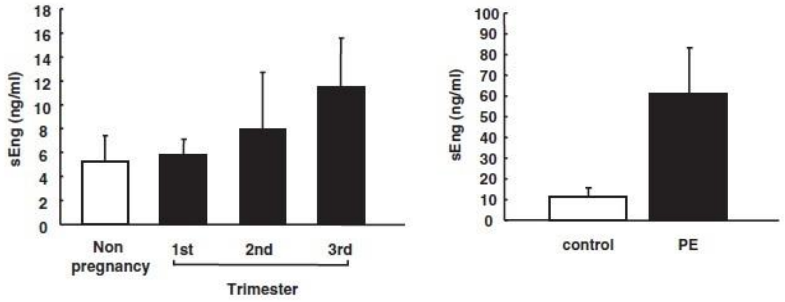


Figure 9. Left: The concentration of sEng in non-pregnant women and during 1st, 2nd and 3rd trimester of healthy pregnancy. Right: The concentration of sEng in controls and in women with PE (adapted from Masuyama et al. 2007).

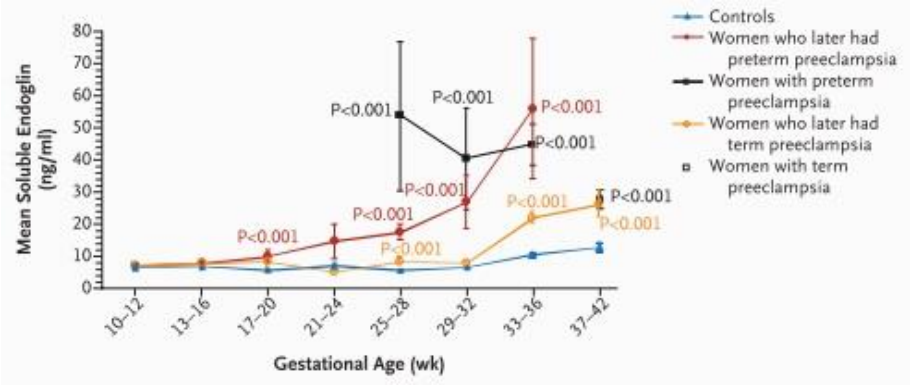


Figure 10. The levels of soluble endoglin according to gestational age in controls and in women with PE (adapted from Levine et al. 2006)

Based on the levels of sEng, PE cannot be predicted during the first trimester screening, as sEng begins to slightly rise only at 17 to 20 weeks of gestation in women who later developed preterm PE. So the concentration of sEng in maternal circulation may have a considerable diagnostic potential but only later during pregnancy, as shown in Figure 10. Then, high levels of sEng together with low levels of PAPP-A (pregnancy-associated protein A) are the most predictive markers of early onset PE (Allen et al. 2014). Circulating sEng increases at 6 – 10 weeks before the clinical signs of PE (Venkatesha et al. 2006), thus together with sFlt1 and PlGF increasing at 5 weeks before the clinical signs (Levine et al. 2004), the prediction of PE may be more sensitive and specific. A predictive test measuring these three markers may well be a part of the prevention of preeclampsia-induced mortality and morbidity.

3.4 An increased level of adiponectin to balance the endothelial dysfunction

Adiponectin is one of the cytokines (adipocytokines) secreted by adipose tissue. Its role in PE was examined by Masuyama et al. by analyzing the correlation with sEng in controls and preeclamptic patients. They found that there is a significant positive correlation between adiponectin and sEng in women with PE but not in healthy controls. Also, only in PE women a negative correlation of sEng and the leptin to adiponectin ratio was found. It was previously

reported that the adiponectin levels are substantially higher in PE pregnancies compared to normal pregnancies, and that there is a significant correlation of sFlt1 and PlGF levels with adiponectin in a group of PE patients (Suwaki, Masuyama, and Nakatsukasa 2006). Adiponectin has strong pro-angiogenic, anti-diabetic, anti-atherogenic and anti-inflammatory properties (Ouchi et al. 2003), therefore its deficiency may lead to endothelial dysfunction and hypertension. And in PE women, the increased level of circulating adiponectin may then be a physiological response in order to balance the endothelial dysfunction caused by placenta-derived angiogenic factors (Masuyama et al. 2007).

The data collected by Masuyama et al. also indicate that the response to alterations in circulating angiogenic factors is particulary strong in women with preexisting alterations in insulin sensitivity. Women with low concentrations of PlGF in the first trimester of pregnancy are at higher risk for developing PE and this risk is even higher in those women who have low concentrations of a substite marker of insulin resistance, the sex hormone-binding globulin (SHBG), which has an inverse relationship to the level of insulin in the serum (Strain et al. 1994; Masuyama et al. 2007).

Also, the deficiency of adiponectin may increase the endothelial dysfunction caused by a lower level of sEng. Demonstrated that the level of sEng has a negative correlation to the leptin to adiponectin ratio in PE women, pregnant women with high insulin resistence may suffer from PE because of a relatively low sEng concentration. Thereofore, the association of hypoadiponectinemia and a high leptin to adoponectin ratio is suggested to be a risk factor for PE (Masuyama et al. 2007).

3.5 PAPP-A regulates fetal growth and its levels are lower during PE

As stated earlier, pregnancy-associated protein A (PAPP-A) together with soluble endoglin were found to be the most predictive biomarkers of early onset of PE (Allen et al. 2014). Low levels of PAPP-A in the first trimester of pregnancy are associated with many adverse pregnancy complications – spontaneous fetal loss, low birth weight, stillbirth, preterm birth, gestational hypertension, and preeclampsia (association with PE shown in Figure 11). On the contrary, high levels of PAPP-A did not show any association to adverse obstetric outcomes (Dugoff et al. 2004).

PAPP-A was identified as a metalloprotease that cleaves insulin-like growth factor binding protein-4 (IGFBP4). It is produced by the syncytiotrophoblast in the placenta, which is the main source of PAPP-A throughout gestation, and together with other markers regulates the trophoblast invasion and fetal growth. It was previously located in various human cells e. g. fibroblasts, osteoblasts, vascular smooth muscle cells, endometrial stromal cells, decidual cells or ovarian follicular fluid and it is thought to be important in local proliferative responses such

as wound healing, bone remodeling, atherosclerotic plaque development and fetal development (Lawrence et al. 1999; J. C. Irwin et al. 1999).

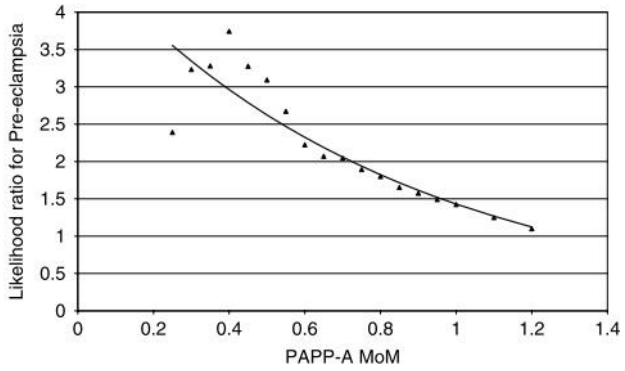


Figure 11. The likelihood ratio for PE based on PAPP-A concentration in maternal serum. Symbols represent the individual point estimates, and the line represents the best fit to the data. Adapted from (adapted from Spencer et al. 2008).

Low levels of PAPP-A are associated with higher levels of IGFBP4 and also low levels of IGF, as PAPP-A is an IGF-dependent protease. Insulin-like growth factors are known to regulate fetal development and trophoblast invasion by controlling the uptake of glucose and aminoacids. Therefore, it is widely acknowledged that there is a reasonable relation between low PAPP-A in the first trimester and pregnancy disorders associated with defective trophoblast invasion (Dugoff et al. 2004).

However, the usage of PAPP-A as a single predictive marker of PE has been proved to be insufficient as its detection rate of the later-developed PE was only 15% (Kevin Spencer, Cowans, and Nicolaides 2008). But together with other markers - maternal serum free β-human chorionic gonadotropin (β-hCG), fetal nuchal translucency (NT) thickness and maternal age, PAPP-A has been widely used during the first trimester screening for detecting chromosomal abnormalities, especially trisomies 21, 18 and 13 (Kagan, Wright, and Valencia 2008).

Generally speaking, the concentrations of PAPP-A are substantially lower in women with early onset PE than in women with late onset PE. The PAPP-A-related patient-specific risk for developing PE can be strongly modified by maternal variables (parity, ethnicity, previous PE etc.) together with the measurement of uterine artery pulsatility index (UtA-PI). Ideally, serum PAPP-A, UtA-PI and information of maternal variables should all be combined and processed in appropriate algorithm to give the best estimation of the particular risk of PE (Kevin Spencer, Cowans, and Nicolaides 2008; L. C. Y. Poon et al. 2009). Though, the concentration of PAPP-A is not predictive for the severity of PE (Atis, Tandogan, and Aydin 2011).

3.6 Unclear levels of the IGF-binding proteins protease ADAM12 during PE

One of the markers that varies during early pregnancy and thus might be suitable for the first trimester screening is another syncytiotrophoblast protein that cleaves insulin-like growth factor-binding protein 3 and 5 (IGFBP3 and IGFBP5) called ADAM12, a disintegrin and metalloprotease 12 (Loechel et al. 2000; Laigaard, Sorensen, and Placing 2005), also known as meltrin α (Primakoff and Myles 2000). ADAM12 has a similar role as PAPP-A, that is cleaving IGF binding proteins and hence releasing free IGF for the cells and promote their growth. Reduced

concentrations of ADAM12 have been found during the first trimester in pregnancies with trisomy 21, trisomy 18 and also other rare aneuploidies. These levels then raised above controls in the second trimester (Laigaard, Sorensen, and Frohlich 2003; Laigaard, Christiansen, and Frohlich 2005; Kevin Spencer, Cowans, and Stamatopoulou 2007).

However, the concentration pattern in the patients with PE has not been found yet, as the scientific research has not been united so far in the question of ADAM12 concentration during preeclamptic (or even normal) pregnancies. Some studies reveal that the level of ADAM12 is significantly increased in women with PE during the first trimester already (Gack, Marmé, and Marmé 2005; Leslie Myatt et al. 2012), while other researchers claim that the level of ADAM12 is significantly decreased in preeclamptic women and/or women with IUGR fetuses (Laigaard, Sorensen, and Placing 2005; Cowans and Spencer 2006). Finally, Spencer at al. demonstrate that during the first trimester, the level of ADAM12 was reduced in women who later developed PE and was even more reduced in those delivering before 35 weeks of gestation. But, on the other hand, in the second trimester the level of ADAM12 was increased in women developing PE and was higher than in controls (Kevin Spencer, Cowans, and Stamatopoulou 2008), giving the same pattern as patients with trisomy pregnancies.

Assuming that the level of ADAM12 is decreased, Spencer et al. also suggest that the reduced concentration of both proteases (ADAM12 and PAPP-A) during preeclamptic gestations may result in increased level of IGF-binding proteins so that free IGF is not available at the cell receptor level to stimulate the trophoblast invasion of the decidua and fetal growth (Kevin Spencer, Cowans, and Stamatopoulou 2008).

3.7 The level of important fetal hormon α-FP is more relevant later in pregnancy

Alpha-fetoprotein (α-FP, AFP) is a fetal equivalent of albumin synthesized in the yolk sac and fetal liver. It goes across the fetal membranes and through the placenta into maternal circulation. Increased maternal serum levels of AFP may be the consequence of increased transfer from the fetal to the maternal circulation due to a placental damage, which may be the issue in PE (Beta et al. 2011).

Primarily, AFP is a major fetal hormone that originates from the fetal liver, therefore its concentration throughout pregnancy increases according to gestational age. Maternal age, parity and child sex have no effect on AFP concentrations in the first trimester, while it is strongly inversely associated with maternal prepregnancy body mass index (BMI) (Lagiou et al. 2007; Chen et al. 2010). The method of conception (in vitro fertilization, conception by ovulation drugs or spontaneous conception), preexisting diabetes mellitus, smoking and racial origin also seem to affect the level of AFP (F. Bredaki, Wright, and Akolekar 2011; F. E. Bredaki et al. 2015). AFP

was suggested to have anti breast cancer properties and also seems to have a physiological role in duration of gestation (Lagiou et al. 2007).

Generally speaking, the level of AFP is more informative in the second and third trimester of pregnancy (KIMWALLER et al. 1996; Lei et al. 2004; Smith et al. 2006; Huang et al. 2010). It has been discovered that high maternal serum levels of AFP in the second trimester of gestation are associated with and increased risk of adverse perinatal outcome, if there is no fetal abnormality (WALLER, LUSTIG, and SMITH 1993). High maternal circulating levels are then likely to reflect a defective placentation. Together with low levels of PAPP-A at 10 to 14 weeks of gestation, high levels of AFP at 15 to 21 weeks of gestation are also associated with adverse perinatal outcome, e. g. stillbirth, preterm birth or SGA infant (Smith et al. 2006).

In preeclampsia screening, AFP has not shown to have a satisfactory predictive value. As stated above, the level of AFP in maternal serum is being changed by various specific maternal factors – and on top of that, some of the factors are modifiable (smoking, BMI). It is therefore not easy to define the border for the risk of PE. Likewise, Ay et al. confirmed that previous studies have been exceptionally inconsistent about the value of AFP for the prediction of PE, and his results reported only modest efficiency in the prediction of PE as well, although it validated that the levels of AFP are significantly higher in preeclamptic gestations (Ay et al. 2005).

3.8 Free β-hCG elevating in the second and third trimester

β-human chorionic gonadotropin (β-hCG) is secreted by the trophoblast cells in the placenta during gestation (Kang, Farina, and Park 2008). Similarly as AFP, the level of free β-hCG is also affected by maternal individual characteristic, e. g. gestational age, weight, racial origin, method of conception and cigarette smoking (Wright, Spencer, and Kagan K 2010). However, together with other markers (e. g. PAPP-A), free β-hCG is currently regularly measured during the first trimester screening for trisomy 21, Down's syndrome, as its proven marker (Mikat et al. 2012). Its application for the prediction of PE in the first trimester has been more problematic.

There are studies which claim that the concentrations of free β-hCG in the sera of preeclamptic pregnancies do not differ from uncomplicated pregnancies (Kevin Spencer et al. 2005; Asvold, Eskild, and Vatten 2014). However, other studies are in agreement with the finding that the free β-hCG level during the first trimester is lower in women who subsequently develop PE (Ong et al. 2000; Mikat et al. 2012). Most recent study of Jelliffe-Pawlowski et al. showed that women with low levels of PAPP-A or high total hCG (not free β-hCG) in the first trimester were at more than 3-fold increased risk of early onset severe PE. These two routinely collected first trimester screening markers (PAPP-A and hCG) provided quite specific risk information for the prediction of PE in this study (Jelliffe-Pawlowski et al. 2015). In addition to that, a study of Norwegian scientists gives evidence that high β-hCG (both free and total)

concentration in the first trimester is associated with reduced risk for preterm PE, while high β-hCG in the second trimester is associated with increased risk for preterm PE. Furthermore, in the third trimester, high levels of β-hCG are associated with increased risk for term PE (Asvold, Eskild, and Vatten 2014).

To conclude, free β-hCG is more important during the second and third trimester screening, as its levels elevate significantly at that time in women who subsequently developed PE (Ay et al. 2005; Kang, Farina, and Park 2008; Asvold, Eskild, and Vatten 2014). At the end of the pregnancy, at 38 to 40 weeks, β-hCG (not specified whether free or total) levels are substantially higher in preeclamptic women (Basirat, Barat, and Hajiahmadi 2006).

3.9 Higher levels of inhibin A and activin A in women later developing PE

Inhibin A is secreted in the syncytiotrophoblast (McCluggage, Ashe, and McBride 1998) and its concentration in maternal serum is increased in the first trimester in women destined to develop PE (Akolekar, Minekawa, et al. 2009). However, the mechanism of the inhibin A elevation is not clear. Several theories have been postulated before (Muttukrishna et al. 1997; Silver, Lambert-Messerlian, and Reis 2002; Manuelpillai, Schneider-Kolsky, and Thirunavukarasu 2003) but are constantly being remodeled by current research (K Spencer, Yu, and Savvidou 2006; Akolekar, Minekawa, et al. 2009). Several associations of the level of inhibin A with other markers (such as sEng) have been observed (Allen et al. 2014), but not satisfactory explained.

An increased level of inhibin A in early pregnancy is associated with both early and late onset of PE (before or after 34 weeks of gestation) (Allen et al. 2014). The detection rate of screening for late PE by inhibin A is only 13.7 - 16.8 % (at false positive rate 5 – 10 %), but it increases to 55.8 % in screening in combination of maternal obstetric history and characteristics. Moreover, the screening of combination of inhibin A with uterine artery pulsatility index (UAPI), the detection rate was 88 % for early PE and 42 % for late onset, which is particulary important as the early onset PE is more associated with maternal and perinatal mortality and morbidity (Akolekar, Minekawa, et al. 2009).

Activin A is a glycoprotein hormone also synthetized in the trophoblast and its role is to stimulate the outgrowth of cytotrophoblast cells into surrounding matrix, in particular to stimulate the formation of cytotrophoblast columns by regulating the differenciation of villous cytotrophoblast into extravillous cytotrophoblast cells. Activin binding protein, follitatin, blocks this activity (Caniggia 1997). Similarly to inhibin A, the levels of activin A are also increased in women with PE, which may reflect the dysfunctional placentation and the compensatory mechanism of the placenta to further promote the trophoblastic invasion (L. C. Y. Poon et al. 2010). Interestingly, plasma inhibin A was higher only in women with PE and not in women with

gestational hypertension (GH), while activin A increased both in pregnancies developing PE and pregnancies with GH.

The exact functions of both activin and inhibin in pregnancy and the underlying mechanism of their increase in PE are uncertain (Akolekar et al. 2009). Older studies give evidence that both have autocrine and paracrine roles in the trophoblast layer in the placenta (Muttukrishna et al. 1997b). In summary, both inhibin A and activin A may play only a supporting role in the prediction of PE so far, further investigation in their function is needed.

3.10 Placental protein 13, the marker of pure fetal/placental origin

Placental protein 13 (PP13, also known as galectin 13) is the only currently known marker that is of pure fetal/placental origin (Huppertz 2015). It is a homodimer expected to be involved in optimal implantation of the trophoblast and remodelling of maternal spiral arteries (Hromadnikova et al. 2010). Although the exact function of PP13 is not yet explored, especially in the maternal circulation (Huppertz et al. 2013a), an examination of the PP13 impact in an animal model gave evidence of its significant vasodilatory effect. In rats, PP13 also induced angiogenesis and increased elaboration of the arteries supplying the placenta, therefore improving uteroplacental blood flow (Gizurarson et al. 2013).

Interestingly, a mutation in the gene that encodes PP13 was revealed in women with PE, suggesting that it created novel shorter PP13 isoforms that were not detectable by previously used technique. Therefore, a screening for PP13 mRNA expression was made. Nonetheless, the early detection of the PP13 mRNA in maternal plasma was proved not to be a suitable method to predict PE in the end (Hromadnikova et al. 2010).

Basically, low concentration of PP13 in the first trimester of pregnancy is associated with the later development of PE (Chafetz et al. 2007; Hromadnikova et al. 2010; Huppertz et al. 2013b).

Current research foresee PP13 to be the new drug for PE and/or one of the most specific and sensitive biomarkers in the first trimester of pregnancy. Chafetz et al. tested maternal blood for PP13 at 9 to 12 weeks of gestation already, and demonstrated significantly decreased levels of PP13 in women who later developed PE, both early onset and late onset, with 79 % sensitivity and 90 % specifity. This is making the test kit for PP13 especially effective, noninvasive and simple method that may be easily integrated into the first trimester screening for PE (Chafetz et al. 2007). Huppertz et al. suggest that women with low serum levels of PP13 in the first trimester may be given additional dose of PP13 to help their vascular system for pregnancy (Huppertz et al. 2013b).

3.11 P-selectin as a direct marker of platelet activation

P-selectin (also known as GMP-140 and CD62) is a cell-surface adhesion molecule. Its expression was found to be significantly higher in preeclamptic pregnancies, which may play an important role in neutrophil-endothelial hyperadhesiveness and contribute to vascular complications in PE (WANG et al. 1998). A substantial correlations between platelet counts, plasma thrombin-antithrombin complex levels and the level of P-selectin were found in women with PE (Halim et al. 1996).

P-selectin is a molecule that is mobilized to the plasma membrane of the platelets and it is exposed on the platelet surface after activation and therefore may directly act as a marker of platelet activation (Yoneyama et al. 2001). Platelet-derived microparticles exposing P-selectin are significantly elevated in PE (Lok et al. 2007).

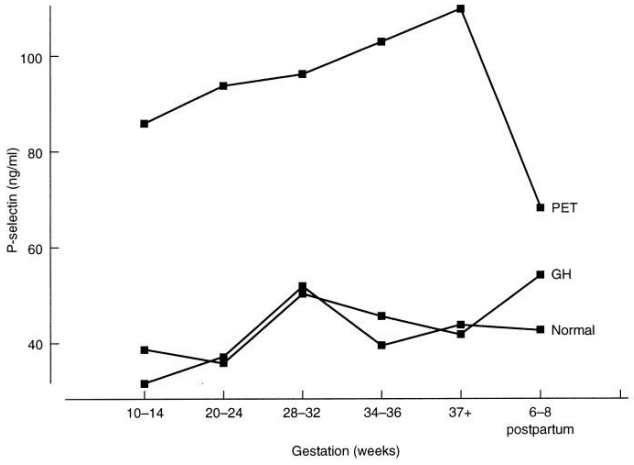


Figure 12. The mean plasma P-selectin concentration in women with preeclampsia (PET), gestational hypertension (GH) and controls (Normal). (adapted from Bosio et al. 2001)

Platelets adhere to damaged endothelium, turn activated and lead to the activation of coagulation and the formation of thrombus. P-selectin regulates the initial interactions between activated platelets and leukocytes and between leukocytes and the endothelium (Vandendries, Furie, and Furie 2004). Higher levels of P-selectin in PE may lead to enhanced recruitment of leukocytes and thus enhanced inflammation and coagulation (Lok et al. 2007). Yoneyama et al. also give evidence of the increase of P-selectin in preeclamptic pregnancies, and establish adenosine to be an effective platelet activation suppressor (Yoneyama 2001).

P-selectin may definitely serve as an early marker for the prediction of PE, as its concentration is elevated significantly at 10 to 14 weeks of gestation in women who later developed PE (shown in Figure 12), with the sensitivity of the test 80 % and the specificity 91.2 % in the first trimester screening. These numbers are indicating better performance of P-selectin than any other reported predictive marker so far (Bosio et al. 2001).

3.12 Renin-angiotensin system dysregulation in preeclampsia

In an experiment of Zhou et al., the infusion of angiotensin II (Ang II) into pregnant mice increased the level of circulating sFlt-1, resulting in the inhibition of endothelial cell migration. Ang II was also found to stimulate sFlt-1 production in human vilii explants and cultured

trophoblast but it did not stimulate the production in endothelial cells. Zhou et al. suggested that the trophoblast is therefore the primary source of sFlt-1 throughout pregnancy. Futhermore, the intense elevation of sFlt-1 in preeclampsia may be the consequence of the renin-angiotensin system dysregulation (C. C. Zhou et al. 2007). This findings were confirmed by latter studies (LaMarca, Parrish, and Wallace 2012; Sahay et al. 2014).

3.13 Non-physiological increase of Pentraxin-3 level during PE

Pentraxins were described as cytokine-inducible genes or molecules that are expressed in specific tissues. The first described pentraxin is well-known C-reactive protein (CRP). Another member of pentraxin family is pentraxin-3 (PTX3). The production of PTX3 is induced by primary inflammatory signals such as IL-1, IL-6 or TNF-α and it stands at the crossroads of immunity, inflammation, extracellular matrix construction and female fertility (Garlanda et al. 2005). PTX3 was shown to be a good prediction marker of PE, as its levels are increased at 11 to 13 weeks of gestation already. But, the levels of PTX3 were significantly higher only in women who developed early onset PE, not in women with later onset PE (Akolekar, Casagrandi, et al. 2009). Cetin et al. demonstrated that PTX3 levels are higher in all healthy pregnant women compared to non-pregnant and these levels do not change during pregnancy. Besides, the PTX3 concentrations are significantly highter in PE women, with no significant difference between mild and severe PE (Cetin et al. 2006). Hence, the increase in PTX3 concentrations is evident in the first trimester already but the underlying mechanism of this increase remains unclear (Akolekar, Casagrandi, et al. 2009).

3.14 Doppler sonography as a meaningful biophysical marker

As described earlier, the vascular system in the uterus transforms dramatically during pregnancy to provide a satisfactory level of blood supply to the fetus. Doppler ultrasound is a non-invasive method to assess the uteroplacental circulation. In case of PE (or other complications caused by impaired placentation), an impaired invasion of the maternal spiral arteries is reflected in increased uterine artery pulsatility index (Ut-PI).

The first trimester value of Ut-PI was indicated to be affected by exact gestational age at the screening, maternal weight or racial origin. Because of measuring by a sonographer, it may be difficult to compare the measured rates as there is a certain chance of manual error. Strict rules are provided to achieve a reliable measurement and every sonographer has to be certified by Fetal Medicine Foundation in London. In combination with mathematical modelling to include maternal factors (age, weight etc.), the detection rate of PE requiring delivery before 34 weeks of gestation reaches 95 % at false positive rate of 10 % (L. C. Poon and Nicolaides 2014). The

combination of ultrasound with maternal characteristics was proved to be beneficial also by Yu et al., confirming it to be an exceptionally good predictive model for PE (Yu et al. 2005).

To sum up, apart from a possible fault due to the human factor, Doppler ultrasound seems to be a powerful tool in best specifying the risk of PE in the first trimester already.

4. Immunological viewpoint of preeclampsia

This chapter will focus on the role of immune system in the development of PE, as there are several theories considering the immune system to be centrally implicated in the pathophysiology of preeclamsia, suggesting that the endothelial dysfunction is the result of an inappropriate maternal immune response agains the fetus. This assumption would partially explain reduced rates of PE in secondary compared to primary pregnancies, as the result of a regulatory memory imprinted in the first pregnancy that sustains anergy to fetal antigen (Rowe et al. 2012).

4.1 Current research of the immunology of PE summarized by Visser et al.

In 2007, Visser et al. made an extensive review of the knowledge up to then about the role of immune system in PE. According to this study, the current research is rather controversial. For example, it has been assumed that women with PE show excessive inflammatory response by cytokine overproduction and thus Th1 type immunity. But on the other hand, the sources of the increased levels of pro-inflammatory cytokines have not been entirely determined. Some researches suggest that the source is placenta, others suggest activated leukocytes.

Similar situation is in the research of the level of cytokines themselves. TNF-α (tumor necrosis factor α) is one of the major pro-inflammatory cytokine that can be found in human placental and uterine cells throughout gestation. However, several studies reported elevated maternal circulating TNF-α level, and several reported decreased level or no difference between preeclamptic and healthy pregnancies. INF-γ (interferon γ) is another important cytokine in the cytotoxic immune response, and while one group of researchers reported its continuous increase in PE, other researchers fail to reproduce this finding.

Overall, the role of the immune system in PE is a tempting issue to be inquired, but since even normal pregnancy already requires considerable immunological adaptations, a strict inclusion criteria may be obtained to eliminate the study design discrepancies among the researchers and to make it easier to compare their results. (Visser, van Rijn, and Rijkers 2007).

4.2 The immune system in healthy pregnancy

The mechanisms to protect the fetus from maternal immune response have evolved, so that although being allogeneic (half the fetal genes are paternally derived, the placenta and embryo

are therefore „semi-allograft“ to maternal immune system), the fetus can survive in a genetically diverse environment and no reaction similar to ones after tissue transplantation happens.

Basically, there are two functional forms of immune reactivity – Th1 reactions (cell-mediated immunity) and Th2 reactions (humoral immunity), both mediated by T cells (T lymphocytes) that produce cytokines, the main regulators of the immunological response. Th1 cells promote strong cell-mediated responses by different cytokines than Th2 cells, which are involved mainly in the regulation of humoral response. In pregnancy, cytokines are produced not only by local T cells, in fact the major source of Th2 cytokines were found to be non-lymphoid placental and fetal tissues, particulary the trophoblast (Chaouat 1999; Challis et al. 2009). The balance between the Th1 and Th2 activity is strongly shifted toward Th2 reactions in healthy pregnancy (also know as „Th2 phenomenon“) to protect the fetus from pro-inflammatory reaction. However, pathologic pregnancies are associated with the exact opposite activity (shown in Figure 13), that is the lack of the Th2 cytokines instead of the Th1 (LIN et al. 1993; MARZI et al. 1996; Challis et al. 2009).

Several specific immunological adaptations established during pregnancy will now be briefly introduced. A rapid rejection induced by T-cells of the fetuses occured when pregnant mice were treated by an inhibitor of indoleamine-2,3-dioxygenase (IDO), which is an enzyme that catalyzes the degradation of tryptophan. This enzyme is synthetized by trophoblast and macrophages. By this experiment, Munn et al. determined that by catabolizing tryptophan, an essential amino acid for rapidly dividing cells, the conceptus suppresses the T-cell driven local inflammatory responses and thus defends itself against rejection (Munn 1998; Mellor et al. 2001).

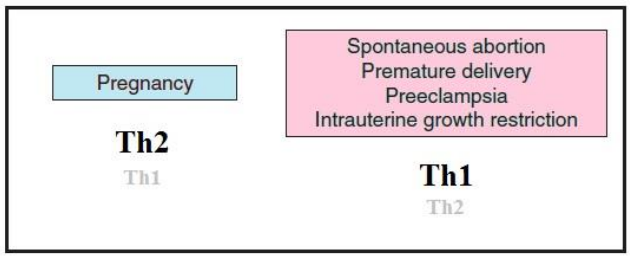


Figure 13. The change in the maternal dominant immune response during normal pregnancy and during pathological pregnancy (adapted from Challis et al. 2009)

Another fetal protective mechanism is the NK-cells (natural killer) inhibition. NK cells are present in maternal uterus and express inhibitory receptors for HLA-G (human leukocyte antigen G). HLA-G is a major histocompatibility complex class I (MHC I) molecule that is expressed by cytotrophoblasts at the fetal-maternal interface. By binding HLA-G to the inhibitory receptors on NK cells, cytotrophoblast inhibits the immune reaction against the fetus and induces maternal tolerance of the fetal tissue (Rouas-Freiss et al. 1997; Fuzzi et al. 2002).

The trophoblast tissue and maternal decidua also produce corticotropin-releasing hormone (CRH) and mainly Fas ligand (FasL), a pro-apoptic cytokine. Local maternal T cells that become activated express Fas receptor that binds to FasL, and this binding subsequently leads to

maternal T cell apoptosis. Therefore, trophoblast also plays a role in maintenance of pregnancy (Makrigiannakis et al. 2001).

Additionally, it was noticed that in case that a woman had an autoimmune disease, its course could be improved during pregnancy, then relapsed again after delivery. Thus, there are not only local changes in the immunity specifics during gestation, general immune system restructuring must occur as well.

Regulatory T cells (T-Reg) were found to be required for the maternal immune system to tolerate the fetal allograft, as they suppress maternal immune responses against fetus rather than responses against male-specific minor histocompatibility antigens (Aluvihare, Kallikourdis, and Betz 2004). T-Reg are characteristically CD4+CD25+ and are crucial for inhibition of inappropriate activation of both Th1 and Th2 immune responses against self antigen (Sakaguchi et al. 2001). The level of T-Reg is constantly growing throughout gestation and it was proved that this increase is not driven by alloantigen, therefore their presence is understood to be important for the suppression of maternal immune aggression. T-Reg can also induce dendritic cells to produce IDO and as stated above, this expression promotes maternal-fetal tolerance (Somerset et al. 2004; Aluvihare, Kallikourdis, and Betz 2004).

The trophoblast also expresses lack of major histocompatibility complex molecules (MHC) class I and II, which literally „hides“ the placenta from maternal immune system. MHC class I classical molecules (HLA-A, HLA-B, HLA-C) are highly polymorphic and are expressed on almost all somatic cells. But the trophoblast also expresses a specific combination of non-classical MHC I molecules - previously mentioned HLA-G and also HLA-E that both act as ligands for inhibitory receptors present on NK cells and macrophages (Blaschitz, Hutter, and Dohr 2001).

4.3 Inflammatory response in PE

During PE, a shift toward Th1 immune predominance is registered, thus the cascade of inflammatory cytokine production is iniciated and intensified. This shift is also characteristic in other pregnancy complications such as IUGR, spontaneous abortion and preterm delivery (Figure 13) (Challis et al. 2009).

One of the alterations during PE is the lack of HLA-G (Hara et al. 1996; Yie et al. 2004; Rizzo et al. 2009) resulting in insufficient inhibition of NK cells. Another deficiency can be found in the level of placental IL-10, an anti-inflammatory substance, therefore associated with increased maternal antifetal immunity (Hennessy et al. 1999; Makris et al. 2006). During normal pregnancy, the fetal particles in maternal circulation are taken up by monocytes and dendritic cells, which results in higher levels of TNF-α and IL-12, but low levels of IL-18 and INF-γ (Schiessl 2007). However as stated earlier, the levels of fetal microparticles in maternal circulation are significantly higher in PE pregnancies, causing increased levels of IL-18 which in

turn stimulate the production of INF-γ by NK cells resulting in an intense systemic inflammatory response (Cockell et al. 1997; Holzgreve 1998; Schiessl 2007). The hypoxia affecting the pregnancy results in increased levels of hypoxia-inducible factor HIF-1α and HIF-2α. HIF-1α is regulated through the expression of TGF-β3 that was also previously shown to be highly increased during PE (Caniggia et al. 2000; Rajakumar 2000).

The relevance of the immunological predictive factors is demonstrated in the next chapter.

4.4 Immunological markers of PE

4.4.1 The disrupted levels of cytokines in PE

Many of the cytokines are in different levels during normal pregnancy and PE. In the first trimester already, IL-6, IL-18, IL-23 and anti-cardiolipin antibodies IgG (ACLA-G) were found to be significantly higher in women with PE. IL-15 level was also elevated but only in women who later developed either severe form of PE or PE in combination with lupus syndrome in the third trimester (Andrea Kestlerová et al. 2014). The level of IL-18 was also significantly increased both in maternal sera and placentas in the investigation of Huang et al., measured after delivery (Huang et al. 2005). IL-18 alone has the capacity to induce Th2 immune responses, but together with IL-12, IL-18 works in synergy to promote Th1 responses. The IL-18 to IL-12 ratio is significantly lower in severe PE cases than in normal pregnancies but in mild PE cases, the IL-18/IL-12 ratio resembles the ratio in normal ones. That suggests that elevated IL-18 secretion and decreased IL-12 secretion may induce Th2 dominance in normal pregnancy, yet higher secretion of both IL-18 and IL-12 may cause Th1 dominance in women with severe PE (Sakai et al. 2004).

The levels of IL-6, IL-8, TNF-α and leptin were also found to be significantly increased when compared to healthy pregnant and non-pregnant women. The level of IL-10 decreased significantly in preeclamptic women, which is compatible with the fact that IL-10 is an inhibitor of inflammatory cytokines. However, these levels were measured in the third trimester (Sharma, Satyam, and Sharma 2007). In the study of Arikan et al., IL-8 levels were significantly higher in women with severe PE, as well as the levels of C-reactive protein (CRP) which is the protein of an inflammatory response and its levels increase following IL-6. The levels of IL-8 and CRP did not differ significantly in the case of mild PE. Thus, given that these concentrations were measured in the third trimester as well, the study of Arikan et al. may not have considerable diagnostic potential (Cemgil Arikan et al. 2012).

The elevated levels of IgM, IgG and IgA were observed in blood samples collected from women shortly before delivery. Also, some pregnant women were positive for anti-cardiolipin autoantibodies (ACLA). However, only 4 and 6 % of healthy pregnant women were positive (for ACLA of classes IgM and IgG, respectively), while 10% (ACLA IgM) and 18% (ACLA IgG) women

were positive among the preeclamptic study group. The elevated values of ACLA-IgG in the first trimester may indicate the risk of preeclampsia in the further course of pregnancy, while elevation of ACLA-IgM in the first-trimester is connected rather with the other pathologies of pregnancy (gestational hypertension, gestational diabetes mellitus and presence of Group B Streptococcus) (A. Kestlerová et al. 2012).

In conclusion, interleukines and other cytokines seem not to be a first choice options in the early prediction of PE, as their dysbalance reveals better in later stages of pregnancy due to generalized inflammation.

4.4.2 Hypoxia inducible factor 1 α increased along with TGF-β3

Placental sFlt-1 expression is increased by both physiologically and pathologically low levels of oxygen. This increase is mediated through hypoxia inducible factor 1 (HIF-1), a transcriptor factor (Nevo 2006). HIF-1 has two subunits, HIF-1α and HIF-2α. Caniggia et al. determined that the expression of HIF-1α subunit parallels the expression of TGF-β3, an inhibitor of trophoblast differentiation (described earlier). Both molecules are present at high levels during early pregnancy, and the concentrations of both molecules decrease at 9 weeks of pregnancy when the placental levels of oxygen are believed to increase. Thus, the early trophoblast differentiation is partly regulated by the level of oxygen through HIF-1 transcriptor factors and subsequently TGF-β3 (Caniggia et al. 2000; Soleymanlou et al. 2005). Besides, higher rates of PE and IUGR prevalence are also known in high-altitude locations (>2.700 MASL), possibly due to natural hypoxic environment (analysed in Zamudio 2007). Higher rates of HIF-1α in early pregnancy were also found in an animal model of PE, L-NAME (N-nitro-L-arginine methyl ester developed) rats (Kaya et al. 2011). Yet, the usage of HIF-1α as an early predictor of PE has not been fully scrutinized.

4.4.3 Human leukocyte antigen H is not predictive in the first trimester

HLA-G is indicated to be a molecule involved in the acceptance of the semi-allogeneic fetus by maternal immune system during pregnancy. The sufficient expression of HLA-G is positively correlated with successful in vitro fertilization (IVF) and contrarily, the shortage of HLA-G is reported to be associated with pregnancy complications as spontaneous abortion or PE (Rizzo et al. 2009). Steinborn et al. were measuring the levels of soluble forms of HLA-G (sHLA-G1/G5) throughout different stages of gestation. They found that among all women (non-pregnant, healthy pregnant, women with demonstrated PE, women with subsequently developed PE and other groups), the levels of sHLA-G1/G5 were strongly increased in the first trimester and then decreased continuously toward term. Women with strongly decreased levels of sHLA-G1/G5 in the second trimester had an increased risk of PE and/or IUGR. But in the third trimester, the

levels of PE women and healthy women did not deviate significantly (Steinborn et al. 2007). Rizzo et al. studied both soluble forms separately and found that low or even undetectable sHLA-G5 levels at the end of gestation seem to be associated with uncomplicated pregnancy, while higher levels of sHLA-G5 are associated with severe PE, preterm birth and IUGR (Rizzo et al. 2009).

4.5 The role of paternal factors in PE

Intriguingly, the paternal factors seem to be also important in the risk of PE. Among women without PE in the first pregnancy, changing partners resulted in 30% increase in the risk of PE in the subsequent pregnancy compared to women who did not change partners. Reversely, if women with PE in the first pregnancy changed partners afterwards, the risk of PE in the subsequent pregnancy reduced by 30% (Li and Wi 2000). Also, without regard to changing partners, for women with PE in the first pregnancy, the risk of PE increased in the second pregnancy with increasing time interval between pregnancies, and contrarily, the women without PE in the first pregnancy, the risk of PE was decreasing with increasing time interval (Trogstad 2001).

Li & Wi support the assumption that parental HLA sharing may play a role in the etiology of PE. A compatible combination of the polymorfic HLA-C on the trophoblast and its receptors on maternal NK cells called killer immunoglobulin receptors (KIR) seems to be fundamental. It is also presumed that prolonged exposure to paternal antigens may induce a form of immunological memory (Li and Wi 2000; Hiby, Walker, and O'Shaughnessy 2004). Ugolini and Vivier suggest the existence of „memory" NK cells (Ugolini and Vivier 2009), thus given the fact that these cells are present in the decidua (dNK cells, also uNK, uterine NK cells), the immunological mechanism of this partner-specific pattern may be hereby explained and could play the pivotal role of the immune system in the onset of PE (Trowsdale and Betz 2006; James, Whitley, and Cartwright 2010). However, Moffett et al. give evidence that there is still more influence of the maternal rather than paternal genetic contribution in the pathophysiology of PE (Moffett and Hiby 2007).

5. First trimester screening, antenatal care and prediction of PE

5.1 Current first trimester screening in the Czech Republic

Presently, the first trimester screening is mainly the congenital disorders testing to show the chance that the fetus has a certain abnormality – Down's syndrome, Edwards syndrome, Patau syndrome, split spinal cord (diastematomyelia) and other malformations. The markers that are recommended to determine during the first trimester screening are PAPP-A, free βhCG (and possibly PlGF) from blood samples, and measuring the nuchal translucency (Doppler

sonography). The best predictive value is achieved when blood samples are collected at 10 to 11 weeks of gestation and nuchal translucency is measured at 11 to 13 weeks of gestation. This examination is called „the combined test“ and shall be a regular practices of antenatal care.

Also cell free fetal DNA (cffDNA) can be determined from maternal blood from 10 weeks of pregnancy to ascertain the exact karyotype of the fetus (Loucký, Springer, and Šubrt 2015).

5.2 Turning the pyramid of prenatal care and proposed predictive markers of PE

In 2011, professor Kypros H. Nicolaides suggested a fundamental transformation of the prenatal care. He pointed out the fact that the current system with antenatal visits at 16, 24, 28, 30, 32, 34, 36 weeks of pregnancy and then weekly until delivery, was established 80 years ago. High frequency of the visits in the third trimester indicates that most of the complications occur then, and more importantly, that the most adverse outcomes are unpredictable during the first or second trimester. However, this was true back in the 1930's but now, with current data, statistical analysis and good predictive rates of the biochemical and biophysical markers, the frequency of the visits should be highest in the first trimester instead and thus, the traditional pyramid of prenatal care established in 1929 shall be inverted, as shown in Figure 14.

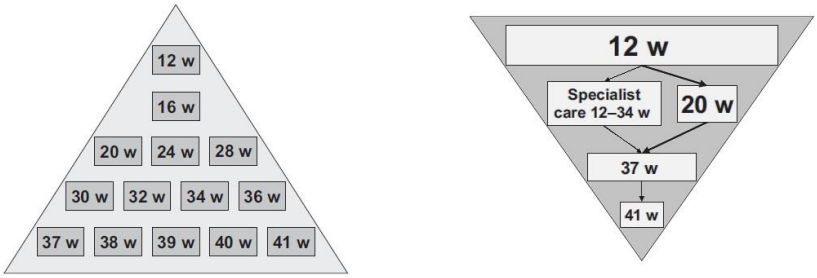


Figure 14.
Left: Traditional pyramid of prenatal care.
Right: Proposed new pyramid of prenatal care; w = weeks
(adapted from Nicolaides 2011)

Regarding PE, Akolekar et al. proved to be successful in the prediction in the first trimester already. The detection rates in their predictive model of screening by a combination of maternal factors and biophysical and biochemical markers (both collected at 11 – 13 weeks) were 91.0 % in case of early PE (before 34 weeks), 79.4 % in case of intermediate PE (34 – 37 weeks) and 60.9 % in case of late PE, all with a false-positive rate of 5 % (Akolekar et al. 2011). The markers used in this predictive model were maternal characteristics and history, UtA-PI, mean arterial pressure (MAP), PAPP-A, PlGF, PP13, inhibin-A, activin-A, sEng, PTX3 and P-selectin.

6. Conclusion

Despite extensive research, the cause of PE remains elusive. It is thought to be initiated with pathological alterations in the placental tissue and subsequent hypoxia and oxidative stress. This leads to the release of necrotic and aponecrotic trophoblast factors into maternal circulation, culminating and resulting in systemic inflammatory response of the mother. The stress and detrimental signalling factors that are overexpressed in the placenta during PE have been thoroughly investigated in the recent decades. This raises the possibility that by therapeutically blocking these signals and pathways, the onset of PE could be prevented.

Current medical therapy focuses on the symptoms of PE and establishing the best predictive screening model of PE in pregnancy as early as possible. Some of the most predictive early markers of PE are already being routinely determined during the first trimester screening to assess the risk of certain chromosomal aberrations and congenital anomalies, hence the determination of the risk of PE does not seem to be an impracticable extension of the antenatal care.

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