CARDIOVASCULAR FUNCTION AND BIOMARKERS IN WOMEN WITH PREECLAMPSIA

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آوْرُاً بِإِنْسَمَآءِ رَبِّكُ الَّذِي خَلَقَ جَدْرَ النَّاسِ مِنْ عَلَقٍ آوْرُاً وَرَبِّكُ الْكَرِيمُ
الذِّي عَلَّمَ بِالْقُلُوْمِ عِلْمَ أَلْهَ السَّمَاوَاتِ وَالْأَرْضِ. (Quran)

Read: In the name of thy Lord Who createth, Createth man from a clot. Read: And thy Lord is the Most Bounteous, Who teacheth by the pen, Teacheth man that which he knew not.

(Quran)
To the soul of my father and to Kurdistan which has not achieved its liberty until now
ABSTRACT

Background: Preeclampsia (PE) is a multisystem disorder peculiar to human pregnancy and characterized by the onset of hypertension and proteinuria after the 20th week of gestation. The pathophysiology of this disorder is still not clear. PE is not only associated with significant maternal and fetal morbidity and mortality during the index pregnancy but also with higher risk of cardiovascular disease later in life.

Overall aim: To study PE-related changes in the function of cardiovascular system and to measure the levels of different biomarkers of PE during pregnancy and after delivery.

Papers I and II: Eighteen women with a history of PE and 17 age-matched controls were enrolled one year after the index pregnancy. All underwent non-invasive ultrasound examination of the brachial artery for evaluation of flow-mediated vasodilatation (FMD). Ambulatory blood pressure and plasma concentrations of lipoproteins, inflammation markers, adhesion molecules, glucometabolic and hemostatic factors, thrombin generation and the levels of microparticles were determined during specific menstrual phases. Women with a history of PE had lower FMD, higher systolic, diastolic and mean arterial pressure during daytime, and a higher degree of insulin resistance. In addition they had a higher total amount of thrombin and platelet-derived microparticles, with no variation during follicular and luteal phases.

Papers III and IV: Thirty-five pregnant women with PE and 30 with normal pregnancy were examined during pregnancy and 3-6 months after delivery. Transthoracic echocardiography and Doppler tissue imaging were performed, and FMD of the brachial artery was examined. The blood levels of amino-terminal pro-brain natriuretic peptide (NT-pro-BNP), C-reactive protein, cystatin C, troponin I and inflammatory and angiogenic markers were measured. Women with PE showed structural and functional alterations in left ventricle and left atrium. They had a higher ratio of early transmitral diastolic flow velocity to early diastolic myocardial velocity (E/E’), higher levels of NT-pro-BNP and cystatin C, and lower glomerular filtration rate as estimated using cystatin C both during pregnancy and at follow-up. In addition, the lateral E/E’ ratio and NT-pro-BNP were higher in women with early-onset, severe PE than in those with late PE. The FMD was decreased in the preeclamptic group at inclusion and at follow-up. Pentraxin 3 (PTX3) and ratio of soluble fms-like tyrosine kinase-1 (sFlt-1) to placent growth factor (PIGF) were elevated in women with PE pregnancy. Furthermore FMD was lower and PTX3 and ratio of sFlt-1/PIGF were higher in women with early-onset, severe PE than in those with late PE.

Conclusion: Women with a history of PE had persisting identifiable abnormalities of vascular function as well as signs of hypercoagulability and excess platelet-derived microparticles, during both follicular and luteal phases of the menstrual cycle. PE was associated with alterations in the left ventricular structure and function, impaired endothelial function and elevation of cardiovascular biomarkers. These alterations persisted months after delivery and were more clearly visible in women with early-onset and severe PE. In addition, elevated inflammatory and antiangiogenic markers were present in those women, and were especially pronounced in early-onset PE.

Key words: preeclampsia, endothelial function, FMD, thrombin, microparticle, menstrual cycle, ventricular diastolic function, echocardiography, NT-pro-BNP, cystatin C, CRP, inflammation, hemostasis, Pentraxin 3, angiogenesis, pregnancy, follow-up.
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IV. Rafik Hamad R, Eriksson MJ, Larsson A, Bremme K. 
Impaired endothelial function and elevated Pentraxin 3 in early-onset preeclampsia.  
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ABBREVIATIONS

A wave  Flow velocity during atrial contraction
ANCOVA  Analysis of covariance
ANOVA  Analysis of variance
BMI  Body mass index
CAD  Coronary artery disease
CD61 + MP  Platelet derived microparticles
CRP  C-reactive protein
CV  Coefficient of variation
CVD  Cardiovascular disease
DBP  Diastolic blood pressure
DTI  Doppler tissue imaging
E  Early transmitral diastolic flow velocity
E’  Early diastolic myocardial velocity
ELISA  Enzyme-linked immunosorbent assay
FBF  Forearm blood flow
FMD  Flow-mediated vasodilatation
FS  Fractional shortening
GFR  Glomerular filtration rate
GTN  Glycerin trinitrate
HDL  High density lipoprotein
HELLP  Hemolysis and elevated liver enzymes and low platelets
HOMA  Homeostasis model assessment
HR  Heart rate
ICAM-1  Intercellular adhesion molecule-1
IGFBP  Insulin-like growth factor binding protein
IGF-I  Insulin-like growth factor I
IVRT  Iso-volumic relaxation time
IVSd  Interventricular septum thickness in diastole
LA  Left atrial
LAAes  Left atrial area at end-systole
LADes  Left atrial end-systolic diameter
LDL  Low density lipoprotein
LV  Left ventricle / Left ventricular
LVDd  Left ventricular end-diastolic diameter
LVH  Left ventricular hypertrophy
LVMi  Left ventricular mass index
MAP  Mean arterial pressure
MP  Microparticles
NS  Non significant
NT-pro-BNP  Amino-terminal pro-brain natriuretic peptide
PAI  Plasminogen activator inhibitor 1
PE  Preeclampsia
PIGF  Placental growth factor
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PTX3</td>
<td>Pentraxin 3</td>
</tr>
<tr>
<td>PWTd</td>
<td>Left ventricular posterior wall thickness in diastole</td>
</tr>
<tr>
<td>RWT</td>
<td>Relative wall thickness</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>sFlt-1</td>
<td>Soluble fms-like tyrosine kinase-1</td>
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<tr>
<td>TGA</td>
<td>Thrombin generation assay</td>
</tr>
<tr>
<td>TNFRI</td>
<td>Tumor necrosis factor receptor 1</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular adhesion molecule-1</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>Vascular endothelial growth factor-A</td>
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<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
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INTRODUCTION

Definition of preeclampsia

Preeclampsia (PE) is a multisystem disorder peculiar to human pregnancy and characterized by the onset of hypertension and proteinuria after the 20th week of gestation. PE complicates approximately 5-10% of nulliparous pregnancies and is among the top three causes of maternal death both in developed and developing countries (Dildy, Belfort, and Smulian 2007; Luo et al. 2007; Valensise et al. 2008). Every year, over four million women develop the disorder worldwide (Lyall F 2007). It is defined as hypertension (systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg measured at least 4 hours apart) with proteinuria (either ≥ 300 mg per 24 hours or ≥ 2+ by dipstick on 2 or more occasions 4 hours apart) (Sibai, Caritis, and Hauth 2003). But this simple clinical definition gives little idea of the complexity of the disorder. The clinical findings of PE can manifest as a maternal syndrome (with hypertension, and proteinuria) or as a fetal syndrome (with growth restriction, prematurity, placental abruption and death) or both. Additional symptoms and complications may be associated with PE. Cerebral manifestations include headache, confusion, visual disturbances or loss of eyesight, paralysis, coma, seizures and stroke. Other manifestations are, for example, nausea, liver damage, hematoma and hepatic rupture, renal failure, pulmonary edema, thrombocytopenia, coagulopathy, hemolysis, elevated liver enzymes and low platelets (HELLP syndrome) (Sibai, Dekker, and Kupferminc 2005). Still, the etiology of preeclampsia is unknown, and delivery is the only definitive treatment to ensure the wellbeing of the mother and the fetus. Fetal syndrome contributes to the increasing proportion of iatrogenic preterm deliveries and infants who are classed as small-for-gestational-age (Chandiramani and Shennan 2008).

History of preeclampsia

Hippocrates first described this condition when he wrote “convulsion takes place from either repletion or depletion”. He observed the sudden and unexpected appearance of maternal grand-mal seizures, which occur when PE progresses to eclampsia, a term derived from the Greek word for lightning (Schlembach 2003). In the beginning, PE was confused with epilepsy and hysteria and it was not described as a separate entity until 1739 (Schlembach 2003). This disorder was previously called toxemia of pregnancy, because it was believed that there were toxins in the body of pregnant women. It is considered “a disease of primiparity”, as it is twice as common among women who are expecting their first child (Trupin, Simon, and Eskenazi 1996; Luo et al. 2007; Bellamy et al. 2007), and Chesley considered that primigravida women were 6 to 8 times more susceptible to develop PE than multigravida women (Chesley 1980).
Pathophysiology of preeclampsia

Although PE is one of the leading causes of maternal morbidity and preterm delivery worldwide, life threatening for both the mother and child, and despite extensive research into the pathogenesis of PE, its etiology remains unknown (Schlembach 2003). Recent evidence suggests there may be several underlying causes or predispositions leading to endothelial dysfunction and causing the signs of hypertension, proteinuria and edema. It is obvious that no single mechanism is responsible for this syndrome (Schlembach 2003; Powers et al. 2008; Quinton, Cook, and Peek 2007). PE is characterized by widespread dysfunction of the endothelium in the mother (Bellamy et al. 2007; Schlembach 2003; Stefanovic et al. 2009). This disease occurs exclusively in pregnant humans (Schlembach 2003).

During the past decade, numerous pathophysiologic abnormalities have been suggested to explain the mechanisms that lead to PE. Among these suggested mechanisms are impaired trophoblast differentiation and invasion, placental and endothelial dysfunction, immunological maladaptation to paternal antigens, and exaggerated systemic inflammatory response (Sibai, Dekker, and Kupferminc 2005; Roberts et al. 1989; Sankaralingam et al. 2006). Because the disorder is heterogeneous, the pathogenesis can differ in women with different risk factors. For example, the pathogenesis in nulliparous women may be different from that in women who have previously had preeclampsia, women with pre-existing vascular disease, pre-existing diabetes, or women carrying more than one fetus (Sibai, Dekker, and Kupferminc 2005).

The most popular theory describes the pathogenesis of preeclampsia as a two-stage process (Roberts 2000; Newstead, von Dadelszen, and Magee 2007). The first stage is the failure of maternal spiral arteries (uteroplacental arteries in pregnancy) to undergo normal remodeling, adequate for the fetal demand. In normal pregnancy the syncytiotrophoblast from the placenta invades the vascular luminal wall of uterine spiral arteries, resulting in these arteries losing their inner elastic lamina and vascular smooth muscle. Their diameter increases fourfold to create an intervillous blood supply which has high capacitance, low resistance and is unresponsive to vasoactive stimuli. These changes extend to the inner third of myometrium. In PE, especially early-onset PE, the vascular remodeling of maternal spiral arteries is limited to the superficial decidua, and the myometrial segments remain narrow and undilated (Newstead, von Dadelszen, and Magee 2007; Maynard et al. 2005) as illustrated in Figure 1.

This is one of many factors that may cause poor placentation. In the second stage, fetal demands exceed the uteroplacental supply, resulting in uteroplacental mismatch. When this happens, many products are released into the maternal circulation, causing maternal endothelial dysfunction, vasospasm, activation of the coagulation cascade, ultimately leading to all the end-organ complications that occur in PE (Newstead, von Dadelszen, and Magee 2007) as shown in Figure 2.
Figure 1: The vascular endothelium in normal pregnancy and preeclampsia.


Figure 2: Pathogenesis of the maternal syndrome of preeclampsia.

Early and late-onset preeclampsia

The degree of maternal hypertension, the amount of proteinuria, and the presence or absence of laboratory abnormalities are highly variable (ranging from mild to severe), with a variable time of onset. The manifestations of PE can develop before 34 weeks of gestation (early onset), at or after 34 weeks (late onset), during labor, or postpartum (Egbor et al. 2006). The concept of early and late PE is nowadays accepted. It has been suggested that these two entities have different etiologies (Huppertz 2008; Sibai 2008; Valensise et al. 2008; von Dadelszen, Magee, and Roberts 2003; Wikstrom et al. 2007). Early-onset preeclampsia (before 34 weeks) is commonly associated with abnormal uterine artery Doppler, fetal growth restriction and adverse maternal and neonatal outcomes (Valensise et al. 2008; Sibai 2008). Late-onset PE (after 34 weeks) is mostly associated with normal or slight increase uterine resistance index, a low rate of fetal involvement, and more favorable perinatal outcomes (Valensise et al. 2008; Sibai 2008). Early-onset PE is associated with a higher rate of maternal death (MacKay, Berg, and Atrash 2001), growth-restricted fetuses (Odegaard et al. 2000) and placental pathology (Moldenhauer et al. 2003; Sebire, Goldin, and Regan 2005) than late-onset preeclampsia.

It had been found that women with severe, very early onset PE, before 24 gestational weeks, had an increased risk of PE in future pregnancies, with about 50% recurrence rate (Gaugler-Senden et al. 2008), and similar rate was described by Sibai et al. (Sibai, Mercer, and Sarinoglu 1991). These women were also at increased risk of cardiovascular disease, because of hypertension, higher levels of lipoprotein(a) and microalbuminuria, which are well established independent risk factors of atherosclerotic disease (Gaugler-Senden et al. 2008; Bellamy et al. 2007; Irgens et al. 2001; Smith, Pell, and Walsh 2001).

As regards the health of children born to mothers who had PE, in addition to increased risk of intrauterine growth retardation and prematurity, the short outcome studies of newborns showed increased encephalopathy (Badawi et al. 1998; Impey et al. 2001), due to PE being an inflammatory condition, and increased occurrence of fetal cerebral vasoconstriction (Impey et al. 2001). Furthermore, there was a higher incidence of febrile seizures due to prematurity (Vestergaard et al. 2003), an increased risk of a variety of morbid conditions, such as endocrine, nutritional, and metabolic and diseases of the blood and blood forming organs (Wu et al. 2009), and an increased risk of epilepsy in children born after 37 weeks of gestation (Wu et al. 2009). The long-term outcome studies have reported a higher risk of childhood hypertension (Tenhola et al. 2006; Palti and Rothschild 1989) and increased risk of diabetes type 1 (Dahlquist, Patterson, and Soltesz 1999).

Risk factors for preeclampsia

The risk factors of PE are illustrated in Figure 3. Nulliparity is one risk factor for PE (Belo et al. 2008; Bellamy et al. 2007; Luo et al. 2007; Trupin, Simon, and Eskenazi 1996). Several of the risk factors for PE are similar to those for cardiovascular disease (with the exception of smoking). It is therefore suggested that some women who develop PE have pre-existing endothelial dysfunction (Lyall F 2007). They are vulnerable to even mild inflammatory stress or increased anti-angiogenic response. The source of this stress
Endothelium and pregnancy

The endothelium is the cell layer lining the internal surface of blood vessels and in a person weighing 70 kg, it covers an area of approximately 700 m² and weighs between 1 and 1.5 kg (Luscher and Barton 1997). The endothelium is responsible for an extensive array of highly specialized homeostatic functions. It plays an important role in control of blood pressure, blood flow, angiogenesis, coagulation, fibrinolysis, vessel patency, and local inflammatory responses. Under normal conditions the endothelium maintains a vasodilator, antithrombotic and anti-inflammatory state (Celemajer 1997). These functions are achieved through the release of endothelium-derived relaxing and contracting factors, thromboregulatory molecules, growth factors, and neutrophil adhesion molecules (Petty and Pearson 1989; Granger et al. 2001; Gilbert et al. 2008). Impaired endothelial function contributes substantially to cardiovascular disorders such as hypertension, atherosclerosis and PE. The vascular endothelium plays an important role in the cardiovascular adaptation to pregnancy and in the pathogenesis of PE (Lyall F 2007).

In normal pregnancy, endothelial function is improved; therefore a significant increase in brachial artery diameter and flow-mediated vasodilatation (FMD) during pregnancy has been found by many researchers (Dorup, Skajaa, and Sorensen 1999; FaberSwensson, O’Callaghan, and Walters 2004; Sierra-Laguado, Garcia, and Lopez-Jaramillo 2006; Saarelainen et al. 2009).
Role of endothelium in preeclampsia

PE is a multisystem disorder affecting virtually every organ and system in the body, with hypertension and proteinuria, the traditional diagnostic features, representing two phases of a complex pathophysiological process. The common pathological feature of the disease, whether in the decidual vessels of the placental bed, renal microvasculature, liver, heart or cerebral circulation, is vascular endothelial damage and dysfunction (Lyall and Greer 1996; Stefanovic et al. 2009; Ouyang et al. 2009; Newstead, von Dadelszen, and Magee 2007).

It has been suggested that factors released from the placenta in response to ischemia lead to endothelial dysfunction of the maternal circulation (Roberts, Taylor, and Goldfien 1991; Roberts et al. 1989; Sankaralingam et al. 2006). Evidence that endothelial dysfunction is an early event in PE suggests that it may be a cause, rather than a result, of the pregnancy-specific disorder. Additionally, in women who develop PE, pre-existing maternal factors such as chronic hypertension, diabetes, and hyperlipidemia may predispose the maternal endothelium to further damage (Roberts, Taylor, and Goldfien 1991; Roberts et al. 1989).

Many markers of endothelial dysfunction have been reported in women who develop PE, suggesting that PE is an endothelial cell disorder (Granger et al. 2001). An imbalance between pro and anticoagulatory forces is found in PE as increases in proteins of the coagulation cascade have been reported in women with PE (Granger et al. 2001). Plasma levels of cell adhesion molecules are also significantly elevated in women who develop PE. These include vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin (Granger et al. 2001). Elevations in ICAM-1 were detected as early as at 18 weeks gestation, thus suggesting that markers of endothelial dysfunction may serve as predictors of PE during pregnancy (Granger et al. 2001).

In summary, endothelial dysfunction may serve as a causative factor in PE and is not just a result of the disorder. Many markers of endothelial dysfunction may function as predictors of the syndrome, as many are significantly elevated weeks before clinical manifestations become apparent (Granger et al. 2001).

Role of inflammation in preeclampsia

Leukocytosis occurs during normal pregnancy (Belo et al. 2005; Rebelo et al. 1995; Pitkin and Witte 1979). Leukocytosis is considered to be evidence of an increased inflammatory response during normal pregnancy (Canzoneri et al. 2009; Sacks et al. 1998; Germain, Sacks et al. 2007), as the inflammatory marker C-reactive protein (CRP) rises during pregnancy compared to the non-pregnant state (von Versen-Hoeynck et al. 2009; Picklesimer et al. 2008; Watts et al. 1991), and is further elevated during labor (Watts et al. 1991). This mild systemic inflammatory activity as seen during normal pregnancy usually does not do any damage; on the contrary: it is beneficial as protection against infectious microorganisms.

Inflammation is thought to contribute significantly to the pathophysiology of PE, including endothelial and placental dysfunction (Redman and Sargent 2005). CRP is an important component of the innate immune system and is primarily produced by the liver as an acute phase reactive protein in response to inflammatory stimuli (Black, Kushner, and Samols 2004). Elevated serum CRP provides a sensitive biomarker of
chronic systemic inflammation, and is an independent predictor of future cardiovascular events (von Versen-Hoeynck et al. 2009; Ridker 2007; Black, Kushner, and Samols 2004). Moreover, elevations in CRP are both associated with and precede PE, although this has not been a consistent finding (Garcia et al. 2007; Paternoster et al. 2006; Teran, Escudero, and Calle 2005).

Pentraxin 3 (PTX3) belongs to the same family as C-reactive protein, and is expressed by endothelial cells, monocytes, macrophages, and fibroblasts exposed to inflammatory conditions (Garlanda et al. 2005). As an inflammatory marker, PTX3 is more sensitive than CRP. PTX3 blood levels are low (<2 ng/ml in humans) in normal conditions but during endotoxic shock, sepsis, and other inflammatory conditions they increase rapidly (peak at 6–8 h) and dramatically (200–800 ng/ml) (Garlanda et al. 2005). There is limited data on the role of PTX3 in normal pregnancy as well as in PE (Cetin et al. 2006; Rovere-Querini et al. 2006).

Role of angiogenic-antiangiogenic factors in preeclampsia

Angiogenesis is a process of growth of new capillaries from pre-existing microvasculature. Pregnancy induces angiogenesis, trophoblast invasion, and vascular remodeling and an optimal balance between angiogenic and antiangiogenic factors is necessary for a functional placenta (Levine et al. 2004). Vascular endothelial growth factor-A (VEGF-A) is important not only in angiogenesis but also in the maintenance of endothelial cell health in the basal state. Although the function of placental growth factor (PIGF) is still poorly defined, it appears to act synergistically with VEGF-A, and may be necessary for wound healing and angiogenesis in ischemic tissues (Maynard et al. 2005; Wikstrom et al. 2007; Ouyang et al. 2009).

There is evidence that an imbalance between substances that promote angiogenesis, such as VEGF-A and PIGF, and antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFlt-1, also known as a soluble VEGF receptor-1), is involved in the pathophysiology of PE (Wikstrom et al. 2008; Wolf et al. 2005; Levine et al. 2004; Goldman-Wohl and Yagel 2009; Maynard et al. 2005). The imbalance is evident from the poor placental vascularization that characterizes this disease (Lambert-Messerlian et al. 2009). The source of excess circulating sFlt-1 protein in PE is thought to be the placenta (Sela et al. 2008) since there is a dramatic fall in circulating levels of sFlt-1 after the delivery of the placenta (Maynard et al. 2003; Wikstrom et al. 2008), but previous studies show that sFlt-1 is also produced by cells outside the placenta such as endothelial cells (Hornig et al. 2000; Kendall, Wang, and Thomas 1996).

Binding of sFlt-1 to VEGF and PIGF deprives maternal vascular endothelium of essential angiogenic factors and causes systemic endothelial cell dysfunction (Maynard et al. 2003; Levine et al. 2004; Koga et al. 2003). Furthermore, the fact that sFlt-1 secretion is higher in first versus second pregnancies may account in part for the increased risk of PE among nulliparous women (Wolf et al. 2005). Other studies showed that first trimester serum levels of PIGF and sFlt1 may identify women at high risk of PE (Levine et al. 2004; Thadhani et al. 2004; Taylor et al. 2003). Elevated sFlt-1 levels and reduced PIGF levels can be observed several weeks prior to the development of PE, particularly PE developing at less than 27 weeks gestation, in high-risk women with previous PE or chronic hypertension (Sibai et al. 2008). In addition, increased sFlt1:PIGF ratios are suggested to predict the onset of PE (Levine et al. 2006; De Vivo et al. 2008).
These angiogenic–antiangiogenic factors may have a causal role in mediating the link between failure of trophoblast invasion and systemic maternal endothelial cell dysfunction. These proteins may therefore be a target for therapeutic interventions.

**Coagulation in preeclampsia**

Normal pregnancy is associated with major changes in blood coagulation and fibrinolysis (Arnout J. 2003; Bremme 2003). There is a more procoagulant state during pregnancy due to increased concentration of fibrinogen and coagulations factors V, VII, VIII, IX, X, XII and von Willebrand factor, whereas free and total protein S decreases (Arnout J. 2003; Bremme 2003). In addition, increased levels of fibrinogen and von Willebrand factor has been found in formerly preeclamptic patients 2-5 years after the index pregnancy (He et al. 1999).

During PE, coagulation is further activated compared to normal pregnancy, as reflected by increased thrombin generation, platelet activation, and fibrin deposition in renal and placental vasculature (VanWijk, Boer et al. 2002). It has been also found that patients with PE have a higher median plasma thrombin–antithrombin complex concentration than healthy pregnant women (Chaiworapongsa et al. 2002). The thrombin generation assay (TGA) has been described as a powerful and reliable test to screen for thrombophilic risk factors (Hezard et al. 2007).

It is important to emphasize that the notion of a direct correlation between PE and thrombophilia remains controversial and uncertain: prospective studies have not demonstrated a relationship between thrombophilia and obstetric complications (Silver and Warren 2006).

Microparticles (MP) are small, procoagulant membrane vesicles, which bud off from the cell surface and are released into the circulation during apoptosis or activation of blood cells or endothelial cells (VanWijk et al. 2003; Lok et al. 2008). It has been found that MP isolated from the blood of patients with myocardial infarction induce endothelial dysfunction in vitro (VanWijk et al. 2003). The contribution of MP to the development of PE, however, is still not completely understood. In preeclamptic patients, the numbers of granulocyte and lymphocyte-derived MP (VanWijk et al. 2003) and placenta-derived MP (Lok et al. 2008), were increased compared to normotensive controls, whereas the platelet-derived MP (Toth et al. 2007; Lok et al. 2008) and the total numbers of MP were reduced in preeclamptic patients (Lok et al. 2008). Six weeks postpartum after a preeclamptic pregnancy, MP numbers were similar to those of normotensive women (Lok et al. 2008). The reported vascular effect of MP in preeclamptic pregnancy is still unclear.

**Metabolic changes in preeclampsia**

Normal pregnancy produces profound metabolic alterations. Hyperinsulinemia and hyperlipidemia serve to support the metabolic needs of the conceptus (Carpenter 1993; Villa et al. 2009). A state of insulin resistance has also been recognized (McIntyre et al. 2009). Insulin sensitivity, estimated by hyperinsulinemic-euglycemic clamp technique, decreases during normal pregnancy (Catalano et al. 1991). The causal mechanism underlying this insulin resistance remains unclear although many hormonal changes have been described, such as increased levels of human placental lactogen, placental growth hormone, cortisol, estrogens, progesterone and prolactin (McIntyre et al. 2009).
PE has many characteristics in common with the metabolic syndrome. The metabolic changes that occur in normal pregnancy are exaggerated in PE (Villa et al. 2009; Stefanovic et al. 2009). Women with the metabolic syndrome have higher risk of cardiovascular disease (Hannaford, Ferry, and Hirsch 1997; Irgens et al. 2001; Smith, Pell, and Walsh 2001). It has been found that pre-pregnancy weight is strongly predictive of the risk of developing PE (Sibai et al. 1995).

A clear inverse relationship between glucose level and FMD is described, particularly in healthy subjects with “high normal” glycemia (Thomas et al. 2004) and the degree of glycemia correlates positively with increased cardiovascular risk (Sasso et al. 2004). There are some indications that insulin resistance may play a role in the pathophysiology of hypertension in preeclamptic patients (Pouta et al. 2004; Negrato et al. 2009; Stefanovic et al. 2009; Wolf et al. 2002). In addition, increased insulin resistance early in pregnancy might independently be associated with subsequent PE (Wolf et al. 2002). Lipoprotein abnormalities have been shown to be involved in the pathogenesis of PE and proatherogenic lipid profiles have been demonstrated in women months before they showed clinical signs of PE (Baker et al. 2009; Hubel et al. 2000).

All of these proatherogenic changes in the lipid profile are also found in cardiovascular disease and in patients at high risk for coronary artery disease.

Cardiac changes during preeclampsia

Normal pregnancy

The maternal cardiovascular system undergoes profound physiological changes during pregnancy; the aim is to ensure that the mother can meet the metabolic demands of the growing conceptus (Lyall F 2007).

In normal pregnancy the fall in the peripheral vascular resistance, evident as early as at 5 weeks of gestation, is almost completed by 16 weeks, at the end of the first trimester. It falls by 40% compared with non-pregnant women (Robson et al. 1989; Andrietti et al. 2008; Chapman et al. 1998). By 24 weeks of gestation, cardiac output increases to 45% above non-pregnant levels due to greater stroke volume, higher heart rate and decreased peripheral vascular resistance (Robson et al. 1989; Andrietti et al. 2008; Bamfo et al. 2008; Simmons, Gillin, and Jeremy 2002; Chapman et al. 1998). Blood volume increases gradually over gestation reaching a 40% increase by term (Thornburg et al. 2000). Maternal blood pressure falls and reaches a nadir at around 20 weeks of gestation and progressively increases thereafter (Robson et al. 1989; Chapman et al. 1998). During pregnancy, the heart undergoes remodeling similar to that observed in athletes with increases in chamber dimensions and left ventricular (LV) wall thickness (Thornburg et al. 2000; Simmons, Gillin, and Jeremy 2002). These changes are summarized in Figure 4.

<table>
<thead>
<tr>
<th></th>
<th>Normal pregnancy</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Systemic vascular resistance</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>←→</td>
<td>↑↑</td>
</tr>
<tr>
<td>Intravascular volume</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Left ventricular mass</td>
<td>↑</td>
<td>↑↑</td>
</tr>
</tbody>
</table>

Figure 4. Hemodynamic changes in normal and preeclamptic pregnancy
**Preeclampsia**

During the preclinical phase, maternal left ventricular (LV) systolic function is increased and peripheral resistance is decreased. However, with the onset of clinical symptoms cardiac output falls and peripheral resistance rises (Bamfo et al. 2008; De Paco et al. 2008). Placentalation may directly or indirectly precipitate these events and there is some evidence for an association between maternal hemodynamic abnormalities and impaired placentalization, with consequent development of PE and intrauterine growth restriction (Bamfo et al. 2008).

Lang’s study showed that myocardial contractility is normal in PE: serial comparisons from before delivery until 4 weeks after delivery showed no changes in myocardial contractility. Furthermore, it was demonstrated that the decrease in overall left ventricular performance noted in patients with pregnancy-induced hypertension reflects a mechanically appropriate response to increased after-load rather than a cardiomyopathic state (Lang et al. 1991). The data describing the ventricular diastolic function in PE are contradictory (Simmons, Gillin, and Jeremy 2002; Bamfo et al. 2008).

Chronic systemic hypertension exposes the heart to long-lasting pressure overload and induces important functional changes in the left ventricle (LV) and left atrium (LA): LA reservoir function increases and LA conduit function decreases, while LA ejection force increases (Ingec, Yilmaz, and Gundogdu 2005). However, the effect of the acute pressure overload associated with PE on the LA function is not well known. Ingec et al. found that the mechanical function of LA did not change and concluded that short-lasting pressure overload is not capable of inducing changes in LA (Ingec, Yilmaz, and Gundogdu 2005).

Considering the severity of preeclampsia’s potential consequences, it is surprising that only limited information exists regarding its hemodynamic characteristics. Even more disturbing is the fact that the studies often draw completely different conclusions, classifying PE as anything from a vasoconstrictory hypo-perfusion disorder to a low peripheral resistance, high cardiac output state (Lang et al. 1991; De Paco et al. 2008; Bosio et al. 1999). These contradictory conclusions might be explained by the patients included in the various studies having different severity and duration of the disease and different clinical treatments (Bosio et al. 1999).

**Biomarkers of cardiac function**

Natriuretic peptide (BNP) is produced predominantly in the ventricular myocardium in response to cardiomyocyte stretch to counterbalance volume or pressure overload (Eggers et al. 2009). BNP is elevated in different cardiovascular pathologies, including left ventricular hypertrophy, arrhythmias, coronary disease, and heart failure (Chong et al. 2004; Abdullah et al. 2005; Campbell 2008). Studies have provided data that plasma BNP, even in the absence of heart failure, has a prognostic value for future cardiovascular events (McKie et al. 2006). In addition, each 1 SD increase in log BNP levels, may increase the risk of death, heart failure, atrial fibrillation, stroke or transient ischemic attack (Wang et al. 2004). Myocardial damage is associated with elevated levels of troponins (Ammann et al. 2004; Eggers et al. 2008). The importance of troponin measurements in PE remains controversial (Joyal et al. 2007; Fleming et al. 2000).
Preeclampsia and future cardiovascular risk

Many studies have found that women with a history of PE have a 2- to 4-fold increased risk of developing hypertension, coronary artery disease, or stroke and venous thromboembolism up to 14 years after the index pregnancy (Bellamy et al. 2007; Ness and Hubel 2005; Sibai, el-Nazer, and Gonzalez-Ruiz 1986). Among women in their fifties, the risk of hypertension 25 years after pregnancy has been shown to be considerably higher (48.5%) for those who had preeclamptic pregnancies than for those who had normotensive pregnancies (22%) (Diehl et al. 2008).

Cardiovascular disease (CVD) is the cause of death for just over 50% of women worldwide (Newstead, von Dadelszen, and Magee 2007). Most deaths are due to coronary heart disease (23%), and stroke (18%) (Newstead, von Dadelszen, and Magee 2007). Nearly two thirds of women who die suddenly of coronary heart disease have no previously recognized CVD.

Epidemiological data indicate that women with PE are more likely to develop CVD later in life (Harskamp and Zeeman 2007; Hjartardottir et al. 2004; Jonsdottir et al. 1995). The increase in mortality from CVD generally becomes evident 2 to 3 decades after pregnancy in women with a history of non-recurring PE, substantially later than women who have had PE more than once (Ileakis, Reddy, and Roberts 2007).

Pregnancy acts as a stress test for the mother. During pregnancy, almost every organ of the mother’s body has to work harder in order to meet the demands of the developing fetus. Syndromes related to pregnancy, so called “gestational syndromes”, develop when an organ system is unable to meet the increased physiological demands of pregnancy. In general these demands become greater as pregnancy progresses and therefore gestational syndromes are more common in the third trimester. Delivery induces remission, but it is only transient. Some or all of the components of the clinical syndrome reappear in later life when the cumulative effects of ageing diminish the reserves of an already vulnerable organ (system), or the age-related return to a pro-atherogenic state triggers the metabolic syndrome that leads to cardiovascular disease and diabetes mellitus (Williams 2003).

It may be the case that PE results from an increased susceptibility to metabolic stress (Dunne et al. 2003) or alternatively, PE may itself induce long-term metabolic and vascular abnormalities that increase overall risk for CVD later in life (Craici, Wagner, and Garovic 2008). If the underlying maternal predisposition to vascular disease is present and the metabolic stress of pregnancy causes this predisposition to vascular disease to be manifested as PE during pregnancy, then after delivery, when the metabolic stress disappears, the women return to a normotensive state. But with time this metabolic stress again becomes manifest as CVD later in life (Newstead, von Dadelszen, and Magee 2007; Sattar and Greer 2002) (Figure 5). Therefore, it seems that women who have a predisposition for the metabolic syndrome are more likely to develop PE during pregnancy and more likely to develop hypertension, obesity, atherosclerosis, and diabetes mellitus type 2 later in life, which eventually results in CVD (Williams 2003).
The mechanism underlying the long-term effects of PE is complex and probably multifactorial, including features of the metabolic syndrome (including hyperinsulinemia, dyslipidemia and obesity) (Kaaja 1998), inflammatory response, a hypercoagulable state, in addition to endothelial dysfunction (Craici, Wagner, and Garovic 2008; Kaaja 1998; Ilekis, Reddy, and Roberts 2007).
THE GENERAL AIM OF THE STUDY

The general aim of the thesis was to study the changes in the cardiovascular function in women with preeclampsia during pregnancy and during early and late postpartum.

The specific aims were:

- To assess the changes in vascular endothelial function during the onset of clinical symptoms of preeclampsia, and during a follow-up period lasting up to one year after delivery, in otherwise healthy women.

- To assess the changes in cardiac morphology and function at onset of clinical symptoms of preeclampsia and after delivery, in women without any previous cardiac disease.

- To evaluate the ambulatory blood pressure and thrombotic state one year after preeclampsia in previously healthy women.

- To compare the endothelial function and the thrombotic state during the follicular and luteal phases of menstruation one year after preeclampsia.

- To measure cardiac, inflammatory, antiangiogenic, endothelial and metabolic biomarkers during onset of clinical symptoms of preeclampsia and up to one year after delivery.
MATERIAL AND METHODS

All women included in this study were non-smoking primipara and were admitted to the Department of Obstetrics at Karolinska University Hospital Solna, Stockholm (Figure 6). The diagnosis of PE was made according to the recommendations of the International Society for Study of Hypertension in Pregnancy (Davey and MacGillivray 1988). The control group was recruited from healthy women with normal pregnancy who delivered during the same time period. The local ethics committee approved the study protocol, and all women gave informed consent to their participation.

In study I and II the participants were examined one year after delivery. Participants were identified through retrospective review of the records of all deliveries at the Department of Obstetrics over a two-year period. The total number of deliveries during this period was 10981 and the incidence of PE was 4.4% (n = 483) and for severe PE approximately 1%. The women included in our preeclamptic group had a history of PE, but otherwise were healthy, non-smokers and young primipara who had normal blood pressure, normal menstrual cycles, no hormonal therapy for 6 months before the study, no other drug treatment, and breast-feeding was terminated at the time of the examination. In addition, all other diseases such as diabetes mellitus, gestational diabetes, coagulation disorders, renal diseases, and chronic hypertension were excluded. Given these strict criteria we were able to include 18 women.

The control group consisted of healthy women with a record of normal, uncomplicated pregnancies, recruited from the same register as the preeclamptic group. Each woman in the preeclamptic group was matched by age and parity and as much as possible to the same date of delivery. The mean time from the index pregnancy to inclusion in both groups was 15±3 months. The clinical characteristics are shown in Table 1.

Figure 6. Distribution of participants in study I-IV
Preeclamptic patients consisted of those who developed early-onset and severe PE, necessitating preterm delivery < 34 gestational weeks (8/18; 44%); and those who developed PE and delivered at ≥ 34 gestational weeks, late PE subgroup (10/18; 56%). Eight of the women in the late PE subgroup had severe PE during the index pregnancy and two had mild PE.

Table 1. Clinical characteristics of women with a history of preeclampsia and healthy controls during pregnancy and one year after delivery.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>PE</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>During pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67±10</td>
<td>65±9</td>
<td>0.51</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25±4</td>
<td>23±3</td>
<td>0.14</td>
</tr>
<tr>
<td>SBP (12 weeks) (mmHg)</td>
<td>114±9</td>
<td>115±11</td>
<td>0.91</td>
</tr>
<tr>
<td>DBP (12 weeks) mmHg</td>
<td>67±9</td>
<td>69±7</td>
<td>0.72</td>
</tr>
<tr>
<td>Smoker</td>
<td>6%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Random glucose (mmol/L)</td>
<td>4.4 ±0.62</td>
<td>5 ±0.75</td>
<td>0.50</td>
</tr>
<tr>
<td>Previous history of hypertension or CAD</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of hypertension</td>
<td>6%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2088 ±945</td>
<td>3634 ±581</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>1 year after delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>30±4</td>
<td>31±4</td>
<td>0.63</td>
</tr>
<tr>
<td>Parity</td>
<td>1</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Oral contraceptive pills</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Regular menstruation</td>
<td>18</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>Lactation</td>
<td>0%</td>
<td>0%</td>
<td>NS</td>
</tr>
<tr>
<td>Time pp (months)</td>
<td>15±3</td>
<td>15±3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70±1</td>
<td>64±8</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 ±5</td>
<td>22 ± 3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>81±12</td>
<td>74 ±7</td>
<td>0.06</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.82 ± 0.05</td>
<td>0.83 ± 0.04</td>
<td>0.49</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>111 ±10</td>
<td>103 ±8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>74 ±9</td>
<td>65 ±5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

All values in Mean ±SD.
BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, CAD: coronary artery disease, pp: postpartum. NS: non significant.

In study III and IV the women were included during pregnancy and followed 3-6 months after delivery. The pregnant women, who were admitted to the Department of Obstetrics at the Karolinska University Hospital, Stockholm, Sweden from October 2004 to November 2007, were screened for participation. The total number of deliveries during that period was 14258 deliveries, about 5000 deliveries/year. Four hundred and eleven pregnant women with diagnosis of PE delivered at our clinic, corresponding to approximately 3% of all deliveries. The inclusion criteria for these studies were non-smoking nullipara with spontaneous, single pregnancy complicated with untreated newly developed PE. One hundred and seventy of the 411 fulfilled the inclusion criteria. Of these 135 were excluded based on the following exclusion criteria: unable to undergo echocardiography (n=39) due to clinical instability, on antihypertensive drugs (n=16), unwilling to participate (n=20), logistic reasons (n=26), chronic disease
(n=14), smokers (n=5), pregnancy by in vitro fertilization (n=2) or egg donation (n=3), multiple pregnancies (n=8) or extreme obesity (n=2). Given this we were able to include 35 women as PE group. The control group (n=30) was recruited from healthy women with normal pregnancy, who delivered during the same time period. The control group matched the preeclamptic group with regard to age, parity and no-smoking habits. The clinical characteristics are shown in Table 2. Preeclamptic patients were divided into two subgroups 1) those who developed early-onset and severe PE, necessitating preterm delivery < 34 gestational weeks (8/35; 23%); and 2) those who developed PE and delivered at ≥ 34 gestational weeks, late PE subgroup (27/35; 77%). The women in the PE group had severe PE, 13 had organ involvement, 2 had HELLP, one developed eclampsia and only 7 women had mild PE in the late subgroup.

Table 2. Clinical characteristic of the study groups at inclusion and follow-up.

<table>
<thead>
<tr>
<th></th>
<th>PE (n=35)</th>
<th>Control (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At 12 weeks of gestation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>31 (5)</td>
<td>31 (4)</td>
<td>0.99</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82 (22)</td>
<td>73 (17)</td>
<td>0.074</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166 (8)</td>
<td>167 (7)</td>
<td>0.422</td>
</tr>
<tr>
<td>Family history of hypertension (%)</td>
<td>14%</td>
<td>16%</td>
<td>0.821</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>119 (2)</td>
<td>117 (2)</td>
<td>0.284</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>72 (2)</td>
<td>68 (1)</td>
<td>0.086</td>
</tr>
<tr>
<td><strong>At inclusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>35 (4)</td>
<td>33 (4)</td>
<td>0.573</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>2.01 (0.04)</td>
<td>1.90 (0.04)</td>
<td>0.083</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>154 (2)</td>
<td>117 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>99 (2)</td>
<td>76 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>118 (2)</td>
<td>90 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery at gestational age (week)</td>
<td>36 (4)</td>
<td>40 (2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2563 (979)</td>
<td>3293 (411)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.89 (0.04)</td>
<td>1.80 (0.04)</td>
<td>0.0897</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>121 (2)</td>
<td>112 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>77 (2)</td>
<td>73 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>92 (2)</td>
<td>86 (2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The values are in Mean (SE) and percentage. BSA: Body surface area, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure.

Endothelial function

Brachial artery flow velocity, endothelium-dependent (FMD) and endothelium-independent glycerin trinitrate (GTN)-induced dilatation were examined according to Celermajer et al. (Celermajer et al. 1992). The measurements were made non-invasively using a high-resolution ultrasound scanner (Acuson 128 XP/10c; Siemens, Mountain View, California, USA) with a 7-MHz linear array transducer, as shown in Figure 7 and described (Lundman et al. 2001). To minimize variability we used a special clamp to keep
the probe in the same position during the investigation, and all studies were performed
by one experienced operator. All analyses of the ultrasound images and measurements of
brachial artery diameters were performed manually off-line by one investigator who was
unaware of the case/control status (study I and II).

Figure 7. Measurement of flow-mediated vasodilatation using the non-invasive ultrasound
technology.
The brachial artery was scanned longitudinally 1-10 cm above the elbow. Reactive hyperemia
was obtained by forearm artery occlusion with 12.5 cm blood pressure cuff inflation (A). The
diameter of the brachial artery was measured at rest, at maximal dilatation after hyperemia and
after administration of 0.4 mg sublingual nitroglycerin (GTN) (B).

Four consecutive late diastolic frames, taken co-incidentally with the R-wave on the elec-
trocardiogram, were analyzed at rest (baseline) and subsequent to different provocations.
The average diameter of the four frames was calculated. Blood flow was calculated from
Doppler velocity, vessel diameter and heart rate (HR). The increase in blood flow following
reactive hyperemia is presented as percentage of basal flow values. The within-individual
variations in our laboratory between two determinations of FMD performed during the
same day and between determinations made on separate days are 0.88 ± 0.82% and
3.3 ± 2.7%, respectively (Lundman et al. 2001).
In study IV no GTN was administered and the FMD was calculated by automatic analysis of
the sequence of the ultrasound scans by dedicated off-line software as shown in Figure 8.
Figure 8. The ultrasound images and automatical measurements of the diameters of the brachial artery for the calculation of FMD.

Ambulatory blood pressure measurement (ABPM)

Participants were equipped with an automatic BP device (Space Lab, Redmond, WA) for a 24-hour ABPM. Readings were made every 20 minutes between 06.00 h and 21.00 h, every 30 minutes between 21.00 h and 23.00 h, and hourly from 23.00 h to 06.00 h. Cuffs of appropriate sizes were used. The left arm was used except when the subject was left-handed. The reading and editing of data provided by the recorder were analyzed. ABPM values were calculated as mean BP/24-hours, mean systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and HR daytime (07.00-22.00 h) and nighttime (22.00 h-07.00 h), maximum and minimum BP and percentage of recordings >140/90 mmHg during day and night. BP was considered abnormal if it exceeded 140/90 mm Hg during daytime and 125/75 mmHg at night (O’Brien et al. 2003). According to European guidelines “high normal” BP is considered below 140/90 mmHg (Tanne 2004).

Thrombin generation assay

The thrombin generation assay (TGA) was performed according to the modification of Hemker’s method (Hemker et al. 2006) and as described by Varadi et al. (Varadi et al. 2003) (Figure 9). Ten microliters of a tissue factor phospholipid solution (TF/PL) (Technoclone, Vienna, Austria) was added to 50 µL of 1 mM thrombin peptide substrate Z-Gly-Gly-arg-amc (Bachem, Budendorf, Switzerland) and calcium 15 mM CaCl₂ giving the final concentration of 1.79 pM for TF and 0.32 µM for PL in the reaction. The reaction was started by addition of patient plasma sample or control. Forty microliters of patient or control plasma was added to 50 µL substrate and 10 µL TFPL. Dilution factor of plasma in the reaction is 2.5. The continuous splitting of the peptides substrate, which releases a fluorophore, was monitored every minute for 90 minutes in a BIO-
Rangeen Rafik Hamad

TEK FLx800 microplate fluorescence reader. Thrombin generation was expressed in RFU (relative fluorescence units) and converted to thrombin concentrations (nM) using a reference curve prepared by purified thrombin. Characteristic parameters (lag phase, thrombin max, thrombin potential at 60 minutes, max-slope, peak time), describing thrombin formation calculated from the assay are outlined in Figure 9. The inter-assay coefficient of variation for the positive control is between 6 and 8.7% and the intra-assay coefficient of variation is between 1 and 6.2%.

**Figure 9. Parameters used in thrombin generation test.**
The parameters of thrombogram: A-Lag phase (min). B-Thrombin max (nM). C-Thrombin potential (RFU) (= area under the curve)(nM xmin). D-Max-slope (RFU/min). E-Peak time (min).
(Modified with permission from Hemker et al. 2006)

**Microparticles (MP)**

Mouse antibodies against human CD61 (FITC) and CD62E (PE) were from BD Biosciences (CA, USA). Mouse anti-human CD45 (FITC) was from Myltenyi Biotec (Utrecht, NL) and Glycophorin A-PE was from Immunotech (via Beckman Coulter). Annexin A5 (FITC conjugated) was from VPS Diagnostics (Nexins Research, The Hoeven, NL) and FITC conjugated anti-TF was purchased from Sanquin Reagents (Amsterdam, NL). Isotypic control IgG (FITC or PE) was from BD Biosciences (CA, USA). Flow count beads were from Beckman Coulter (Mijdrecht, NL).

**Analysis of microparticles**

MP samples were measured essentially as described by Keuren et al (Keuren et al. 2006). Cellular origin was determined via binding of specific antibodies against platelets (CD61, 1:50 dilution), leukocytes (CD45, 1:50 dilution), erythrocytes (glycophorin A, 1:25 dilution) and endothelial cells (CD62E, 1:50 dilution). For MP surface characterization we used annexin A5 (1 µg/mL in the presence of 3 mM CaCl₂) and monoclonal antibodies against TF (10 µg/mL). Before analysis the mixtures were incubated for 15 min in the dark at 37 °C. Samples were analyzed in a Coulter Epics XL flowcytometer (Beckman Coulter, Mijdrecht, Netherlands). Forward (FSC) and side scatter (SSC) were set at logarithmic gain and triggering was set at FSC. Fluorescence thresholds for monoclonal antibodies were set in terms of binding of isotype-matched control antibody (IgG). Fluorescence threshold for annexin A5 was set in terms of binding of annexin A5 in
filtered HEPES buffer containing 0.1 mmol/L EDTA. The percentage of positive MP was estimated by placing a marker at the upper edge of the control fluorescence distribution so as to include 95% of the counts as negative for the specific dye, annexin A5 or antibody binding.

To determine MP concentration, flow count beads were added in a 1:10 dilution to the sample. Flow count beads (10 µm) fall in a size gate different from the MP and were identified by fluorescence measured in the FL-4 channel. MP concentration was calculated with the following equation:

\[ \text{microparticle concentration} = \frac{\text{bead concentration} \times (\text{microparticle count}/\text{bead count})}{(\text{microparticle count}/\text{bead count})} \]  

(Keuren et al. 2006).

The intra-assay and inter-assay variation of the MP-assay are 10 and 8% respectively. These have been measured as intra-day assay variation and inter-day assay variation. The samples in the study II were measured on 5 consecutive days.

**Echocardiography**

*Two-dimensional Doppler echocardiography*

All ultrasound examinations were performed with a Vivid 7 ultrasound equipment (General Electric, Horten, Norway) equipped with Doppler tissue imaging (DTI) capabilities. The two-dimensional, M-mode and Doppler echocardiography according to the guidelines of the American Society of Echocardiography were acquired and stored digitally. Standard echocardiographic measurements included LV end-diastolic dimensions (LVDD), end-diastolic wall thickness of the interventricular septum (IVSd) and left ventricular posterior wall (PWTd). The fractional shortening (FS) was calculated from M-mode recordings (Sahn et al. 1978). The LV mass was calculated according to the Penn formula (Devereux et al. 1986). The LV mass index (LVMI) was calculated by the indexation of LV mass by height\(^2.7\) (de Simone et al. 1992). The relative wall thickness (RWT) was calculated according to the formula RWT= (IVS + PWT)/LVDD, to classify the LV hypertrophy (LVH) and geometric pattern (concentric LVH, RWT >0.45; eccentric LVH, RWT <0.45). Transmitral, pulmonary venous flow velocities were acquired with pulse Doppler. The velocities of early transmitral diastolic flow velocity (E) and flow velocity during atrial contraction (A), its ratio (E/A), and deceleration time and isovolumic relaxation time (IVRT) were measured. Left atrial end-systolic diameter (LADes) was measured in a parasternal long-axis view and the maximal LA area (LAArea es) was traced at end-systole in a four-chamber view.

*Doppler tissue imaging*

After completion of the conventional echocardiography, pulsed Doppler DTI was recorded in the apical four-chamber view at the septal and lateral part of the mitral annulus. A 3-mm sampling volume was used. Early diastolic myocardial velocity (E) of the DTI recorded Doppler signal was used to calculate E/E' ratio as an estimate of the LV filling pressure (Figure 10).
Biochemical analysis

Study I

The major fasting plasma lipoproteins: very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) were determined by a combination of preparative ultracentrifugation and the precipitation of apolipoprotein B-containing lipoproteins followed by lipid analysis (Carlson 1973). Fibrinogen was determined as described by Claus (Clauss 1957), with reagents from Instrumentation Laboratory (Lexington, Massachusetts, USA). Lipoprotein (a), plasminogen activator inhibitor 1 and tissue plasminogen activator were determined with kits from Biopool (Umea, Sweden). Von Willebrand factor antigen was measured with an enzyme-linked

Figure 10. Example of pulsed Doppler recordings of mitral inflow and pulsed Doppler tissue imaging at inclusion and at follow-up in a patient with preeclampsia.

E – flow velocity during early filling, A – flow velocity during atrial contraction, É – tissue velocity during early filling and À – during atrial contraction at septal and at lateral mitral annulus
immunosorbent assay (ELISA) using antibodies from Dako A/S (Glostrup, Denmark). Activated factor VII concentration was determined in a clotting assay using soluble recombinant truncated tissue factor (Clauss 1957). Intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 and soluble (s) E-selectin were measured using ELISA from R&D Systems Inc. (Minneapolis, Minnesota, USA), and insulin and intact proinsulin were determined using ELISA from Dako (Cambridgeshire, UK). C-reactive protein was determined by particle-enhanced immunonephelometry with high sensitivity using a Behring Nephelometer II (Dade Behring, Marburg, Germany). C-peptide, insulin-like growth factor I (IGF-I), insulin-like growth factor binding protein (IGFBP) 1 and IGFBP 3 were measured by an Immulite Analyzer (Diagnostic Products Corp., Flanders, New Jersey, USA). Free IGF-I was measured using an ELISA (Diagnostic Systems Laboratories Inc., Webster, Texas, USA). The degree of insulin resistance was estimated by homeostasis model assessment (HOMA= fasting glucose/insulin/22.5) (Matthews et al. 1985; Bonora et al. 2000).

Study III
C-reactive protein (reagent: 6K2601, Abbott Laboratories, Abbott Park, IL, USA), cystatin C (reagent: 1014, Gentian, Moss, Norway) and troponin I (reagent: 2K41, Abbott Laboratories) were analyzed on an Architect Ci8200 analyzer (Abbott Laboratories). The cystatin C calculated glomerular filtration rate (GFR) in mL/min/1.73 m² was calculated according to \[= 79.901 \times (\text{cystatin C mg/L})^{-1.4389}\] as described by Flodin and co-workers (Flodin et al. 2007). The reference values were: <5 mg/L for CRP, <1.20 mg/L for cystatin C, >75 mL/min/1.73 m² for GFR and <0.022 µg/L for troponin I. NT-pro-BNP was analyzed on a Modular E 170 (Roche Diagnostics, Mannheim, Germany), the reference value in women <50 years of age is <150 ng/L. The total coefficient of variation (CV) of the assays was 0.8% at 8 mg/L for CRP, 1.1% at 1.25 mg/L for cystatin C, 4.6% at for troponin I at 0.26µg/L and 2.2% at 262 ng/L for NT-pro-BNP. The limit of detection for the CRP method was 0.2 mg/L. The assays were performed at the Department of Clinical Chemistry and Pharmacology, Uppsala University Hospital, Uppsala. The laboratory participates in the external quality assurance program for plasma proteins organized by the Swedish external quality assurance organization Equalis (Uppsala, Sweden).

Study IV
Samples were analyzed using commercially available ELISA kits (placental growth factor PlGF, DY264; vascular adhesion molecule 1 VCAM-1/CD106, DY809; intercellular adhesion molecule ICAM, DY720; vascular endothelial growth factor VEGF-A, DY293B; Tumor necrosis factor receptor 1, 2 TNFRI/TNFRSF1A, DY225; TNFRII/ TNFRSF1B, DY726; Pentraxin3/TSG-14, DY1826; soluble fms-like tyrosine kinase-1 (sFlit-1)/Flt-1, DY321, R&D Systems, Minneapolis, MN). The assays had inter-assay coefficient of variation (CV) of approximately 7% or less.
Statistical analysis

Results are presented as mean and standard deviation (SD) for normally distributed variables and as median and interquartile range (P25-P75) for skewed variables. Group comparisons were performed with Student’s t-test, the Mann-Whitney U test, the chi-square test or Fisher’s exact test, as appropriate (study I and II). In study III and IV, a two-way repeated measures analysis of variance (ANOVA) using Procedure Mixed in SAS® (System 9.1, SAS Institute Inc., Cary, NC, USA) was performed to establish whether there were any differences in variables between pregnancy and postpartum. Time and Group were related to the main effects. Time was related to the difference between pregnancy and follow-up across the two groups and Group was related to the difference between the groups across time. When the two-factor interaction, Group*Time, was significant, simple main effects tests were done, i.e. examination of the effects of one factor while the other factor is held fixed. In the ANOVA model we took into consideration the between-subject heterogeneity of variances as well as patients with missing data. Correlations were assessed by calculation of Pearson correlation coefficients or Spearman rank correlation coefficients. Analysis of covariance was used to adjust for effects of prognostic factors. A p value < 0.05 was considered to be statistically significant.
RESULTS

Study I

Endothelial function
The ultrasound examination of the brachial artery showed diminished FMD% in the preeclamptic group: 2.5 ± 2.9 compared with the controls: 10.3 ± 2.0 (p < 0.0001) and glyceryl trinitrate-induced vasodilatation (GTN%) in preeclamptic group: 19.8 ± 5.5 compared to control 25.8 ± 6.5 (p < 0.001), as shown in Figure 11.

![Figure 11. Flow-mediated-dilatation (FMD) and glyceryl trinitrate-induced vasodilatation (GTN) in women with a history of preeclampsia (FMD-PE, GTN-PE) and controls (FMD-CTRL, GTN-CTRL) one year after delivery.](image)

Ambulatory blood pressure measurements
The 24-hour ABPM showed higher day values of systolic (SBP) and diastolic blood pressure (DBP) as well as mean arterial blood pressure in the preeclamptic group, as shown in Table 3.
Maximal systolic blood pressure measured during the index pregnancy correlated inversely with FMD measured one year after pregnancy in the preeclamptic group (r = −0.470; p < 0.05). Women with FMD less than 2% at follow-up all delivered a child who was small for gestational age.
Table 3. 24-hour ambulatory blood pressure measurement in women with a history of preeclampsia (PE) and in controls one year after delivery.

<table>
<thead>
<tr>
<th></th>
<th>PE (n = 18)</th>
<th>Control (n = 17)</th>
<th>Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day readings (mean values)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123 ± 9</td>
<td>116 ± 9</td>
<td>+1.27; +13.93 #</td>
<td>&lt;0.05 #</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>81 ± 6</td>
<td>76 ± 7</td>
<td>+1.19; +10.29 #</td>
<td>&lt;0.05 #</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>95 ± 6</td>
<td>90 ± 7</td>
<td>+1.29; +10.55 #</td>
<td>&lt;0.05 #</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>82 ± 9</td>
<td>77 ± 8</td>
<td>-0.75; +10.59</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Night readings (mean values)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>105 ± 9</td>
<td>99 ± 12</td>
<td>-1.71; +13.11</td>
<td>0.13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>66 ± 7</td>
<td>62 ± 8</td>
<td>-1.45; +9.47</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>79 ± 6</td>
<td>75 ± 9</td>
<td>-1.22; +9.70</td>
<td>0.12</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>67 ± 9</td>
<td>64 ± 7</td>
<td>-2.53; +9.04</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.
# Adjusted for BMI.

**Biochemical analyses**
The degree of insulin resistance was estimated by homeostasis model assessment (HOMA). Insulin resistance was calculated to 1.3 (1.1 – 2.1) {median (interquartile range)}, in the PE group and 1.0 (0.7 – 1.3) in the healthy control group (p <0.01) and when adjusted for BMI was still significant (p< 0.05). There were no significant differences in other metabolic markers: cholesterol, triglycerides, LDL, HDL, VLDL, lipoprotein, proinsulin, IGF-1, IGFBP-1, IGFBP-3, free IGF-1, or C-peptide. We investigated biochemical markers of endothelial activation (ICAM-1, VCAM-1, E-selectin), inflammation (hs-CRP), and hemostatic function (fibrinogen, plasminogen activator inhibitor 1, tissue plasminogen activator, v WF, activated factor VII), but no significant differences were found between the groups.

**Study II**

**Endothelial function at different menstrual phases**
In formerly preeclamptic subjects, FMD (%) was decreased in the luteal phase, 2 (0.9─4), compared to controls 10 (9─11) (p<0.0001); GTN (%) was also decreased, 21 (18─23) compared to the controls, 27 (23─32) (p<0.001), but the difference in FMD and GTN between follicular and luteal phase was not significant (p=0.98 and p=0.20, respectively).

**Thrombin generation assay and Microparticles**
The women with previous PE produced a higher total amount of thrombin as calculated from max-slope, thrombin max and thrombin potential (p= 0.01, <0.05 and 0.01) as shown in Table 4. Analysis of covariance (ANCOVA) showed that blood pressure did not explain these increased levels of thrombin in women with PE (p=0.40). Platelet derived MP, (CD61+MP), tended to be higher in the PE (p=0.07) and it was well correlated with thrombin max in the preeclamptic group (r ≥ 0.45, p=0.006). In addition, CD 61+MP was correlated with the maximum diastolic blood pressure measured during the index
Results

pregnancy in the preeclamptic group ($r = 0.48$, $p=0.05$). There was no detectable variation in levels of thrombin and microparticles during the menstrual phases.

Table 4: Thrombin generation assay and microparticles in women with a history of preeclampsia and controls in follicular and luteal phases.

<table>
<thead>
<tr>
<th></th>
<th>PE (n=18)</th>
<th>Control (n=17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular Phase</td>
<td>Luteal Phase</td>
<td>Follicular Phase</td>
</tr>
<tr>
<td>Max-slope (RFU/min)</td>
<td>2383 (1867–3200)</td>
<td>2222 (1717–2778)</td>
<td>2158 (1339–2650)</td>
</tr>
<tr>
<td>Thrombin max (nM)</td>
<td>230 (179–311)</td>
<td>211 (159–271)</td>
<td>220 (137–271)</td>
</tr>
<tr>
<td>Thrombot (RFU)</td>
<td>42295 (39097–47058)</td>
<td>41603 (36756–46922)</td>
<td>38601 (32890–43303)</td>
</tr>
<tr>
<td>CD61+MP (per µL)</td>
<td>157 (0-5158)</td>
<td>1528 (0-5640)</td>
<td>231 (0-1926)</td>
</tr>
</tbody>
</table>

All values in median (IQ).

$P_1 =$ differences between women with history of preeclampsia and controls

$P_2 =$ difference between follicular and luteal phases

$CD61+MP =$ platelet derived microparticles.

Study III

Echocardiographic variables

Variables describing cardiac dimensions and structure were significantly higher in the preeclamptic group both at inclusion and at follow-up. Systolic LV function was within the normal range; nonetheless, it was significantly lower in the preeclamptic group during pregnancy but not at follow-up. Women with PE had longer IVRT, a higher peak mitral flow velocity during atrial contraction (A wave), and lower E/A ratio, both at inclusion and at follow-up. They also had higher $E/E'$ ratio measured at the septal and lateral part of the mitral annulus and larger left atrial dimensions compared to the women with normal pregnancies, and these differences persisted after delivery, Table 5.

Tabel 5. Diastolic function parameters in the study groups at inclusion and 3-6 months follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnancy</td>
<td>Follow-up</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.29 (0.07)</td>
<td>1.58 (0.07)</td>
<td>1.54 (0.07)</td>
</tr>
<tr>
<td>E/E' septal</td>
<td>10.92 (0.38)</td>
<td>9.08 (0.40)</td>
<td>7.49 (0.40)</td>
</tr>
<tr>
<td>E/E' lateral</td>
<td>8.23 (0.43)</td>
<td>5.86 (0.44)</td>
<td>5.72 (0.20)</td>
</tr>
<tr>
<td>LAArea es (cm²)</td>
<td>19.37 (0.49)</td>
<td>16.22 (0.50)</td>
<td>15.89 (0.52)</td>
</tr>
<tr>
<td>LAD es (cm)</td>
<td>3.76 (0.07)</td>
<td>3.44 (0.07)</td>
<td>3.42 (0.07)</td>
</tr>
</tbody>
</table>

The values are in Mean (SE).
Biomarkers
The levels of NT-pro-BNP in the preeclamptic and control group during pregnancy and at follow-up are illustrated in figure 12.

![Figure 12. Levels of NT-pro-BNP (ng/L) during pregnancy and at follow-up in the study groups.](image)

Early-onset and late preeclampsia
In the early-onset subgroup the highest readings of MAP were significantly higher (125 (4) mm Hg) than in the late subgroup (116 (2) mm Hg), p=0.035. The E/E’ ratio at the lateral position in the early-onset subgroup [11.32 (1.29)] was also significantly higher than in the late subgroup [7.41 (0.39)], p = 0.0004. Likewise, the level of NT-pro-BNP in the early-onset subgroup was significantly higher than in the late subgroup (1243 (583) ng/L versus 254 (57) ng/L), p=0.005.

Correlations
There were significant relationships between NT-pro-BNP and several echocardiographic variables analyzed by Spearman’s correlation as shown in Table 6. However, using multivariate analysis, we found that only the LVMI was significantly correlated to NT-pro-BNP and expressed 45% of the total NT-pro-BNP variation in the preeclamptic group (R²:0.581, p <0.0001). NT-pro-BNP was also significantly correlated with E/E’ septal and E/E’ lateral in the entire group at inclusion (Spearman r (r_s):0.39, p= 0.0019, and (r_s):0.42, p= 0.0008 respectively). This relationship was not significant in the PE group alone.

| Table 6. Spearman’s correlations in the preeclamptic group at inclusion. |
|-----------------|-----------------|-----------------|
|                 | NT-pro-BNP      |                 |
|                 | r_s             | P value         |
| LVMI            | 0.652           | <0.0001         |
| LVM             | 0.583           | 0.001           |
| LVDD            | 0.485           | 0.0057          |
| RWT             | 0.06            | 0.748           |
| LAArea es       | 0.319           | 0.081           |
| LAD es          | 0.460           | 0.009           |
| SBP             | 0.395           | 0.028           |
| Gestational age | -0.355          | 0.0499          |

$r_s$ = Spearman’s correlation
Study IV

The FMD was lower in the preeclamptic group than in healthy controls both at inclusion and follow-up (p=0.043), but there was no significant change of FMD between inclusion and follow-up in any of the groups (p=0.45). The levels PTX3, sFlt-1, ratio sFlt-1/P1GF were significantly higher and P1GF was lower in the preeclamptic group than in healthy controls as shown in Table 7. These markers also changed significantly between inclusion and follow-up within each group (Table 7).

Table 7: The values of FMD, inflammatory and angiogenic markers in the study groups at inclusion and follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At inclusion</td>
<td>Follow-up</td>
<td>At inclusion</td>
</tr>
<tr>
<td>FMD (%) ¤</td>
<td>8.49 ± 0.71</td>
<td>7.82 ± 0.74</td>
<td>10.07 ± 0.71</td>
</tr>
<tr>
<td>PTX3 (ng/ml)</td>
<td>22.64 (18.56-26.34)</td>
<td>6.18 (4.62-8.35)</td>
<td>13.17 (8.55-16.54)</td>
</tr>
<tr>
<td>sFlt-1 (pg/ml)</td>
<td>48468 (42893-67341)</td>
<td>1714 (1013-2153)</td>
<td>18948 (12749-35727)</td>
</tr>
<tr>
<td>P1GF (pg/ml)</td>
<td>33 (22-52)</td>
<td>31 (21-46)</td>
<td>170 (68-375)</td>
</tr>
<tr>
<td>Ratio sFlt-1/P1GF</td>
<td>1407(897-2397)</td>
<td>48 (30-78)</td>
<td>105 (34-546)</td>
</tr>
</tbody>
</table>

¤Values are in mean ± SE. All other values are in median (IQR).

At follow-up there were no significant differences in the levels of PTX3, sFlt-1, ratio sFlt-1/P1GF or P1GF between the groups (Table 8).

Table 8: Pairwise comparisons of PTX3, sFlt-1, P1GF and ratio sFlt-1/P1GF in preeclampsia and healthy controls at inclusion and 3-6 months follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Preeclampsia (PE)</th>
<th>Control (C)</th>
<th>Mean Difference (PE–C)</th>
<th>SE</th>
<th>P value</th>
<th>95 % Confidence Interval for Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At inclusion</td>
<td>Follow-up</td>
<td>10.001</td>
<td>1.947</td>
<td>&lt;0.0001</td>
<td>6.098-14.905</td>
</tr>
<tr>
<td>PTX3 (ng/ml)</td>
<td>Follow-up</td>
<td></td>
<td>0.093</td>
<td>0.864</td>
<td>0.915</td>
<td>-1.643-1.729</td>
</tr>
<tr>
<td>sFlt-1 (pg/ml)</td>
<td>At inclusion</td>
<td>Follow-up</td>
<td>31642</td>
<td>3624</td>
<td>&lt;0.0001</td>
<td>24383-38901</td>
</tr>
<tr>
<td>P1GF (pg/ml)</td>
<td>At inclusion</td>
<td>Follow-up</td>
<td>-293</td>
<td>107</td>
<td>0.008</td>
<td>-507-79</td>
</tr>
<tr>
<td>Ratio sFlt-1/P1GF</td>
<td>Follow-up</td>
<td></td>
<td>22</td>
<td>21</td>
<td>0.304</td>
<td>-21-64</td>
</tr>
<tr>
<td>sFlt-1/P1GF</td>
<td>At inclusion</td>
<td>Follow-up</td>
<td>1281</td>
<td>236</td>
<td>&lt;0.0001</td>
<td>806-1755</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td></td>
<td>11</td>
<td>66</td>
<td>0.864</td>
<td>-122-145</td>
</tr>
</tbody>
</table>

SE: standard error
Early-onset and late preeclamptic subgroups

The FMD was significantly lower in the early-onset subgroup, than in the late preeclamptic subgroup, both at inclusion (6.09 ± 1.31% versus 9.11 ± 0.67%) and at follow-up (5.58 ± 1.43% versus 8.49 ± 0.72%), \( p = 0.0177 \). There was a significant difference in FMD between these two subgroups and the healthy control group (\( p = 0.022 \)) as shown in Figure 13.

![Figure 13. The levels of FMD% in the subgroups: early, late preeclampsia and control during pregnancy and 3-6 months at follow-up.](image)

The levels of PTX3 at inclusion were higher in the early-onset than in the late PE subgroup (31 ± 5 ng/ml versus 20 ± 1 ng/ml, \( p = 0.002 \)), but not at 3-6 months follow-up (\( p = 0.537 \)). The ratio of sFlt-1/P1GF was also higher at inclusion in the early-onset subgroup (4284 ± 1502) than late subgroup (1844 ± 427, \( p = 0.039 \)) (Figure 14) but not at follow-up \( p = 0.358 \).

![Figure 14. The levels of PTX3 and Ratio of SFlit-1/P1GF (Log10) in early-onset, late PE and control during pregnancy.](image)


**DISCUSSION**

**Endothelial function in preeclampsia**

We have found impaired endothelial function, expressed as diminished FMD of the brachial artery, in women who were preeclamptic during pregnancy compared to a healthy control group. Impairment was seen at an early stage of PE during pregnancy, at short-term follow-up 3-6 months after delivery (study IV) and at mid-term follow-up one year after delivery (study I-II).

PE is associated with vasoconstriction and is recognized to be a syndrome characterized by profound dysfunction of the vascular endothelium (Cockell and Poston 1997; Chambers et al. 2001; Svedas et al. 2002; Lopez-Jaramillo et al. 2008). The presence of endothelial dysfunction in relatively high-risk patients predicts the subsequent development of PE (Garcia et al. 2007; Takase et al. 2003; Savvidou et al. 2003; Germain, Romanik et al. 2007). We have found impairment of endothelial function in early stages of developing PE (study IV), and this is in line with previous studies (Yoshida et al. 2000; Takata, Nakatsuka, and Kudo 2002; Germain, Romanik et al. 2007; Garcia et al. 2007; Takase B 2003; Savvidou et al. 2003). The endothelial dysfunction has also been demonstrated in women examined after delivery. We found persistent decrease in FMD one year after delivery (study I and II). It seems that signs of impaired endothelial function may be observed for many years after preeclamptic pregnancy. Chambers et al. and Agatisa et al. showed presence of decreased endothelial function at a median of 3 years after PE (Chambers et al. 2001; Agatisa et al. 2004). Endothelial dysfunction might contribute to the pathogenesis of cardiovascular sequelae (Agatisa et al. 2004; Chambers et al. 2001; Hamad et al. 2007). In study I and IV, FMD measured after delivery correlated with the systolic blood pressure measured during pregnancy, indicating more pronounced endothelial dysfunction in severe PE.

**Methodological considerations**

There are different methods of studying the endothelial function in peripheral arteries. We have studied endothelial function by measuring endothelium-dependent vasodilatation and have used hyperemia to induce flow-mediated dilatation in the brachial artery. The changes in brachial artery diameters were measured non-invasively by ultrasound technique. This method is preferable to use particularly in pregnant women because it is non-invasive, easy to perform and well tolerated by the subjects.

There are other methods to measure endothelium-dependent vasodilatation such as venous occlusion plethysmography for measurement of forearm blood flow (FBF) before and after acetylcholine infusion in the brachial artery. This method mainly evaluates vasodilatation in resistance arteries. Endothelial dysfunction appears to be related to major cardiovascular risk independently of whether the measurement technique employs FMD or plethysmography (Lind 2008). However, it has been reported that endothelium-dependent vasodilatation studied by plethysmography and FMD are not related and that the results might therefore contain different information.
concerning endothelial function (Lind et al. 2000; Lind, Hall, and Johansson 2002). These two techniques evaluate endothelium-dependent vasodilatation in different parts of the vasculature and following different stimuli (Lind, Hall, and Johansson 2002). The invasive forearm technique evaluates endothelium-dependent vasodilatation mainly in resistance vessels in skeletal muscle, while the brachial artery ultrasound technique measures vasodilatation in a conduit artery. Furthermore, the FMD technique uses shear stress as the stimulus for vasodilatation, whereas plethysmography uses a receptor-dependent mechanism. It should also be emphasized that one of the techniques measures blood flow, while the other evaluates a change in artery diameter. As these two techniques obviously evaluate different properties of the endothelium, it is suggested to include both of these established techniques in the evaluation of endothelium-dependent vasodilatation in future studies (Lind, Hall, and Johansson 2002).

A third, non-invasive, technique is based on pulse wave analysis and vasodilatation with a β-2 agonist. As with the brachial ultrasound technique, pulse wave analysis is quick and does not require arterial cannulation. However, like the FBF technique, it mainly measures vasodilatation in resistance vessels. This method does not evaluate endothelium-dependent vasodilatation specifically, but rather vasodilatation in general (Lind, Hall, and Johansson 2002).

**Inflammation in preeclampsia**

We found significantly elevated levels of PTX3 in the preeclamptic group during pregnancy (study IV). CRP was higher than in controls, but the difference did not reach statistical significance, neither during pregnancy, nor 3-6 months (study III) or one year after pregnancy (study I).

Several factors are known to be involved in the pathophysiology of PE: impaired trophoblast invasion, abnormal genetic polymorphism, vascular endothelial cell activation, and immune intolerance by the maternal immune system (Schiessl 2007; von Versen-Hoeynck et al. 2009). In addition, an exaggerated systemic inflammatory process has been described (Schiessl 2007; von Versen-Hoeynck et al. 2009). CRP is an important component of the innate immune system and is primarily produced by the liver as an acute phase reactive protein in response to inflammatory stimuli (Black, Kushner, and Samols 2004). An elevated serum CRP is a biomarker of chronic systemic inflammation, and an independent predictor of future cardiovascular events (Black, Kushner, and Samols 2004). CRP as a marker of inflammation is increased in normal pregnancy (von Versen-Hoeynck et al. 2009) without further increase in preeclamptic pregnancy (Stefanovic et al. 2009; Cetin et al. 2009; von Versen-Hoeynck et al. 2009; Portelinha et al. 2008; Gaugler-Senden et al. 2008). Our findings in study I and III are in line with those studies. We have shown that the CRP is higher during pregnancy in both groups compared to values after delivery, with no statistically significant changes between the groups, as illustrated in study III.

Pentraxin-3 (PTX3) is an inflammatory molecule that belongs to the same family as C-reactive protein. It is considered to be a more sensitive marker than CRP and is expressed by endothelial cells, monocytes, macrophages, and fibroblasts exposed to inflammatory conditions (Garlanda et al. 2005; Suliman et al. 2008). Few studies have measured the levels of PTX3 during PE (Cetin et al. 2006; Rovere-Querini et al. 2006). In study IV, we
found higher PTX3 levels in preeclamptic pregnancy and – for the first time – detectable levels 3-6 months after delivery. Furthermore, PTX3 was inversely correlated with FMD. These changes may explain the association between PE and the increased risk of CVD later in life.

**Coagulation in preeclampsia**

We have found that women with a history of PE, examined one year after preeclamptic pregnancy, had a significantly elevated total amount of thrombin and a slightly increased number of platelet derived microparticles, which were correlated with the thrombin levels (study II). However, we found no significant differences in levels of fibrinogen, von Willebrand factor, activated factor VII, plasminogen activator inhibitor 1, or tissue plasminogen activator between women with prior PE and healthy controls (study I).

Normal pregnancy is characterized by a procoagulant state. PE further increases coagulability compared to normal pregnancy, as reflected by increased thrombin generation, platelet activation, and fibrin deposition in renal and placental vasculature (VanWijk, Boer et al. 2002). This suggests that activation of the coagulation cascade is a feature of this complication of pregnancy. In study II we found that even one year after a preeclamptic pregnancy, thrombin levels were still significantly higher in the preeclamptic group than in the controls, which is in agreement with previous findings from our research group in a study of women 6-15 months after preeclamptic pregnancy (Bremme and Blomback 1996). These findings indicate a chronic persistent procoagulant state that may increase the risk of thromboembolic complications later in life. However, there was no difference in the levels of the hemostatic factors as plasminogen activator inhibitor 1, tissue plasminogen activator, in women with prior PE described in study I, while our research group has previously found elevated levels of plasma fibrinogen and von Willebrand factor at longer follow-up a mean of 4.5 years after the index pregnancy (He et al. 1999).

Microparticles (MP) are small membrane vesicles that are released from various cells and are involved in initiating and propagating the activation of coagulation. MP may modulate or reflect several of the key processes in PE, including inflammation, coagulation, platelet activation, and endothelial dysfunction (Toth et al. 2007). The data about the levels of MP in PE are conflicting: there are reports to support both increased and decreased MP levels (VanWijk, Nieuwland et al. 2002; Gonzalez-Quintero et al. 2004; Bretelle et al. 2003; Lok et al. 2008). In study II we observed a trend towards higher levels of platelet derived microparticles (CD61+ MP) in women with a history of PE compared to controls, which is in line with some studies (VanWijk, Nieuwland et al. 2002; Gonzalez-Quintero et al. 2004) and these MP were correlated with the thrombin levels in women with prior PE. These higher values of MP could exert procoagulant or pro-inflammatory effects that hypothetically might favor cardiovascular problems later in life.
Biomarkers

We have measured various biomarkers in our four studies; many of them are discussed under separate subheadings. Here we will focus on metabolic and antiangiogenic markers in PE. In study I, we presented evidence that insulin resistance measured by homeostasis model assessment (HOMA) is higher in women with prior PE. In study III, we found in the preeclamptic group higher cystatin C and lower cystatin C calculated GFR at inclusion and 3-6 months after delivery. In study IV, we have shown elevated levels of PI GF, sFlt-1 and ratio of sFlt-1/PIGF during pregnancy in preeclamptic group.

Pregnancy is a state of increased insulin resistance; insulin is regarded as a regulator of blood pressure during pregnancy. It had been indicated that insulin resistance and relative glucose intolerance are associated with an increased risk of new-onset hypertension in pregnancy, particularly PE, which supports the hypothesis that insulin resistance may play a role in the pathogenesis of PE (Negrato et al. 2009). Moreover, a higher degree of insulin resistance determined by HOMA has been shown early in pregnancy, before the onset of clinical manifestations of the pregnancy-induced hypertension (Sierra-Laguado et al. 2007). Hamasaki suggested that women with gestational hypertension were in a state of low systemic sensitivity to insulin (Hamasaki et al. 1996).

We showed higher fasting plasma insulin levels and high HOMA (study I), indicating that insulin resistance persists one year after delivery in women with PE, which is in agreement with earlier reports (Nisell et al. 1999; Wolf et al. 2004), as well as with two long-term follow-up studies (Laivuori, Tikkanen, and Ylikorkala 1996; Norden Lindenberg and Hanson 2000). Women with the metabolic syndrome may be at even greater risk of cardiovascular disease (Hannaford, Ferry, and Hirsch 1997; Irgens et al. 2001; Smith, Pell, and Walsh 2001); this may be another mechanism explaining why women who have previously had PE appear to have an increased risk of CVD.

It has been shown that serum concentrations of the antiangiogenic sFlt-1 are increased and those of the angiogenic PI GF are decreased in women with PE (Hirashima et al. 2005; Verlohren et al. 2009) and that the sFlt-1/PIGF ratio may be of value for prediction of PE (Hirashima et al. 2005; Verlohren et al. 2009). Our findings (study IV) are in line with the above studies.

Cardiac changes in preeclampsia

In study III, we showed that significant changes in LV size and wall thickness, impairment of diastolic LV function and left atrial enlargement occurred in nulliparous pregnant women, with increased after-load due to PE in comparison with normal pregnancy. These changes were associated with an increase in plasma concentrations of NT-pro-BNP and cystatin C both during pregnancy and after delivery. We have shown for the first time that the ratio of transmural E wave velocity to myocardial E´ velocity (E/E´), measured by DTI, remained significantly higher in the preeclamptic group than in the normal pregnancy group several months after delivery.

Preeclampsia represents a model of acute pressure overload that may induce dramatic changes in the LV structure and function (Novelli et al. 2003; Simmons, Gillin,
Jeremy 2002). The cardiac changes we described in study III are in line with previous studies (Simmons, Gillin, and Jeremy 2002; Borghi et al. 2000; Valensise et al. 2006; Ingec, Yilmaz, and Gundogdu 2005).

The LV systolic function has been studied extensively (Atkins et al. 1981; Hunter and Robson 1992), while in fact the diastolic dysfunction precedes the systolic dysfunction in pregnancy (Hirota 1980; Yamamoto, Redfield, and Nishimura 1996). Since loading conditions change significantly during pregnancy, less load-dependent variables are more suitable for assessment of cardiac function in pregnant women (Bamfo et al. 2007; Fok et al. 2006). DTI is a new echocardiographic technique which provides variables less dependent on preload and estimates of LV filling pressures. The ratio of transmitral E wave velocity to myocardial E velocity measured by DTI, E/E′, has been validated and widely accepted as a non-invasive surrogate measure of LV filling pressure in the general population, in patients with hypertension (Ceyhan et al. 2008), as well as in PE (Bamfo et al. 2008). In addition, it has been suggested to be a useful and more reliable index for evaluating the LV diastolic function than E/A ratio alone. We found higher E/E’ ratio in the PE group at the lateral and septal margin of the mitral annulus, which indicates that the higher LV filling pressures occurring in this group are due to pressure overload. We have shown for the first time that these ratios were still significantly higher in the PE than in the control group several months after delivery.

In multivariate analysis, LVMI was a significant predictor and expressed 45% of the total NT-pro-BNP variation at inclusion in study III. Thus we believe that the elevated NT-pro-BNP in PE might reflect LV stress and sub-clinical dysfunction.

**Severity of preeclampsia**

In study III and IV, we had divided our preeclamptic group into two subgroups: 1) those who developed early-onset and severe PE, necessitating pre-term delivery < 34 gestational weeks; and 2) those who developed PE and delivered at ≥ 34 gestational weeks, late PE subgroup, according to previously described criteria (Stepan et al. 2007; Huppertz 2008). We preferred this classification as it distinguishes very clearly between the early-onset, severe form of PE, requiring premature delivery of the fetus, and the late and mild form of PE.

In study III, the early-onset subgroup had more strongly impaired diastolic function, as they had higher E/E’ ratios at the lateral position, and even further elevated NT-pro-BNP compared to the subgroup with late PE. In study IV, the early-onset subgroup had further impaired endothelial function, with lower FMD. Furthermore, the inflammatory (PTX3) and anti-angiogenic (ratio of sFlt-1/P1GF) biomarkers were more elevated in this subgroup. These observed changes demonstrate clearly that women who develop early-onset PE have more severely impaired cardiovascular function than those who develop PE late or not at all. This impairment may explain why women with early-onset PE are at increased risk for CVD later in life.

**Clinical implications**

From a clinical perspective, the findings in our study may change the management of preeclamptic women during pregnancy, by prompting use of such biomarkers as NT-pro-BNP and PTX3 to identify “high-risk” subjects, i.e. the group with early-onset
and severe PE. These pregnancies are more strongly associated with not only fetal complications but also maternal complication, than pregnancies involving late form of PE. Besides identifying the group of pregnant women with the highest risk, our findings may shift the method of management towards earlier termination of the pregnancy. Labor would be induced due to maternal indications, as these high-risk women have pronounced impairment of cardiac function, excessive inflammatory reaction and endothelial dysfunction. A more aggressive primary prevention may be considered by attempting to optimally control blood pressure, weight, blood glucose, and lipids. The finding of that, the level of blood pressure during pregnancy is related to endothelial dysfunction after delivery, may indicate that more aggressive therapeutic measures are required. Using NT-pro-BNP in women with previous preeclampsia may encourage more frequent use of echocardiography to determine changes in cardiac structure and function. Future studies will be needed to show whether a reduction in level of NT-pro-BNP may reduce mortality from CVD.

**Timing of monitoring and follow-up**

Our findings in studies I-IV might suggest that the management of women with PE, particularly those with early-onset PE, could be improved by measuring levels of inflammatory and anti-angiogenic markers. The association we found between hypertension and endothelial dysfunction indicates that it is important also to follow up those who developed severe PE with organ involvement owing to serious vascular deterioration. Hyperinsulinemia, dyslipoproteinaemia and higher mean blood pressures have been found many years after preeclamptic first pregnancy (Hubel et al. 2000); an increased risk of death from ischemic heart disease and cerebrovascular events, up to about 50 years after a hypertensive pregnancy have been also reported (Arnadottir et al. 2005; Bellamy et al. 2007; Harskamp and Zeeman 2007; Irgens et al. 2001; Jonsdottir et al. 1995). Atherosclerosis is a chronic progressive condition that originates in early life and is accelerated in the presence of risk factors. Since preeclampsia has similar risk factors, a preeclamptic pregnancy might hasten the decision to put in primary preventive measures directed against CVD. Young women with preeclampsia represent an obvious target for prophylactic measures. These measures are certainly needed at an earlier age than usual for this risk group of women, to prevent CVD.

**Future direction**

Effective and cheap screening is needed for further improvement in care of high-risk women who may develop preeclampsia, as are reliable prophylactic measures. Although there have been minor advances, further trials and research are needed in this field. Even more research is needed to identify and prevent the recurrence of preeclampsia, by focusing on the high-risk population who has had preeclampsia. Management of preeclampsia at present focuses on controlling blood pressure, but only through better and more comprehensive understanding of the pathogenesis of the disease will we succeed in finding more effective treatment.
In summary, this thesis aids in identifying the group of preeclamptic women who are most vulnerable and run the highest risk of life threatening maternal prenatal and perinatal manifestations. Many strategies are required to ensure that these women deliver at the optimal time and also for follow-up after delivery. These strategies include monitoring their blood pressure, blood glucose and cardiovascular function, and measuring related biomarkers. The use of cardiac (NT-pro-BNP), inflammatory (PTX3) and antiangiogenic biomarkers (ratio of sFlt-1/PlGF) is needed to identify a high risk group of PE patients, as are prophylactic and therapeutic measures such as adjusting life-style, diet and exercise habits to prevent the late sequelae of cardiovascular disease in this group.
CONCLUSIONS

- The endothelial function was impaired during onset of clinical symptoms of PE and impairment persisted 3-6 months and one year after delivery (study I, II and IV). Furthermore, the endothelial impairment was more evident in the early-onset and severe subgroup than in the late subgroup of PE (study IV).

- PE caused significant changes in cardiac structure and function, with left atrial and ventricular enlargement, increase in wall thickness and impairment of diastolic left ventricular function. The changes in diastolic function were still present after delivery and these changes were more clearly observed in the patients with early-onset and severe PE (study III).

- One year after delivery ambulatory blood pressure was higher (study I), total thrombin was elevated and there was a trend towards higher levels of platelet derived microparticles (study II) in patients with PE than in controls. In addition, the blood pressure measured 3-6 months after delivery was higher in the preeclamptic group (study III).

- There were no significant differences between follicular and luteal menstrual phases in the endothelial function nor in the thrombotic state one year after PE (study II).

- PE was associated with elevated levels of cardiac (NT-pro-BNP levels), inflammatory (PTX3) and antiangiogenic (ratio of sFlt-1/PlGF) biomarkers during pregnancy and 3-6 months after delivery (study III and IV) and also with increased metabolic factor (HOMA) (study I). There was no increase in endothelial biomarkers (ICAM-1, VCAM-1, Selectin E) during pregnancy or at one year follow-up (study IV and I). The activity of cardiac, inflammatory and antiangiogenic biomarkers seem to correlate with the severity of PE.
Future Perspectives

During last decades the differences in cardiovascular outcomes between women and men have been increasingly recognized. However, the gender difference in the prevalence of risk factors for cardiovascular disease is still an ongoing area of research. Preeclampsia is a pregnancy-related disorder with multi-system and varied manifestations, and a leading cause of maternal and fetal morbidity and mortality. It has been shown that there is an association between preeclampsia and an increased risk of future cardiovascular disease. Through research, our knowledge about this association successively improves. This knowledge may have implications for the acute management of preeclampsia and even determine certain therapeutic measures for regular and close cardiovascular follow-up of this target group of young women.

We have plans to study the metabolic and hemostatic state of women during preeclampsia-complicated pregnancy as well as after delivery, in the same material as in study III and IV. In addition, we have started a long-term follow-up study intended to cover about 10 years after an episode of preeclampsia, in the same material as in study I and II. We are studying the vascular endothelial function by measuring the flow-mediated dilatation and by pulse wave analysis, in addition to 24-hour ambulatory blood pressure measurement, echocardiography and measurement of various biomarkers. Furthermore we have been collecting samples from women with previous early-onset and severe preeclampsia about 3 months after delivery, as this group has more evident vascular changes and even more risk for developing cardiovascular disease in the future. These studies might result in a successful method for monitoring this risk-group and make the women aware about their increased risk for cardiovascular diseases and even establish certain prophylactic and therapeutic measures. In addition to these plans for the future, we intend to study Pentraxin-3 expression in vessels of maternal tissue such as myometrium, fat tissue and even placental biopsy. We are also performing a follow-up study of children born to mothers who had preeclampsia during pregnancy, because preeclampsia is also associated with cardiovascular risk factors later in the life of the child (Vatten et al. 2003; Tenhola et al. 2006).

Therefore, pregnancy can be seen as a cardiovascular and metabolic test, which may also predict the risk for cardiovascular and metabolic diseases later in life (Craici, Wagner, and Garovic 2008; Williams 2003). Because of the long-term effect of PE, there is need for earlier cardiovascular risk assessment and considering preventive therapies at an earlier age. It is suggested that active assessment of cardiovascular risk up to 6 months postpartum may lead to earlier identification of cardiovascular risk and thus perhaps help encourage lifestyle modification (Magee and von Dadelszen 2007; Chandiramani and Shennan 2008). Women who have had preeclampsia need to be monitored closely and make lifestyle changes that will reduce the risk of cardiovascular disease in their future life.
Svensk sammanfattning


Övergripande mål: Att hos kvinnor med PE studera förändringar i hjärt- och kärlfunktion och för att mäta halterna av olika biomarkörer under graviditeten och efter förlossningen.


Arbete III och IV: 35 gravida kvinnor med PE och 30 friska gravida kontroller undersöktes under graviditet och 3-6 månader efter förlossningen. Transthorakal ekokardiografi och Doppler tissue imaging, FMD av brachialis utfördes. Blodkonzentrationen av amino-terminal pro-brain natriuretic peptide (NT-pro-BNP), C-reactivt protein, cystatin C, troponin I och nivåer av inflammatoriska och angiogena markörer mättes. Kvinnor med PE hade ett högre värde av septal och lateral kvot mellan tidig diastolisk mitralisflödeshastighet / tidig dikostolisk vävnadhastighet i myokardiat (E / É), högre nivåer av NT-pro-BNP, cystatin C och lägre cystatin C-beräknat GFR både under graviditet och vid uppföljning. Dessutom E / É kvoten och NT-pro-BNP var högre hos gravida kvinnor med tidig debut av än hos de som blev förlösta vid eller efter graviditetsvecka 34. FMD var lägre i gruppen med tidig-debut PE vid inklusion och vid uppföljning. Pentraxin 3 (PTX3) och kvoten av fm-like tyrosine kinase-1 (sFlt-1) till placental growth factor (P1GF) var förhöjda hos kvinnor med PE under graviditet. Dessutom FMD var lägre och PTX3 och kvoten sFlt-1/P1GF var högre i tidig-debut PE.

Slutsats: Kvinnor som drabbats av PE har kvarstående identifierbara avvikelser i kärlfunktionen liksom tecken på hyperkoagubilitet och högre nivåer av trombocytframställda mikropartiklar, både under follikulär och luteal fas av menstruationscykeln. PE var förändad med förändringar i hjärtstruktur och funktion, nedsatt endotelfunktion och förhöjning av hjärtbiomarkörer, de här förändringarna fanns fortfarande flera månader efter förlossningen och var mer tydliga hos patienter med tidig-debut och svår PE. Dessutom hade dessa kvinnor förhöjda inflammatoriska och antiangiogena markörer, och detta var särskilt uttalat i tidig-debut PE.
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References


Borghi, C., D. D. Esposito, V. Immordino, A. Cassani, S. Boschi, L. Bovicelli, and E.


References


References


Rangeen Rafik Hamad


Lyall, F., M Belfort. 2007. Pre-eclampsia etiology and clinical practice: Cambridge University


Novelli, G. P., H. Valensise, B. Vasapollo, G. Larciprete, G. Di Pierro, F. Altomare,


