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Endothelial dysfunction in the pathogenesis of pre-eclampsia

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1. ABSTRACT

There are many theories regarding the ultimate cause of pre-eclampsia, and nowadays it is thought that the mechanism of pathogenesis is most likely multifactorial. The pathophysiology probably involves both fetal or placental and maternal factors. The most likely relevant factors in the pathogenesis are the abnormal development of the placenta, systemic endothelial dysfunction or cell activation, and an imbalance between pro-angiogenic and anti-angiogenic proteins with a predominance of anti-angiogenic factors. In women with pre-eclampsia, placental tissue overproduces two main anti-angiogenic proteins which enter into the maternal circulation: soluble Fms-such as tyrosine kinase 1 (sFlt1 or sVEGFR1) and soluble endoglin (sEng). Moreover, these patients have low circulating blood levels of two pro-angiogenic peptides: placental growth factor (PlGF) and vascular endothelial growth factor (VEGF). Adequate levels of CECs (circulating endothelial cells), EPCs (endothelial progenitor cells) and microparticles most likely play an important part in the development and regulation of vascularization in pregnancy but the exact role of these cells and micropatticles in the pathogenesis of pre-eclampsia is unknown. Some imbalances in these levels are associated with endothelial insufficiency.

2. INTRODUCTION

Pre-eclampsia is a hypertensive complication affecting 7-10 percent of pregnant women worldwide. It is responsible for approximately 40 percent of all iatrogenic preterm deliveries and is associated with significantly increased rates of perinatal morbidity and newborn mortality. Pre-eclampsia is described clinically as the occurrence of hypertension in women during late pregnancy (usually from the 20th week of gestation or earlier in patients with trophoblastic diseases such as hydatidiform mole or hydrops) with systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg on at least two occasions and urinary protein ≥0.3g in a 24-hour specimen in previously normotensive women (see the Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000) (1). Concomitant signs and symptoms that may develop in women suffering from pre-eclampsia are edema, visual disturbances, headache, renal failure, epigastric pain, low platelet count, and abnormal liver enzyme values. These clinical manifestations result from the microangiopathy of the target organs, namely the brain, kidney, liver, and placenta.

It is important to know the epidemiological risk factors for pre-eclampsia because this knowledge may help

in understanding its pathogenesis. It is well known that pre-eclampsia occurs more frequently in nulliparous women, multiparous women pregnant by a new partner, and women with a personal, family, and particularly paternal family, history of the disease. Obstetrical conditions that cause increased mass of the placenta are associated with a higher than normal risk of developing pre-eclampsia, e.g., multiple pregnancies or hydatidiform mole. Maternal conditions such as chronic hypertension, renal disease, diabetes, obesity, insulin resistance, dyslipidemia, androgen excess, systemic inflammation, and hypercoagulable status (antiphospholipid syndrome and thrombophilia) increase a woman's risk of developing pre-eclampsia. There is some evidence that the reduced exposure to the paternal antigens (resulting from the use of barrier contraception, long inter-pregnancy interval, fertilization with donor sperm, and pregnancy following intracytoplasmic sperm injection) may be the factor that predisposes some women to develop pre-eclampsia. Surprisingly, smoking cigarettes during pregnancy is a behavioral factor that protects against pre-eclampsia (2,3). Additionally, recent studies have shown that pre-eclampsia is a risk factor for cardiovascular diseases in later life (such as hypertension and cerebrovascular diseases) as well as for death from such diseases. This is probably the result of endothelial dysfunction and subsequent atherosclerosis, which may be present for many years following a pre-eclamptic pregnancy (4,5,6).

Despite identification of some predisposing factors, the etiology of this multi-system disorder remains largely unclear. According to recent findings, the development of pre-eclampsia is inherently related to systemic maternal endothelial cell injury and a subsequent decrease in the secretion of endothelium-dependent vasodilators that promotes vasospasm and activates the coagulation cascade.

In this article we consider the pathogenesis of pre-eclampsia with an emphasis on the role of endothelial dysfunction as a core feature of pre-eclampsia.

3. PATHOGENESIS

Recent studies have led to the development of the two-stage model of pre-eclampsia. The first stage— the clinical stage without any clinical features of the disorder— occurs after implantation and is triggered by some abnormalities in the process of placentation that lead to placental hypoxia. However, in this stage there are some abnormalities in the Doppler flow measurements of the uterine arteries that may make it easier to identify patients who have a higher risk of developing pre-eclampsia. The second stage, which represents clinical disease, usually occurs after the twentieth week of gestation and is a result of the release of placental factors due to oxidative stress or hypoxia into the maternal circulation, causing a generalized inflammatory response. These inflammatory factors cause endothelial dysfunction which manifests in damage to various organs of the mother and in the signs and symptoms of pre-eclampsia such as proteinuria, epigastric pain, visual disturbances, headache, shortness of breath, and even seizures (7). The underlying mechanisms contributing to these changes remain unclear and may overlap.

There are many theories regarding the ultimate cause of pre-eclampsia, and nowadays it is thought that the mechanism of pathogenesis is most likely multifactorial. The pathophysiology probably involves both fetal or placental and maternal factors. The most likely relevant factors in the pathogenesis are the abnormal development of the placenta, systemic endothelial dysfunction or cell activation, and an imbalance between pro-angiogenic and anti-angiogenic proteins with a predominance of anti-angiogenic factors. In addition, the increased sensitivity to angiotensin II, circulating syncytiotrophoblast debris, maternal inflammation, immunologic factors, nutritional factors, and genetic susceptibility may also all play a role in the pathogenesis of pre-eclampsia.

The endothelium is the cell layer lining of the blood vessels, but it has important functions in addition to constituting the lining of a vessel wall. These functions include:

(a) control over thrombosis and thrombolysis—normally the endothelium provides a non-thrombogenic surface,
(b) platelet and leukocyte interaction with the vessel wall,
(c) regulation of vascular tone to control blood pressure,
(d) barrier function – it acts as a semi-selective barrier between the lumen of the vessel and the surrounding tissue; increasing the permeability of the endothelial layer (e.g., in cases of chronic inflammation) may lead to swelling of the tissues,
(e) and regulation of vascular growth (angiogenesis).

Endothelial dysfunction can manifest in the following conditions: (a) vasoconstriction, (b) inflammation, (c) leukocyte adhesion, (d) excessive thrombosis and/or excessive vascular proliferation including hypertension and atherosclerosis (8, 9). Nowadays there are many tests for the various endothelial functions, but most of them are invasive and no simple test has been found.

3.1. Abnormal development of the placenta

The placenta is a crucial factor in the pathogenesis of pre-eclampsia and much evidence supports this theory. While the presence of the placenta is essential to the development of the disease, the fetus is not. Data shows that pregnancy with complete hydatidiform mole in the absence of a fetus may still lead to pre-eclampsia. The disease goes into remission after the mole tissues from the uterus have been evacuated by uterine suction or by surgical curettage. Some cases of postpartum eclampsia have been related to the retention of placental tissue in the uterus, and such patients recover immediately following uterine curettage (10). In cases of extra-uterine pregnancy coexisting with pre-eclampsia, the removal of the fetus is not what cures the condition; only delivery of the placenta causes the symptoms to remit (11) and, in fact, will always cure pre-eclampsia.

3.2. Remodeling of the spiral arteries

The process of remodeling of the spiral arteries usually extends from the eighth through eighteenth weeks of gestation. The spiral arteries constitute the terminal branches of the uterine arteries. In an uncomplicated pregnancy, cytotrophoblast cells of the developing placenta migrate through the decidua and part of the myometrium and invade maternal spiral arteries and this invasion results in the replacement of the endothelial layer of those vessels. This invasion by the cytotrophoblast results in the transformation from small muscular, small capacitance, and high resistance arterioles to high capacitance, low resistance systems that are sufficient for delivering the oxygen and nutrients to the placenta and, hence, the growing fetus (12). In pre-eclamptic patients this process is incomplete. The cytotrophoblast invasion is limited to the decidua and the penetration of the myometrial segment is deficient, so in effect the mean external diameter of the deeper myometrial arterioles is only half that of the vessels in a normal placenta. Consequently, the spiral artery remains narrow and undilated, resulting in placental hypoperfusion (13). Over the course of pregnancy, the poor and shallow placentation is associated with some complications, such as placental infarcts, abruption of the placenta, intrauterine fetal death in the second trimester of pregnancy, pre-eclampsia with or without intrauterine growth restriction, intrauterine growth restriction of the fetus, and preterm labor or premature ruptures of the membranes (14).In normal placental development, the cytotrophoblasts alter their adhesion molecule expression from those that are characteristic of epithelial cells (integrins alpha 6/ beta 1, alpha V/ beta 5, E-cadherin) to those character- istic of endothelial cells (integrins alpha 1/ beta 1, alpha V/ beta3, VE-cadherin) in order to promote adequate placental invasion; this process is referred to as pseudovasculogenesis or vascular mimicry (15, 16). Trophoblasts obtained from patients with pre-eclamptic pregnancies do not show this switching of cell-surface molecule. This leads to abnormal placentation followed by ischemia and the release of soluble factors that cause systemic endothelial dysfunction resulting in the pre-eclamptic phenotype (17).

3.3. Hypoperfusion, hypoxia, and ischemia of the placenta

It is not known whether pre-eclampsia is the cause or result of placental hypoperfusion, hypoxia, and ischemia. The existence of a causation between placental hypoperfusion, abnormal placental development, and pre-eclampsia is supported by the fact that in the animal models which have reproduced the condition, some findings of pre-eclampsia have involved mechanically, reducing uteroplacental blood flow (18, 19). Moreover, obstetrical conditions causing an increase in placental mass without a concomitant increase in placental blood flow (for example, in the cases of patients with hydropsfetalis, diabetes mellitus, hydatidiform mole, and multiple gestation) or diseases with vascular insufficiency (for example, hypertension, renal diseases, diabetes mellitus, and thrombophilias) result in relative ischemia and are associated with pre-eclampsia (3, 20- 23). Furthermore, the risk of pre-eclampsia is higher in women living at high altitude (24).

Hypoperfusion followed by hypoxia is also a result of abnormal placental development. Hypoperfusion becomes more pronounced as the pregnancy progresses because, with increasing gestational age, the growing uterus and fetus need enhanced blood flow into the vessels; the abnormal vasculature is insufficient for delivering enough blood and oxygen. There is some data which does examine arteries from the implantation site using an electron microscopy: early pre-eclamptic changes include endothelial damage, insudation of plasma constituents into the vessel walls, proliferation of myointimal cells, and medial necrosis; late pre-eclamptic changes include atherosis, fibrinoid necrosis, thrombosis, sclerotic narrowing of arterioles, and placental infarction. These lesions have not been seen in all cases of pre-eclamptic patients, but some investigators suspect a positive correlation between the severity of the symptoms of the disease and the degree of the lesions (25- 29). The chief factors in the pathogenesis of pre-eclampsia are hypoperfusion, hypoxia, and ischemia, and these lead to the release of many factors by the placenta into the maternal circulation. These factors cause maternal endothelial dysfunction and subsequent systemic signs and symptoms of pre-eclampsia.

3.4. Systemic endothelial dysfunction

There is strong evidence that the placenta of pre-eclamptic women releases factors into the maternal blood stream which may cause endothelial dysfunction and clinical manifestations of pre-eclampsia.

3.4.1. Imbalance between angiogenic and anti-angiogenic factors

In physiological pregnancy, the placentation process requires proper angiogenesis in order to provide for the suitable development of a placenta which can deliver enough oxygen and nutrients into the fetus. In women with pre-eclampsia, placental tissue overproduces two main anti-angiogenic proteins which enter into the maternal circulation: soluble Fms- such as tyrosine kinase 1 (sFlt1 or sVEGFR1) and soluble endoglin (sEng) (30- 32). Moreover, these patients have low circulating blood levels of two pro-angiogenic peptides: placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) (30, 33- 35).

sFlt-1 are splice variants of VEGF receptor 1 (Flt-1). This protein acts as a receptor for VEGF. SFlt-1 inhibits vascular endothelial growth factor and the placental growth factor signaling necessary for the formation of new blood vessels and for promoting their maturation. SFlt-1 binds and reduces free circulating levels of the pro-angiogenic factors VEGF and PlGF.

3.4.1.1. VEGF and PlGF

VEGF is a mitogen for vascular endothelial cells and there is strong evidence that VEGF is a survival factor for endothelial cells. VEGF withdrawal has been shown to result in the regression of vasculature in several physiological and pathological conditions. VEGF also seems to have a direct vasodilatory effect on the systemic vasculature. VEGF is known as vascular permeability

factor because of its ability to induce vascular leakage and the fen-estrated phenotype in certain endothelial cells. Some data suggests that an increase in microvascular permeability to proteins is an essential step in angiogenesis. VEGF stimulates the cellular response by binding to the tyrosine kinase receptors (VEGFR) that are localized on the surface of the cell. The activities of VEGF are mediated by the stimulation of two receptors: VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). VEGFR-2 mediates almost all cellular responses to VEGF. The function of VEGFR-1 is less well-known, but it probably modifies the VEGFR-2 signaling.

PlGF is a member of the VEGF subfamily. Several studies have shown that PlGF is redundant for normal vascular development and maintenance, but contributes to the angiogenic switch in pregnancy, wound healing, tumor growth, and ischemic conditions. P1GF was first discovered in the placenta, and the placental trophoblast is the main source of PlGF during pregnancy. Circulating PlGF reaches its peak in mid-gestation. It can also be detected in the heart, lungs, and in the muscles. PlGF binds and signals through VEGFR-1 (Flt-1), but not VEGFR-2 (KDR/Flk-1).

Although the exact mechanisms that regulate Flt-1 expression are not clear, it appears to be upregulated by hypoxia through the action of hypoxia-inducible factor-1alpha (HIF-1 alpha). Sflt-1 prevents the interactions of VEGF and PlGF with membrane-bound Flt-1 in blood vessels and leads to endothelial dysfunction. Circulating sFlt-1 levels are significantly higher in pre-eclamptic patients than in normal pregnant women, sometimes well before the symptomatic phase of the disease and its concentration levels correlate with the severity of the disease. In turn, concentrations of free VEGF and free PlGF are decreased in pre-eclampsia, even before the presentation of clinical symptoms (30, 31, 33-35).

Several experimental models of pre-eclampsia lend credence to the causative role of sFlt-1 in the pathology of pre-eclampsia. Maynard *et al.* administered sFlt-1 (it binds VEGF and PlGF) or sVEGFR-2 (it binds only VEGF) to both pregnant and non-pregnant rats. Following the administration of either sFlt-1 or sVEGFR-2, the non-pregnant rats developed hypertension, proteinuria, and glomerular endotheliosis, and it was possible to reverse this effect by administering VEGF. After the administration of sFlt, the pregnant rats also develop the pre-eclamptic phenotype. Nevertheless, pregnant rats administered sVEGFR-2 did not develop the pre-eclamptic phenotype (30). This data suggests that PlGF, which is produced by the placenta during pregnancy, may have a protective function against the syndrome when VEGF is antagonized. Other studies show that the chronic administration of VEGF by infusion into rats causes intrauterine growth restriction of the fetus and lower mass of the placenta and leads to oxidative stress followed by vascular endothelial dysfunction (36). Moreover, administration of antibodies against VEGF in non-pregnant mice results in glomerular endothelial damage and proteinuria. In non-pregnant mice, a 50% reduction of VEGF expression leads to glomerular endotheliosis and proteinuria, as in the mice with pre-eclampsia (37). Additionally, cancer patients who are treated with VEGF antagonists may exhibit hypertension, proteinuria, and endothelial dysfunction (38, 39). Experimental animal models of placental hypoxia and ischemia have demonstrated increased production of sFlt-1 by the placenta and resulting increases in maternal circulating levels of sFlt-1 (18, 19). It is clear that hypoxia-induced sFlt-1 derived from the placenta is an important pathologic factor in the development of pre-eclampsia and that manipulation of the levels of sFlt-1, VEGF, or both is a promising target for therapeutic intervention.

The data which was relevant to the human population included the following findings: anti-angiogenic features in the serum of pregnant pre-eclamptic women; high maternal serum concentrations among pre-eclamptic patients; lower concentrations of PlGF and VEGF in women with pre-eclampsia compared with healthy pregnant women; activation of Flt-1 mRNA in the placentas of pre-eclamptic patients, and hypertension, proteinuria, and histological abnormalities of the kidneys similar to those described in pregnant rats who developed pre-eclampsia after the administration of the adenovirus that expresses the sFlt-1 gene (30).

In their nested case-control study, Levine *et al.* reported that in healthy pregnant women the serum PlGF level decreased and sFlt-1 increased constantly through the last trimester; these changes were more pronounced and were detectable earlier in pregnancies with subsequent pre-eclampsia. These changes allow us to predict the onset of pre-eclampsia approximately 5 weeks before the onset of clinical symptoms; lower PlGF levels in early pregnancy predispose a woman to develop early-onset pre-eclampsia; low VEGF concentrations were observed approximately 5 weeks prior to the onset of pre-eclampsia (33.) Similar findings have been reported by other investigators (34, 40). Unfortunately, the ability of the serum sFlt-1 concentration to predict pre-eclampsia still requires prospective, longitudinal studies. Measurements of sFlt-1:VEGF ratio or sFlt-1: PlGF ratio in a urine specimen are considerably better tests for identifying groups of pregnant women with high risk for developing pre-eclampsia. Moreover, a high sFlt-1: PlGF ratio may indicate that a woman is at risk of delivering within two weeks due to severe pre-eclampsia.

Increases in the concentration of sFlt-1 in maternal serum are observed in women who have risk factors for developing pre-eclampsia. In women pregnant with twins, levels of sFlt-1 and sFlt-1/PlGF ratios were twice as high as in women experiencing singleton pregnancies. These changes were not associated with any alterations in the sFlt-1 mRNA levels and concentrations of HIF-1 alpha protein in the twin placentas, but were correlated with a higher placental mass. This has led to the conclusion that the higher risk of pre-eclampsia in women pregnant with twins may be the consequence of increased placental weight which is followed by a major release of sFlt-1 (21). This may explain that while smokers have lower levels of sFlt-1, as a

population group they also have fewer pre-eclampsia cases (41, 42).

Some clinical data presents decreased uteroplacental blood flow in pre-eclamptic women (43). The VEGF and Flt-1 gene transcription is regulated by hypoxia-inducible factor 1 (HIF-1 alpha) (44, 45). In pre-eclamptic patients, placenta levels of HIF-1 alpha and HIF-2 alpha are significantly elevated (46- 48). This can result in the creation of an additional pathway that may play a role in inducing sFlt-1 production.

All these observations indicate the major role played by sFlt-1 in the etiopathogenesis of pre-eclampsia. The trigger factor for increased production of sFLt-1 is unknown but most investigators focus on placental ischemia and hypoxia (49, 50).

3.4.1.2. SEng

Endoglin (CD105) is a homodimeric type I membrane glycoprotein that is expressed in vascular endothelial cells, the syncytiotrophoblasts of a full-term placenta, stromal cells, and hemopoetic cells (51). Endoglin has a role in vascular morphogenesis and is illustrated by the intrauterine death of mice deficient in endoglin due to defects in angiogenesis (52, 53). Mutations in endoglin genes lead to the wasting of capillaries and multiple arteriovenous malformations (54). Moreover, endoglin interacts with endothelial nitric oxide synthase to play a role in the regulation of vascular tone. Endoglin is also an accessory receptor for transforming growth factor-beta (TGF-beta). TGF-beta is a factor involved in angiogenesis that acts by binding with cell surface receptors: TGF-beta type I receptor (TGF-beta I) and TGF-beta type II receptor (TGF-beta II). Soluble endoglin (s-Eng),a truncated form of endoglin, is a cell surface co-receptor for TGF-beta, which binds and antagonizes TGF-beta in the extracellular environment.

S-Eng is an anti-angiogenic factor that shares many features with sFlt-1 and also plays a role in the pathogenesis of the systemic endothelial dysfunction in pre-eclampsia. The precise relationship of s-Flt-1 to s-Eng is unknown, but there is much evidence to confirm its role in the pathogenesis of pre-eclampsia through separate mechanisms. (55)

Venkatesha *et al.* have explored the effect of the adenoviral administration of sEng or both sEng and sFlt-1 in pregnant rats. Rats administered only sEng- expressing adenovirus developed an increase in mean arterial pressure and mild proteinuria. Administration of the sFlt-1-expressing adenovirus alone led to a greater increase in mean arterial pressure and severe proteinuria. In addition, when s-Flt-1 and sEng were administered together, the pregnant rats developed a severe pre-eclampsia-like syndrome with the signs and symptoms typical of human hemolysis—namely, elevated liver enzymes and low platelet syndrome (HELLP syndrome) with decreased platelet counts and elevated lactate dehydrogenase and aspartate aminotransferase. Moreover, intrauterine growth restriction of the fetus, evidence of hemolysis, extensive placental damage, infarction, areas of hepatic ischemia, and necrosis were observed only in the group administered both s-Flt-1 and sEng. Furthermore, non-pregnant rats administered s-Flt-1 and sEng together developed severe vascular damage. This suggests that these factors directly affect vessels and the placenta is not necessary for the generation of disease (32).

In the study by Maharaj *et al.*, the overexpression of sFlt-1 and sEng caused focal vasospasm, hypertension, and increased vascular permeability associated with brain edema which was similar to reversible posterior leukoencephalopathy associated with human pre-eclampsia (56). Moreover, an increased concentration of sEng was demonstrated in rat models of pre-eclampsia with reduced uterine perfusion (57).

Studies assessing the levels of sEng-1 in women with or without pre-eclampsia were similar to the animal studies and supported the role of elevated sEng in the pathogenesis of pre-eclampsia. One group identified that the 65 Da sEng monomer in the placentas of pre-eclamptic patients was fourfold higher than in the placentas from women with uncomplicated pregnancies (58).

The level of SEng is elevated in pregnant women with established pre-eclampsia 2-3 months before the onset of clinical manifestations, especially in severe and preterm (<37g.a.) pre-eclampsia with a peak in sEng at the onset of clinical disease and with a fall after delivery. This suggests that the increase in sEng may be the proper tool for predicting the severity of the disease (41).

Furthermore, in women experiencing normal pregnancy, the sEng levels fall from the first to second trimester without significant changes in the concentrations of sFlt1. In patients with subsequent pre-eclampsia, both sFlt-1 and sEng levels continue to rise from the first to second trimester. These changes may be useful in identifying those patients who have a higher than normal risk of developing pre-eclampsia (59).

There is some data to support the advantages of using a combination of sFlt-1, PlGF, and sEng levels to identify those prone to develop pre-eclampsia. Increased levels of both sEng and sFlt-1/PlGFratio are usually observed prior to the onset of preterm pre-eclampsia (33, 41). The levels of sFlt-1, PlGF, and sEng were also higher in a group of pre-eclamptic women with abruption of the placenta than in a control group (60, 61). Sandrim *et al.* report changes in sFlt-1, PlGF, and sEng concentration in women with pre-eclampsia, and its association with maternal endothelial dysfunction and impaired nitric oxide formation (55, 62).

Other investigators report changes in circulating concentrations of PlGF, sFlt-1 and in the sFlt-1/PlGF ratio before the onset of pre-eclampsia. Women predisposed to develop pre-eclampsia had higher sFlt-1 levels and sFlt-1/PlGF ratio and lower PlGF levels than women with uncomplicated pregnancies. Furthermore, the sFlt-1/PlGF ratio was more tightly associated with risk of preterm (<34 week of gestation) or term pre-eclampsia than either

angiogenic factor alone (63). Verlohren *et al.* showed increased sFlt-1/PlGF ratio in patients with pre-eclmpsia as compared with healthy pregnant women and with patients with chronic and gestational hypertension. The measurement of the sFlt-1/PlGF ratio can differentiate between different forms of hypertensive disorder. Moreover, they observed significantly increased risk for an immediate occurrence of delivery in women with pre-eclampsia with high sFlt-1/PlGF ratio (64).

The fetuses of pre-eclamptic mothers do not have higher levels of sEng and sFlt-1. This means that these fetuses do not develop hypertension and proteinuria because they are not exposed to increasing concentrations of these anti-angiogenic factors (65). All experimental evidence in animals and humans suggests that sEng and sFlt1, both of which cause endothelial dysfunction, may contribute to pre-eclampsia syndrome.

3.4.2. The role of circulating endothelial cells (CECs), endothelial progenitor cells (EPCs), and microparticles in pre-eclampsia

The exact role of CECs, EPCs, and microparticles in the pathogenesis of pre-eclampsia is unknown. Adequate levels of these cells and microparticles most likely play an important part in the development and regulation of vascularization in pregnancy. Some imbalances in these levels are associated with endothelial insufficiency.

3.4.2.1. Circulating endothelial cells (CECs)

CECs were detected in the peripheral blood as early as the 1970s (66.) These are mature endothelial cells of 15 –50 μm in diameter which could be isolated from the blood by flow cytometry or by immunomagnetic techniques using the presence of specific antigens on these cells. The number of CECs in the blood samples of healthy volunteers was low (≈ 5cells/1ml). The levels of CECs increase in some diseases which are related to endothelial damage, including myocardial infarction, small-vessel vasculitis, hypertension, diabetes, chronic kidney failure, septic shock, thrombotic thrombocytopenic purpura, and systemic lupus erythematosus. CECs reflect endothelial status and function and the number of CECs is used to assess vascular damage and, even more, it could be a marker of disease activity (67- 71).

Several studies have assessed the numbers of CECs in women suffering from pre-eclampsia. The first study was performed by Canbakan *et al.* in 2007. This group showed significantly higher levels of CECs in pre-eclamptic patients (13.2 +/- 5.2 cells/ml) than in healthy pregnant women (5.2 +/- 1.4 cells/ml), non-pregnant controls (4.0 +/- 1.8 cells/ml), and non-pregnant hypertensive women (6.9 +/- 0.8 cells/ml). Moreover, there was a significant difference between the hypertensive non-pregnant patients and the non-pregnant control, and in the pregnant women a statistically significant correlation between the number of CECs and systolic and diastolic blood pressures was found (72).

Grundman *et al.* showed statistically significant differences between the amount of CECs before delivery in healthy pregnant women (median: 16 cells/ml; range: 0-100 cells/ml) and women with pre-eclampsia (median: 88 cells/ml; range: 40- 812 cells/ml). Moreover, in pre-eclamptic patients cell numbers declined rapidly after delivery in contrast with the more subtle decline observed in healthy pregnant women following delivery. The number of CECs was correlated with values of systolic blood pressure in women with pre-eclampsia, whereas there was no such correlation in the healthy pregnant group (73).

The findings of another recent study have indicated that the number of CECs in pre-eclamptic women (median: 24.7 cells/ml; range: 2-100 cells/ml) is significantly higher than in normotensive pregnant women (median: 13 cells/ml; range: 1- 40 cells/ml) (74).'

However, when Strijbos *et al.* evaluated the number of CECs both in patients with severe pre-eclampsia and in the healthy pregnant control group, they were unable to detect any statistically significant differences between the study group (median: 5.5 cells/ml; range: 3.3- 7.5 cells/ml) and the control group (median: 3.5 cells/ml; range: 2- 4.8 cells/ml). Additionally, there were no differences in the number of CECs in stabilized as compared to non-stabilized pre-eclamptic patients. The researchers have suggested that endothelial dysfunction and cell activation rather than actual damage occurs in pre-eclamptic patients (75).

CECs are a novel specific and sensitive marker of endothelial dysfunction and injury. They constitute a marker that can also be used for assessing the extent of disease activity. However, the relationship between the number of CECs and disease severity is unknown and would require further investigation. Prospective studies are needed to evaluate the significance of CECs in predicting pre-eclampsia before high blood pressure and proteinuria occur.

3.4.2.2. Endothelial progenitor cells (EPCs)

EPCs are immature precursor cells smaller than 15μm in diameter and derived from the bone marrow and vascular wall that have proliferative potential (76). These cells are mobilized from the bone marrow in conditions such as trauma, burns, arthritis, retinal disease, hemangioma, psoriasis, atherosclerosis, tumor growth, and metastasis (77- 82). The process of EPC identification makes use of flow cytometry (because of surface antigens presence) or *in vitro* colony-forming assays. EPCs are thought to reflect the body's capacity for endothelial repair and to play a role in vascular remodeling and endothelial hemostasis (76). Some evidence suggests that these cells play a role in prenatal and postnatal neovascularization and re-endothelialization and may also serve an important part in the vascularization of the uterine endometrium during embryo implantation and placentation in pregnancy (83). These cells constitute about 25% of the endothelial cells in newly formed vessels (84). Estrogen, through its anti-apoptotic effect, leads to increased numbers of EPCs (85, 86). In conditions related to endothelial dysfunction, such as diabetes mellitus or cardiovascular diseases, the number and function of EPCs are reduced (87, 88). A recent study

suggests the involvement of EPCs in the pathogenesis of pre-eclampsia. The mobilization of EPCs into the circulation is caused by nitric oxide-dependent pathways in response to the stimulation of vascular endothelial growth factors (VEGF), placental growth factor (PlGF), and estrogen (among other factors) (85, 89, 90). Moreover, the mobilization of EPCs may constitute an important mechanism whereby estrogens protect the vascular endothelium during pregnancy, and the pregnancy is related to the mobilization of EPCs from the bone marrow to the peripheral blood (91).

Only a few studies have reported data on circulating EPCs in uncomplicated human pregnancy. Sugawara *et al.* have shown that the number of circulating EPCs increases gradually with gestational age and levels of circulating EPCs correlate with plasma estradiol levels (92). The other study by Luppi *et al.* has shown that EPCs levels are increased during the second and third trimesters of an uncomplicated pregnancy as compared to the first trimester (93). Nevertheless, studies by Matsubara have revealed that the number of EPCs is highest in women in their first trimester of pregnancy and then decreases significantly over the course of pregnancy (94).

There are only three diseases of pregnancy in which EPCs have been assessed: pre-eclampsia, gestational hypertension, and gestational diabetes mellitus. Sugawara *et al.* found a decreased number of EPCs and increased senescence of EPCs in patients who developed pre-eclampsia compared with the healthy pregnant controls (95). Luppi *et al.* also found a significantly lower number of EPCs in women with pre-eclampsia when compared to women in their third trimester of uncomplicated pregnancy (93). However, in the study by Matsubara *et al.*, the number of EPCs in pre-eclamptic women was not significantly different when compared to that of women in the third trimester of uncomplicated pregnancy (94). It is difficult, however, to do a reliable comparison of these studies because of the different methods used to assess EPCs.

The exact role of EPCs in the pathogenesis of pre-eclampsia is still unknown. Probably a deficiency may lead to higher maternal susceptibility to pre-eclampsia. It is still unknown whether a decrease in the number of EPCs in pre-eclamptic women reflects an underlying etiology or is rather the effect of the disease. The data revealing an increasing number of EPCs with gestational age in women with uncomplicated pregnancies suggests that these cells may play an important role in the regulation of placental development and vascular integrity during pregnancy. The creation of a subsequent study which will assess the number and function of EPCs prospectively before the onset of the symptoms of pre-eclampsia is needed.

3.4.2.3. Microparticles (MPs)

MPs are found in the peripheral blood in the form of vesicles which are shed from cell membranes under the influence of activation or apoptosis. These nuclear structures are 0.1 to 1 μm in diameter and contain membrane proteins and cytoplasmic material of the parental cell. Their differentiation from other types of circulating subcellular elements (exosomes, ectosomes, and apoptotic bodies) is based on size, mechanism of formation, and content. Flow cytometry is typically used for detecting MPs in blood samples, and this process is based on vesicle size, the externalization of phosphatidylserine, and the presence of specific types of surface antigens similar to their parental cell antigens (76). The main function of MPs is to mediate intracellular communication which leads to modifications in hemostasis, thrombosis, inflammation, and angiogenesis. In the blood stream MPs act as carrier and transport proteins (growth factors, apoptotic factors, receptors, and others) of RNA and DNA fragments from one cell to another (96) MPs can originate from different types of cells. Most commonly they originate from platelets (identified by the presence of surface antigens: CD41a, CD42b, CD62P), but also from endothelial cells (CD 144, CD62E, CD31), leukocytes (CD45, CD8, CD4, CD14), and erythrocytes (CD235a) (76). Recently, Germain *et al.* have reported circulating microparticles derived from syncytiotrophoblasts (97).

MPs are released into the circulation under conditions of cell stress or damage. There has been some evidence of increased levels of platelet, endothelial, and leukocyte MPs in conditions related to endothelial dysfunction, and this may reflect the degree of the endothelial dysfunction (98- 101). These types of MPs have been shown to be elevated in conditions such as cardiovascular diseases, metabolic syndrome, diabetes, sepsis, antiphospholipid syndrome, thrombotic thrombocytopenic purpura, rheumatoid arthritis, chronic renal failure, systemic lupus erythematosus, and cancer metastasis (96). Moreover, Burger *et al.* have suggested that MPs may exert harmful effects on the endothelium, and have demonstrated that these vesicles promote oxidative stress and expression of cell adhesion proteins in cultured endothelial cells (99).

Endothelial MPs are considered a direct marker of endothelial cell stress or damage. Furthermore, MPs may also reflect endothelial inflammation, increased coagulation, and vascular tone (101). Studies by both Bretelle *et al.* and Alijotas-Reig *et al.* have shown that the number of endothelial and platelet MPs are increased in women with uncomplicated pregnancy compared with non-pregnant healthy women. Moreover, a higher number of these vesicles can be detected in healthy women for two months after they have delivered (102, 103).

Some investigators have assessed levels of plasma endothelial MPs in pregnancies complicated by pre-eclampsia. González- Quintero *et al.* have shown statistically significant higher numbers of plasma endothelial MPs in women with pre-eclampsia as compared with healthy pregnant women. In this study there was no association between plasma endothelial MP levels and the MAP (mean arterial pressure) either in the study group or the control group. Moreover, in pre-eclamptic women no correlation between proteinuria and endothelial MPs was found. Endothelial MPs correlated well with the clinical presentation of pre-eclampsia (104). Reyna- Villasmil *et al.* observed higher concentration of endothelial MPs in eclamptic and severe pre-eclamptic patients. Lower values

were found in mild pre-eclamptic women. There were significant higher values of endothelial MPs in the studied groups (eclamptic/ pre-eclamptic patients)compared with healthy pregnant women (105).

The next study performed by the same group of investigators revealed significant elevations in CD31+/CD41- endothelial MPs in patients with pre-eclampsia when compared to women with gestational hypertension. Also both of these groups showed significantly elevated CD31+/CD41- endothelial MP levels in comparison to the control group. The significant elevations of CD62E+ endothelial MP count were observed in patients with pre-eclampsia as compared with the women with gestational hypertension and the healthy control pregnant group (106).

Alijotas-Reig *et al.* compared MPs in healthy non-pregnant women, healthy pregnant women, and women with pregnancy complicated by pre-eclampsia, HELLP syndrome, or fetal growth restriction. They demonstrated significant increases in endothelial and platelet MPs in healthy pregnant versus healthy non-pregnant women. When they compared the pre-eclamptic women with the group of healthy pregnant women, increased levels of CD31+/CD41 endothelial MPs were detected. Interestingly, they also assayed the differences between the pre-eclampsia group of women and the isolated fetal growth restriction group and observed a tendency toward a lower number of CD31+/CD41 endothelial MPs in the isolated fetal growth restriction group; however, this relationship was not statistically significant (107).

Some clinical or laboratory evidence has revealed that in the presence of higher levels of endothelial MPs, the endothelium-dependent vasodilation is worse (108). It means that endothelial MPs may act as a marker of endothelial dysfunction.

Furthermore, the level of syncytiotrophoblast MPs correlates with the degree of underlying inflammatory and oxidative status in pre-eclamptic patients (96, 109). The women in the group with pre-eclampsia showed higher STBM levels than the women in the healthy pregnancy group. Some data suggests that STBM promotes endothelial and immune cell dysfunction, activates superoxide production in neutrophils, and may stimulate the production and release of pro-inflammatory cytokines through the binding of monocytes (110).

There is currently a need to investigate women in the first trimester of pregnancy followed by serial blood sampling to determine whether endothelial MPs increase before the clinical symptoms of pre-eclampsia. Salomon *et al.* have shown that the number of cell-derived MPs at 24 weeks of gestation does not have predictive value for the subsequent development of vascular diseases specific to pregnancy such as hypertension, preeclampsia, intrauterine growth restriction, and small-for-gestational age live births (111).

We can conclude that MPs reflect vascular injury, endothelial apoptosis, and leukocyte or platelet activation, and also may lead to further vascular injury. The real relationship between pre-eclampsia and circulating MPs should be investigated in well-designed prospective studies in order to assess their prognostic and diagnostic value for pre-eclampsia. The exact pathophysiology of this field may lead to the creation of therapies for modulating the effect of MPs.

4. CONCLUSION

Knowledge about the exact pathophysiology of pre-eclampsia is one of the most important aims of current research in the field of hypertensive disorder during pregnancy.

While considerable interesting and valuable data about the pathogenesis of pre-eclampsia has been brought to light in recent years, much further research is needed before we can fully understand this complex disorder. There is some evidence that circulating levels of anti-angiogenic and angiogenic factors alter several weeks before the onset of pre-eclampsia. We do not, however, have any data about such alterations in CECs, CPCs, and microparticle levels because this field is still so young. Such data would be useful for creating effective pharmacologic therapeutic approaches for the management of pre-eclampsia. This new sight on the pathophysiology of pre-eclampsia has indicated new target points for potential therapies and provides an intriguing subject for future research.

5. REFERENCES

1. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* 183(1), S1-S22 (2000)

2. ACOG Committee on Practice Bulletins- Obstetrics: ACOG practice bulletin: Diagnosis and management of pre-eclampsia and eclampsia. *Obstet Gynecol* 99, 159 (2002)

3. J.R. Barton, B.M. Sibai: Prediction and prevention of recurrent pre-eclampsia. *Obstet Gynecol* 112(2 Pt 1), 359-372 (2008)

4. R.E. Harskamp, G.G. Zeeman: Pre-eclampsia: at risk for remote cardiovascular disease. *Am J Med Sci* 334(4), 291-295 (2007)

5. R. Thadhani, C.G. Solomon: Pre-eclampsia- a glimpse into the future? *N Engl J Med* 359(8), 858-860 (2008)

6. M. Wolf, C.A. Hubel, C. Lam, M. Sampson, J.L. Ecker, R.B. Ness, A. Rajakumar, A. Daftary, A.S. Shakir, E.W. Seely, J.M. Roberts, V.P. Sukhatme, S.A. Karumanchi, R. Thadhani: Pre-eclampsia and future cardiovascular disease: potential role of altered angiogenesis and insulin resistance. *J Clin Endocrinol Metab* 89(12), 6239-6243 (2004)

7. C.W. Redman, I.L. Sargent: Latest advances in understanding pre-eclampsia. *Science* 308(5728), 1592-1594 (2005)

8. P.L. Gross, W.C. Aird: The endothelium and thrombosis. *Semin Thromb Hemost* 26(5), 463-478 (2000)

9. D.D. Wagner, P.S. Frenette: The vessel wall and its interactions. *Blood* 111(11), 5271-5281 (2008)

10. K. Matsuo, S. Kooshesh, M. Dinc, C.C. Sun, T. Kimura, A.A. Baschat: Late postpartum eclampsia: report of two cases managed by uterine curettage and review of the literature. *Am J Perinatol* 24 (4), 257-266 (2007)

11. M.A. Shembrey, A.D. Noble: An instructive case of abdominal pregnancy. *Aust N Z J Obstet Gynaecol* 35(2), 220-221 (1995)

12. Y. Zhou, C.H. Damsky, S.J. Fisher: Pre-eclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest* 99(9), 2152-2164 (1997)

13. J.W. Meekins, R. Pijnenborg, M. Hanssens, I.R. McFadyen, A. van Asshe: A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Br J ObstetGynaecol* 101(8), 669-674 (1994)

14. I. Brosens, R. Pijnenborg, L. Vercruysse, R. Romero: The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. *Am J Obstet Gynecol* 204(3), 193-201 (2011)

15. Y. Zhou, C.H. Damsky, K. Chiu, J.M. Roberts, S.J. Fisher: Pre-eclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. *J Clin Invest* 91(3), 950-60 (1993)

16. K.H. Lim, Y. Zhou, M. Janatpour, M. McMaster, K. Bass, S.H. Chun, S.J. Fisher: Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. *Am J Pathol* 151(6), 1809-1818 (1997)

17. S.J. Fisher: The placental problem: linking abnormal cytotrophoblast differentiation to the maternal symptoms of pre-eclampsia. *Reprod Biol Endocrinol* 2, 53 (2004)

18. A. Makris, C. Thornton, J. Thompson, S. Thomson, R. Martin, R. Ogle, R. Waugh, P. McKenzie, P. Kirwan, A. Hennessy: Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. *Kidney Int* 71(10), 977-984 (2007)

19. J.S. Gilbert, S.A. Babcock, J.P. Granger. Hypertension produced by reduced uterine perfusion in pregnant rats is associated with increased soluble fms-like tyrosine kinase-1 expression. *Hypertension* 50(6), 1142-1147 (2007)

20. K. Duckitt, D. Harrington: Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *BMJ* 330(7491), 565 (2005)

21. Y. Bdolah, C. Lam, A. Rajakumar, V. Shivalingappa, W. Mutter, B.P. Sachs, K.H. Lim, T. Bdolah-Abram, F.H. Epstein, S.A. Karumanchi: Twin pregnancy and the risk of pre-eclampsia: bigger placenta or relative ischemia? *Am J Obstet Gynecol* 198(4), 428 e1-6 (2008)

22. K. Koga, Y. Osuga, T. Tajima, Y. Hirota, T. Igarashi, T. Fujii, T. Yano, Y. Taketani: Elevated serum soluble fms-like tyrosine kinase 1 (sFlt1) level in women with hydatidiform mole. *Fertil Steril* 94(1), 305-308 (2010)

23. B. Sibai, G. Dekker, M. Kupferminc: Pre-eclampsia. *Lancet* 365(9461), 785-799 (2005)

24. S.K. Palmer, L.G. Moore, D. Young, B. Cregger, J.C. Berman, S. Zamudio: Altered blood pressure course during normal pregnancy and increased pre-eclampsia at high altitude (3100 meters) in Colorado. *Am J Obstet Gynecol* 180(5), 1161-1168.(1999)

25. F. De Wolf, C. De Wolf-Peeters, I. Brosens, W.B. Robertson: The human placental bed: electron microscopic study of trophoblastic invasion of spiral arteries. *Am J Obstet Gynecol* 137(1), 58-70 (1980)

26. C.M. Salafia, J.C. Pezzullo, A. Ghidini, J.A. Lopèz-Zeno, S.S. Whittington: Clinical correlations of patterns of placental pathology in preterm pre-eclampsia. *Placenta* 19(1), 67-72 (1998)

27. G. Gerretsen, H.J. Huisjes, J.D. Elema: Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation. *Br J Obstet Gynaecol* 88(9), 876-881 (1981)

28. F. De Wolf, W.B. Robertson, I. Brosens. The ultrastructure of acute atherosis in hypertensive pregnancy. *Am J Obstet Gynecol* 123(2), 164-174 (1975)

29. W.B. Robertson, I. Brosens, H.G. Dixon: The pathological response of the vessels of the placental bed to hypertensive pregnancy *J Pathol Bacteriol* 93(2), 581-592 (1967)

30. S.E. Maynard, J.Y. Min, J. Merchan, K.H. Lim, J. Li, S. Mondal, T.A. Libermann, J.P. Morgan, F.W. Sellke, I.E. Stillman, F.H. Epstein, V.P. Sukhatme, S.A. Karumanchi: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 111(5), 649-658 (2003)

31. K. Koga, Y. Osuga, O. Yoshino, Y. Hirota, X. Ruimeng, T. Hirata, S. Takeda, T. Yano, O. Tsutsumi, Y. Taketani: Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with pre=eclampsia. *J Clin Endocrinol Metab* 88(5), 2348-2351 (2003)

32. S. Venkatesha, M. Toporsian, C. Lam, J. Hanai, T. Mammoto, Y.M. Kim, Y. Bdolah, K.H. Lim, H.T. Yuan, T.A. Libermann, I.E. Stillman, D. Roberts, P.A. D'Amore, F.H. Epstein, F.W. Sellke, R. Romero, V.P. Sukhatme, M. Letarte, S.A. Karumanchi: Soluble endoglin contributes to the pathogenesis of pre-eclampsia. *Nat Med* 12(6), 642-649 (2006) Erratum in: *Nat Med* 12(7), 862 (2006)

33. R.J. Levine, S.E. Maynard, C. Qian, K.H. Lim, L.J. England, K.F. Yu, E.F. Schisterman, R. Thadhani, B.P. Sachs, F.H. Epstein, B.M. Sibai, V.P. Sukhatme, S.A. Karumanchi: Circulating angiogenic factors and the risk of pre-eclampsia. *N Engl J Med* 350(7), 672-683 (2004)

34. B.M. Polliotti, A.G. Fry, D.N. Saller, R.A. Mooney, C. Cox, R.K. Miller: Second-trimester maternal serum placental growth factor and vascular endothelial growth factor for predicting severe, early-onset pre-eclampsia.*Obstet Gynecol* 101(6), 1266-1274 (2003)

35. R.N. Taylor, J. Grimwood, R.S. Taylor, M.T. McMaster, S.J. Fisher, R.A. North: Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. *Am J Obstet Gynecol* 188(1), 177-182 (2003)

36. J.P. Bridges, J.S. Gilbert, D. Colson, S.A. Gilbert, M.P. Dukes, M.J. Ryan, J.P. Granger: Oxidative stress contributes to soluble fms-like tyrosine kinase-1 induced vascular dysfunction in pregnant rats. *Am J Hypertens* 22(5), 564-568 (2009)

37. V. Eremina, M. Sood, J. Haigh, A. Nagy, G. Lajoie, N. Ferrara, H.P. Gerber, Y. Kikkawa, J.H. Miner, S.E. Quaggin: Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 111(5), 707-716 (2003)

38. B.C. Kuenen, M. Levi, J.C. Meijers, A.K. Kakkar, V.W. van Hinsbergh, P.J. Kostense, H.M. Pinedo, K. Hoekman: Analysis of coagulation cascade and endothelial cell activation during inhibition of vascular endothelial growth factor/vascular endothelial growth factor receptor pathway in cancer patients. *Arterioscler Thromb Vasc Biol.* 22(9), 1500-1505 (2002)

39. J.C. Yang , L. Haworth L, R.M. Sherry, P. Hwu, D.J. Schwartzentruber, S.L. Topalian, S.M. Steinberg, H.X. Chen, S.A. Rosenberg: A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 349(5), 427-434 (2003)

40. E. Shibata, A. Rajakumar, R.W. Powers, R.W. Larkin, C. Gilmour, L.M. Bodnar, W.R. Crombleholme, R.B. Ness, J.M. Roberts, C.A. Hubel: Soluble fms-like tyrosine kinase 1 is increased in pre-eclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. *J Clin Endocrinol Metab* 90(8), 4895-4903 (2005)

41. R.J. Levine, C. Lam, C. Qian, K.F. Yu, S.E. Maynard, B.P. Sachs, B.M. Sibai, F.H. Epstein, R. Romero, R. Thadhani, S.A. Karumanchi, CPEP Study Group: Soluble endoglin and other circulating antiangiogenic factors in pre-eclampsia. *N Engl J Med* 355(10), 992-1005 (2006) Erratum in: *N Engl J Med* 355(17), 1840 (2006)

42. R.W. Powers, J.M. Roberts, K.M. Cooper, M.J. Gallaher, M.P. Frank, G.F. Harger, R.B. Ness: Maternal serum soluble fms-like tyrosine kinase 1 concentrations are not increased in early pregnancy and decrease more slowly postpartum in women who develop pre-eclampsia. *Am J Obstet Gynecol* 193(1), 185-191 (2005)

43. N.O. Lunell, R. Lewander, I. Mamoun, L. Nylund, S. Sarby, S. Thornström: Uteroplacental blood flow in pregnancy induced hypertension. *Scand J Clin Lab Invest Suppl* 169, 28-35 (1984)

44. J.A. Forsythe, B.H. Jiang, N.V. Iyer, F. Agani, S.W. Leung, R.D. Koos, G.L. Semenza: Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 16(9), 4604-4613 (1996)

45. H.P. Gerber, F. Condorelli, J. Park, N. Ferrara: Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem* 272(38), 23659-23667 (1997)

46. I. Caniggia, H. Mostachfi, J. Winter, M. Gassmann, S.J. Lye, M. Kuliszewski, M. Post: Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta3. *J Clin Invest* 105(5), 577-587 (2000)

47. A. Rajakumar, K. Doty, A. Daftary, G. Harger, K.P. Conrad: Impaired oxygen-dependent reduction of HIF-1alpha and -2alpha proteins in pre-eclamptic placentae. *Placenta* 24(2-3), 199-208 (2003) Erratum in: *Placenta* 24(6), 711 (2003)

48. A. Rajakumar, K.A. Whitelock, L.A. Weissfeld, A.R. Daftary, N. Markovic, K.P. Conrad: Selective overexpression of the hypoxia-inducible transcription factor, HIF-2alpha, in placentas from women with preeclampsia. *Biol Reprod* 64(2), 499-506 (2001)

49. S. Ahmad, A. Ahmed: Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in pre-eclampsia. *Circ Res* 95(9), 884-891 (2004)

50. T. Nagamatsu, T. Fujii, M. Kusumi, L. Zou, T. Yamashita, Y. Osuga, M. Momoeda, S. Kozuma, Y. Taketani: Cytotrophoblasts up-regulate soluble fms-like tyrosine kinase-1 expression under reduced oxygen: an implication for the placental vascular development and the pathophysiology of pre-eclampsia. *Endocrinology* 145(11), 4838-4845 (2004)

51. F. Lebrin, M.J. Goumans, L. Jonker, R.L. Carvalho, G. Valdimarsdottir, M. Thorikay, C. Mummery, H.M. Arthur

HM, P. ten Dijke: Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *EMBO J* 23(20), 4018-4028 (2004)

52. A. Bourdeau, D.J. Dumont, M. Letarte: A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest* 104(10), 1343-1351 (1999)

53. H.M. Arthur, J. Ure, A.J. Smith, G. Renforth, D.I. Wilson, E. Torsney, R. Charlton, D.V. Parums, T. Jowett, D.A. Marchuk, J. Burn, A.G. Diamond: Endoglin, an ancillary TGFbeta receptor, is required for extra-embryonic angiogenesis and plays a key role in heart development. *Dev Biol* 217(1), 42-53 (2000)

54. S. Maynard, F.H. Epstein, S.A. Karumanchi: Pre-eclampsia and angiogenic imbalance. *Annu Rev Med* 59, 61-78 (2008)

55. V.C. Sandrim, A.C. Palei, I.F. Metzger, V.A. Gomes, R.C. Cavalli, J.E. Tanus-Santos: Nitric oxide formation is inversely related to serum levels of anti-angiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia. *Hypertension* 52(2), 402-407 (2008)

56. A.S. Maharaj, T.E. Walshe, M. Saint-Geniez, S. Venkatesha, A.E. Maldonado, N.C. Himes, K.S. Matharu, S.A. Karumanchi, P.A. D'Amore: VEGF and TGF-beta are required for the maintenance of the choroid plexus and ependyma. *J Exp Med* 205(2), 491-501 (2008)

57. J.S. Gilbert, S.A. Gilbert, M. Arany, J.P. Granger: Hypertension produced by placental ischemia in pregnant rats is associated with increased soluble endoglin expression. *Hypertension* 53(2), 399-403 (2009)

58. C.E. Powe, R.J. Levine, S.A. Karumanchi: Preeclampsia, a disease of the maternal endothelium: the role of anti-angiogenic factors and implications for later cardiovascular disease. *Circulation* 123(24), 2856-2869 (2011)

59. S. Rana, S.A. Karumanchi, R.J. Levine, S. Venkatesha, J.A. Rauh-Hain, H. Tamez, R. Thadhani: Sequential changes in anti-angiogenic factors in early pregnancy and risk of developing preeclampsia. *Hypertension* 50(1), 137-142 (2007)

60. C. Signore, J.L. Mills, C. Qian, K. Yu, C. Lam, F.H. Epstein, S.A. Karumanchi, R.J. Levine: Circulating angiogenic factors and placental abruption. *Obstet Gynecol* 108(2), 338-344 (2006) Erratum in: *Obstet Gynecol* 108(4), 1035 (2006)

61. C. Signore, J.L. Mills, C. Qian, K.F. Yu, S. Rana, S.A. Karumanchi, R.J. Levine: Circulating soluble endoglin and placental abruption. *PrenatDiagn* 28(9), 852-858 (2008)

62. M. Noori, A.E. Donald, A. Angelakopoulou, A.D. Hingorani, D.J. Williams: Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. *Circulation* 122(5), 478-487 (2010)

63. A. Leaños-Miranda, I. Campos-Galicia, I. Isordia-Salas, R. Rivera-Leaños, J.F. Romero-Arauz, J.A. Ayala-Méndez, A. Ulloa-Aguirre: Changes in circulating concentrations of soluble fms-like tyrosine kinase-1 and placental growth factor measured by automated electrochemiluminescence immunoassays methods are predictors of preeclampsia. *J Hypertens* 30(11), 2173-2181 (2012)

64. S. Verlohren, I. Herraiz, O. Lapaire, D. Schlembach, M. Moertl, H. Zeisler, P. Calda, W. Holzgreve, A. Galindo, T. Engels, B. Denk, H. Stepan: The sFlt-1/PlGF ratio in different types of hypertensive pregnancy disorders and its prognostic potential in preeclamptic patients. *Am J Obstet Gynecol* 206(1), 58 e1-8 (2012)

65. A.C. Staff, K. Braekke, G.M. Johnsen, S.A. Karumanchi, N.K. Harsem: Circulating concentrations of soluble endoglin (CD105) in fetal and maternal serum and in amniotic fluid in pre-eclampsia. *Am J Obstet Gynecol* 197(2), 176 e1-6 (2007)

66. F. Dignat-George, J. Sampol: Circulating endothelial cells in vascular disorders: new insights into an old concept. *Eur J Haematol* 65(4), 215-220 (2000)

67. U. Erdbruegger, M. Haubitz, A. Woywodt: Circulating endothelial cells: a novel marker of endothelial damage. *Clin Chim Acta* 373(1-2), 17-26 (2006)

68. M. Haubitz, A. Woywodt: Circulating endothelial cells and vasculitis. *Intern Med* 43(8), 660-667 (2004)

69. F. Dignat-George, J. Sampol, G. Lip, A.D. Blann: Circulating endothelial cells: realities and promises in vascular disorders. *Pathophysiol Haemost Thromb* 33(5-6), 495-499 (2003/2004)

70. C. Holmén, E. Elsheikh, P. Stenvinkel, A.R. Qureshi, E. Pettersson, S. Jalkanen, S. Sumitran-Holgersson: Circulating inflammatory endothelial cells contribute to endothelial progenitor cell dysfunction in patients with vasculitis and kidney involvement. *J Am SocNephrol* 16(10), 3110-3120 (2005)

71. A. Woywodt, F. Streiber, K. de Groot, H. Regelsberger, H. Haller, M. Haubitz: Circulating endothelial cells as markers for ANCA-associated small-vessel vasculitis. *Lancet* 361(9353), 206-210 (2003)

72. B. Canbakan, K. Keven, H. Tutkak, N. Danisman, I. Ergun, G. Nergizoglu: Circulating endothelial cells in pre-eclampsia. *J Hum Hypertens* 21(7), 558-563 (2007)

73. M. Grundmann, A. Woywodt, T. Kirsch, B. Hollwitz, K. Oehler, U. Erdbruegger, H. Haller, M. Haubitz: Circulating endothelial cells: a marker of vascular damage in patients with pre-eclampsia. *Am J Obstet Gynecol* 198(3), 317 e1-5 (2008)

74. F. Mehrabian, S.M. Jazi, S.H. Javanmard, M. Kaviani, V. Homayouni: Circulating endothelial cells (CECs) and E-

selectin: Predictors of pre-eclampsia. *J Res Med Sci* 17(1), 15-21 (2012)

75. M.H. Strijbos, C.A. Snijder, J. Kraan, C.H. Lamers, J.W. Gratama, J.J. Duvekot: Levels of circulating endothelial cells in normotensive and severe pre-eclamptic pregnancies. *Cytometry B Clin Cytom* 78(6), 382-386 (2010)

76. D. Burger, R.M. Touyz: Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. *J Am Soc Hypertens* 6(2), 85-99 (2012)

77. S. Rafii: Circulating endothelial precursors: mystery, reality, and promise. *J Clin Invest* 105(1), 17-19 (2000)

78. M. Gill, S. Dias, K. Hattori, M.L. Rivera, D. Hicklin, L. Witte, L. Girardi, R. Yurt, H. Himel, S. Rafii: Vascular trauma induces rapid but transient mobilization of VEGFR2 (+)AC133 (+) endothelial precursor cells. *Circ Res* 88(2), 167-174 (2001)

79. P. Carmeliet: Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 6(4), 389-395 (2000)

80. P. Carmeliet: Angiogenesis in health and disease. *Nat Med* 9(6), 653-660 (2003)

81. D. Hanahan, J. Folkman: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86(3), 353-364 (1996)

82. J. Folkman: Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 29(6 Suppl 16), 15-8 (2002)

83. T. Asahara, H. Masuda, T. Takahashi, C. Kalka, C. Pastore, M. Silver, M. Kearne, M. Magner, J.M. Isner: Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 85(3), 221-228 (1999)

84. T. Murayama, O.M. Tepper, M. Silver, H. Ma, D.W. Losordo, J.M. Isner, T. Asahara, C. Kalka: Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization *in vivo*. *ExpHematol* 30(8), 967-972 (2002)

85. K. Strehlow, N. Werner, J. Berweiler, A. Link, U. Dirnagl, J. Priller, K. Laufs, L. Ghaeni, M. Milosevic, M. Böhm, G. Nickenig: Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. *Circulation* 107(24), 3059-3065 (2003)

86. A. Iwakura, C. Luedemann, S. Shastry, A. Hanley, M. Kearney, R. Aikawa, J.M. Isner, T. Asahara, D.W. Losordo: Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to re-endothelialization after arterial injury. *Circulation* 108(25), 3115-3121 (2003)

87. M. Vasa, S. Fichtlscherer, A. Aicher, K. Adler, C. Urbich, H. Martin, A.M. Zeiher, S. Dimmeler: Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 89(1), E1-7 (2001)

88. O.M. Tepper, R.D. Galiano, J.M. Capla, C. Kalka, P.J. Gagne, G.R. Jacobowitz, J.P. Levine, G.C. Gurtner: Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 106(22), 2781-2786 (2002)

89. A. Aicher, A.M. Zeiher, S. Dimmeler: Mobilizing endothelial progenitor cells. *Hypertension* 45(3), 321-325 (2005)

90. S. Rafii, S. Avecilla, S. Shmelkov, K. Shido, R. Tejada, M.A. Moore, B. Heissig, K. Hattori: Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. *Ann N Y Acad Sci* 996, 49-60 (2003)

91. A.O. Robb, N.L. Mills, D.E. Newby, F.C. Denison: Endothelial progenitor cells in pregnancy. *Reproduction* 133(1), 1-9 (2007)

92. J. Sugawara, M. Mitsui-Saito, T. Hoshiai, C. Hayashi, Y. Kimura, K. Okamura: Circulating endothelial progenitor cells during human pregnancy. *J Clin Endocrinol Metab* 90(3), 1845-1848 (2005)

93. P. Luppi, R.W. Powers, V. Verma, L. Edmunds, D. Plymire, C.A. Hubel: Maternal circulating CD34+VEGFR-2+ and CD133+VEGFR-2+ progenitor cells increase during normal pregnancy but are reduced in women with pre-eclampsia. *Reprod Sci* 17(7), 643-652 (2010)

94. K. Matsubara, E. Abe, Y. Matsubara, K. Kameda, M. Ito: Circulating endothelial progenitor cells during normal pregnancy and pre-eclampsia. *Am J Reprod Immunol* 56(2), 79-85 (2006)

95. J. Sugawara, M. Mitsui-Saito, C. Hayashi, T. Hoshiai, M. Senoo, H. Chisaka, N. Yaegashi, K. Okamura: Decrease and senescence of endothelial progenitor cells in patients with pre-eclampsia. *J Clin Endocrinol Metab* 90(9), 5329-5332 (2005)

96. C.W. Redman, I.L. Sargent: Circulating microparticles in normal pregnancy and pre-eclampsia. *Placenta* 29Suppl A, 73-77 (2008)

97. S.J. Germain, G.P. Sacks, S.R. Sooranna, I.L. Sargent, C.W. Redman: Systemic inflammatory priming in normal pregnancy and pre-eclampsia: the role of circulating syncytiotrophoblast microparticles. *J Immunol* 178(9), 5949-5956 (2007) Erratum in*: J Immunol* 179(2), 1390 (2007)

98. C. Beyer, D.S. Pisetsky: The role of microparticles in the pathogenesis of rheumatic diseases. *Nat Rev Rheumatol* 6(1), 21-29 (2010)

99. D. Burger, A.C. Montezano, N. Nishigaki, Y. He, A. Carter, R.M. Touyz: Endothelial microparticle formation by angiotensin II is mediated via Ang II receptor type I/NADPH oxidase/ Rho kinase pathways targeted to lipid rafts. *Arterioscler Thromb Vasc Biol* 31(8), 1898-1907 (2011)

100. G.N. Chironi, C.M. Boulanger, A. Simon, F. Dignat-George, J.M. Freyssinet, A. Tedgui: Endothelial microparticles in diseases. *Cell Tissue Res* 335(1), 143-151 (2009)

101. A.S. Leroyer, F. Anfosso, R. Lacroix, F. Sabatier, S. Simoncini, S.M. Njock, N. Jourde, P. Brunet, L. Camoin-Jau, J. Sampol, F. Dignat-George: Endothelial-derived microparticles: Biological conveyors at the crossroad of inflammation, thrombosis and angiogenesis. *Thromb Haemost* 104(3), 456-463 (2010)

102. J. Alijotas-Reig, C. Palacio-Garcia, I. Farran-Codina, C. Zarzoso, L. Cabero-Roura, M. Vilardell-Tarres: Circulating cell-derived microparticles in women with pregnancy loss. *Am J Reprod Immunol* 66(3), 199-208 (2011)

103. F. Bretelle, F. Sabatier, D. Desprez, L. Camoin, L. Grunebaum, V. Combes, C. D'Ercole, F. Dignat-George: Circulating microparticles: a marker of procoagulant state in normal pregnancy and pregnancy complicated by pre-eclampsia or intrauterine growth restriction. *Thromb Haemost* 89(3), 486-492 (2003)

104. V.H. González-Quintero, J.J. Jiménez, W. Jy, L.M. Mauro, L. Hortman, M.J. O'Sullivan, Y. Ahn: Elevated plasma endothelial microparticles in preeclampsia. *Am J Obstet Gynecol* 189(2), 589-593 (2003)

105. E. Reyna-Villasmil, J. Mejia-Montilla, N. Reyna-Villasmil, D. Torres-Cepeda, E. Peña-Paredes, J. Santos-Bolívar, J. Perozo-Romero: Endothelial microparticles in preeclampsia and eclampsia. *Med Clin (Barc)* 136(12), 522-526 (2011)

106. V.H. González-Quintero, L.P. Smarkusky, J.J. Jiménez, L.M. Mauro, W. Jy, L.L. Hortsman, M.J. O'Sullivan, Y.S. Ahn: Elevated plasma endothelial microparticles: pre-eclampsia versus gestational hypertension. *Am J Obstet Gynecol* 191(4), 1418-1424 (2004)

107. J. Alijotas-Reig, C. Palacio-Garcia, I. Farran-Codina, M. Ruiz-Romance, E. Llurba, M. Vilardell-Tarres: Circulating cell-derived microparticles in severe pre-eclampsia and in fetal growth restriction. *Am J Reprod Immunol* 67(2), 140-151 (2012)

108. C.M. Boulanger, A. Scoazec, T. Ebrahimian, P. Henry, E. Mathieu, A. Tedgui, Z. Mallat: Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation* 104(22), 2649-2652 (2001)

109. S. Guller: Role of the syncytium in placenta-mediated complications of pre-eclampsia. *Thromb Res* 124(4), 389-392 (2009)

110. A.S. Aly, M. Khandelwal, J. Zhao, A.H. Mehmet, M.D. Sammel, S. Parry: Neutrophils are stimulated by syncytiotrophoblast microvillous membranes to generate superoxide radicals in women with pre-eclampsia. *Am J Obstet Gynecol* 190(1), 252-258 (2004)

111. O. Salomon, B.Z. Katz, R. Dardik, T. Livnat, D.M. Steinberg, R. Achiron, U. Seligsohn: Plasma levels of microparticles at 24 weeks of gestation do not predict subsequent pregnancy complications. *Fertil Steril* 92(2), 682-687 (2009)

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