From the Department of Women's and Children's Health
Karolinska Institutet, Stockholm, Sweden

# LONG-TERM CARDIOVASCULAR ASSESSMENT IN WOMEN WITH PREECLAMPSIA

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# Long-term cardiovascular assessment in women with preeclampsia

## Thesis for a licentiate degree

By

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“Education is the most powerful weapon which you can use to change the world.”

Nelson Mandela

To my angels, Edisson, Angie, Benjamin & Imad

# Abstract

**Background:** Preeclampsia (PE) is one of the most common medical multi-organ, pregnancy-specific disorder. PE is associated with endothelial dysfunction, elevated blood pressure, and inflammation. In addition to vascular dysfunction, preeclampsia is associated with cardiac remodeling and left ventricular (LV) dysfunction. Whether these cardiovascular changes eventually resolve remains unclear.

**Aims:** This thesis is aimed to study the long-term effects of PE on the cardiovascular system in women with a history of PE-complicated pregnancy

**Material and Methods: Studies I** and **II** examined 15 women with a history of PE (mean age 39±4 years) and 16 matched healthy women with an uncomplicated pregnancy (41±3 years) 10-12 years following the index pregnancy. Assessment of medical and family history, physical examination, anthropometric measurements, and biochemical markers were evaluated. In **Study I** forearm flow-mediated vasodilatation (FMD), pulse wave analyses (PWA), 24-h ambulatory blood pressure measurement (ABPM), plasma concentrations of glucose metabolism, lipid metabolism, inflammatory markers, and vascular function were assessed. In **Study II** echocardiography including Tissue Doppler Imaging and two-dimensional speckle-tracking echocardiography for myocardial strain imaging was used for evaluation of systolic and diastolic, left ventricular (LV), and right ventricular function (RV). LV global strain, atrial size, indices of ventricular-arterial coupling (VAC), and concentrations of N-terminal pro-B-type natriuretic peptide (NT-pro- BNP) were analyzed.

**Results:** In **Study, I** endothelial function assessed as hyperemia, hyperemia-induced FMD, and as responses to glyceryl trinitrate were similar in both groups (+10.7±5.4 % vs +9.0±4.7 %, and +29.1±9.7 % vs +25.1±8.8 %). ABPM showed slightly higher blood pressure (24-h 117±11/75±8 mm Hg vs 112±11/71±9 mm Hg) and reduced systolic and diastolic night/day ratios (0.81±0.06 vs 0.76±0.05, and 0.88±0.04 vs 0.84±0.04; both p=<0.05) in women with a history of PE. They also had an increased body mass index (26±6 vs 23±3 kg/$m^2$; p=<0.05), reduced glucose tolerance (HOMA 10.9±10.8 vs 6.6±3.8 mmol/l x pmol /l, p=<0.05), increased ICAM (102±15vs120±29 µg/ l, p=<0.05), and higher TNF receptor (11.62± 0.24 vs1.91±0.47 µg/ l, p=<0.05) concentrations. In **Study II** there were no significant differences in LV or RV dimensions, systolic function, or global LV strain. Indices of diastolic LV function left and right atrial size, VAC variables, or NT-pro-BNP levels did not differ between the study groups.

The offspring weight was inversely correlated to the maternal central BP (SBP r -0.33, p=0.07, DBP r -0.37, p=0.04) and brachial BP (SBP r -0.34, p=0.062, DBP r -0.40, p=0.029) and LV end-diastolic diameter. The relations for the birth weight centile were generally similar but weak. There was no association between the placenta weight and cardiac indices or VAC.

I sub-group analyses according to the severity of PE; 6 women with early and severe PE had a greater night/day ratio for ambulatory SBP and DBP than 9 women with neither early nor severe PE. The subgroups of PE were similar concerning NT-pro-BNP, cardiac structure and function, and indices of VAC.

**Conclusion:** A long-term follow-up (10-12 years after index pregnancy) of women with a history of PE-complicated pregnancy showed normalized endothelial function despite higher blood pressure and impaired glucose tolerance in comparison to women without a previous PE. Extensive echocardiographic examinations could not demonstrate significant alterations in systolic or diastolic LV function or VAC.

We suggest that pre-existing risk factors may be more important for future cardiovascular complications than myocardial and vascular damage occurring during pregnancy in women with PE. This highlights the importance of early treatment of risk factors, integrating adequate preventive strategies, and long-term surveillance with close monitoring.

# List of scientific papers

I. Östlund E, Al-Nashi M, Hamad RR, Larsson A, Eriksson M, Bremme K, Kahan T. Normalized endothelial function but sustained cardiovascular risk profile eleven years following a pregnancy complicated by preeclampsia. Hypertens Res. 2013;36 (12):1081-7

II. Al-Nashi M, Eriksson MJ, Östlund E, Bremme K, Kahan T. Cardiac structure and function and ventricular-arterial interaction 11 years following a pregnancy complicated with preeclampsia. J Am Soc Hypertens. 2016 ;10(4):297-306

# CONTENTS

# List of abbreviations

| | |
|---|---|
| ABPM | Ambulatory Blood pressure measurment |
| ACOG | American College of Obstetrics and Gynecology |
| AHA | American Heart Association |
| AIx | Augmentation Index |
| BA | Brachial artery |
| BMI | Body mass index |
| BP | Blood pressure |
| CO | Cardiac output |
| CVD | Cardiovascular disease |
| CV | Cardiovascular |
| DBP | Diastolic blood pressure |
| DTI | Doppler tissue image |
| ECG | Electrocardiography |
| Ea | Arterial Elastance |
| ED | Endothelial dysfunction |
| EF | Ejection Fraction |
| ESRD | End-stage renal disease |
| Ev | Left ventricular elastance |
| FMD | Flow-mediated vasodilatation |
| GLS | Global longitudinal strain |
| HDL | High density lipoprotien |
| HDP | Hypertension disease of pregnancy |
| HELLP | Hemolysis, elevated liver enzymes and low platelets |
| HOMA | Homeostasis model assessment |
| HR | Heart rate |
| Hs-CRP | High sensitive-C reactive protien |

| | |
|---|---|
| ICAM-1 | Intercellular adhesion molecule-1 |
| IUGR | Intrauterin growth restriction |
| LA | Left atrium/atrial |
| LDL | Low density lipoprotien |
| LV | Left ventricle/ventricular |
| LVM | Left ventricular mass |
| LVMI | Left ventricular mass index |
| MAP | Mean arterial pressure |
| NT-pro-BNP | Amino-terminal pro-brain natriuretic peptide |
| PE | Preeclapsia |
| PlGF | Placental growth factor |
| PWA | Pulse wave analyses |
| RA | Right atrum/atrial |
| RV | Right ventricle/ventricular |
| SBP | Systolic blood pressure |
| SD | Standard deviation |
| sFLt-1 | Soluble fms-like tyrosine kinase-1 |
| STE | Speckle tracking echocardiography |
| SV | Stroke Volume |
| SVR | Systemic vascular resistance |
| TG | Triglyceride |
| TNF | Tissue necrosis factor |
| VAC | Ventricular arterial coupling |
| VACM | Vascular cell adhesion molecule |
| VEGF | Vascular endothelial growth factor |

# Introduction

Preeclampsia (PE) is one of the most important obstetric emergencies, with high maternal and fetal morbidity and mortality, especially in developing countries [1].

PE is a multiorgan disorder with hypertension and proteinuria being common features. Severe PE can also be associated with placental insufficiency, coagulopathy, multi-organ dysfunction, intrauterine growth restriction (IUGR), and intrauterine fetal death, situations that may require early delivery [2].

Long-term epidemiological studies have shown that women with previous PE have an increased risk of cardiovascular disease (CVD) such as hypertension, ischemic heart disease, stroke, and venous thromboembolism later in life [3]. The risk is even higher for women with more severe forms of PE and twice in women with recurrent PE [4-7]. Furthermore, the prevalence of developing metabolic syndrome, renal disease, and diabetes mellitus is higher in women with previous PE [8, 9].

The rationale for this licentiate thesis is to increase our knowledge on long-term vascular and cardiac changes in women with previous PE in comparison to women with uncomplicated pregnancies. Furthermore, we aimed to study the temporal changes in vascular function from one-year to 11-year follow-up in relation to PE. Understanding the long-term consequences of PE on the cardiovascular system is crucial for identifying women with an increased risk of developing CVD and integrating adequate preventive strategies and appropriate follow-up.

# 1 Background

## 1.1 Maternal cardiovascular function in uncomplicated pregnancy

During pregnancy, almost every organ has physiological and anatomical changes that are not only important for increasing the metabolic demands of the pregnancy but also are needed to meet the developmental needs of the fetus. These changes begin immediately after fertilization and make changes in almost all organ systems, including the cardiovascular system [10].

For most pregnant women, these changes resolve and disappear almost six weeks to three months after delivery [10]. Understanding cardiovascular physiological modification in pregnancy is particularly important in increasing awareness of the pathogenesis of hypertensive disorders of pregnancy, including preeclampsia [6, 11, 12].

## 1.2 Cardiovascular changes during pregnancy:

In a normal pregnancy, several changes occur in the maternal cardiovascular system to alteration in blood pressure (BP) to the needs of pregnancy and prepare for delivery[13]. Cardiovascular adaptation to pregnancy begins as early as after conception and remains throughout pregnancy, with rapid shifts occurring postpartum[11, 13]

### 1.2.1 Heart rate

Maternal heart rate (HR) increases within 5–6 gestational weeks to a maximum increase of 15–20 beats per minute in the third trimester [14, 15]. HR is primarily responsible for the maintenance of cardiac output (CO) late in pregnancy [15]. HR can be affected by the changes in maternal position, with a slight reduction from the supine to the left lateral position [13]. During labor, variation in HR can be observed depending on the uterine contraction, the pain associated with the contraction, and the mother's position [13].

### 1.2.2 Stroke volume

The stroke volume (SV) is the amount of blood ejected from the left ventricle (LV) into the aorta in a single heartbeat. SV depends on the preload and afterload. During pregnancy, the preload increases because of the increase in blood volume, which causes more stretching of the LV myocardium, while the afterload decreases because of maternal arterial vasodilatation and a decrease

in systemic vascular resistance (SVR) [11]. SV increases gradually until the end of the mid-trimester and then declines slightly towards the term [11, 15]. The reduction in SV in the third trimester is coupled to: 1) the growing fetus and the quickly increasing size of the uterus cause the blood flow to the uteroplacental circulation to reach its peak, 2) the enlarging size of the pregnant uterus compresses the vena cava; both reasons can lead to a decrease in preload and a reduction in SV [16].

### 1.2.3 Blood pressure

Changes in BP could be seen as early as the first gestational weeks. As shown in Figure 1 the SBP is usually unchanged or decreases slightly while the diastolic BP and the mean arterial blood pressure (MAP) decrease gradually until they reach nadir by mid-pregnancy, when the BP starts to increase again. The BP returns to or exceeds pre-pregnancy levels by term or in the early postpartum period [11, 14, 15, 17].

MAP is important for the perfusion of organs and defined as the pressure in the artery in one cycle and calculated according to the following equation: MAP=DBP+1/3 (SBP-DPB). The MAP is proportional to the SVR and CO [11, 17] and is positively related to maternal age, inter-gestational period, and body mass index (BMI). In addition, MAP is a predictive marker for PE when it rises at 20 weeks [18].

BP variations are common; both SBP and DBP are slightly higher in the sitting position than the supine position. These variations depend on the SVR and the maternal body position. Maternal position has a major impact on the term. In the supine position, the enlarged maternal uterus causes a potential occlusion of the inferior vena cava, and compression in the aorta. The aortocaval compression syndrome causes a reduction in venous return to the heart, which results in supine hypotension syndrome[14]. Because of this problem, it is preferable to measure the blood pressure in the left-lateral position while the pregnant woman tilts 30 degrees[19].

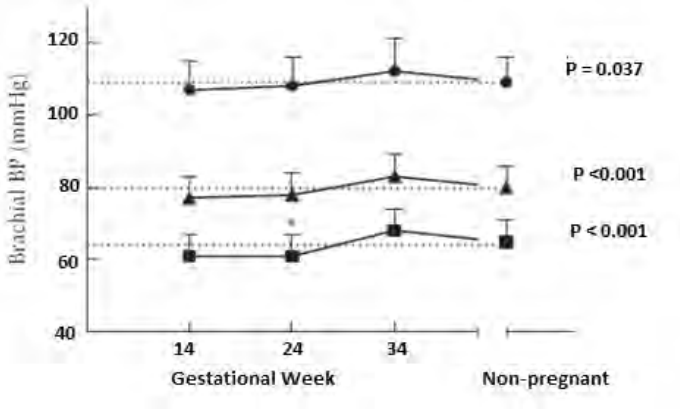


**Figure1:** Changes in BP during normal pregnancy [20]. ▲: mean arterial pressure, ●: Systolic blood pressure, ■: Diastolic blood pressure. Data are presented as mean + SD. *P*-values in graphs relate to trend for change over time, by linear mixed model. Significant differences between values in each trimester and baseline at 9 months postpartum are indicated by asterisks *$P < 0.05$. Reproduced with permission from Johan Wiley & sons.

### 1.2.4 Cardiac output

Cardiac output measures the functional capacity of the heart and is the amount of blood ejected by the LV per minute [11]. CO depends on two parameters: stroke volume and heart rate. Noninvasive methods such as echocardiography are commonly used to assess CO in pregnant women. Maternal CO increases as early as five weeks, steadily into the first trimester, reaching peak levels by the end of the second trimester, then decreases slightly towards term [15, 17].

In early pregnancy, the increase in SV is responsible for the rise in CO. As the pregnancy advances, e.g. in the third trimester, the SV decreases slightly while HR increases, making HR a more dominant factor in maintaining the increasing CO [15, 16]. During labor, the uterine contraction increases the transfer of blood volume from the uteroplacental unit back to the maternal circulation, causing an increasing preload, rising SV, and thus increasing CO [16]. CO decreases rapidly in the early post-partum period, reaching normal values in 6 weeks [13, 16].

### 1.2.5 Systemic vascular resistance

A significant decrease in SVR occurs during pregnancy, resulting in a decrease in BP (primarily DBP) and MAP. Many factors could participate in these changes, mostly hormonal changes, i.e., progesterone and estrogen [13]. Other factors that could contribute to the reduction in SVR are the relaxin hormone and nitric oxide (NO), which have vasodilation effects in the blood vessels [13].

Reduction in the SVR occurs as early as a few weeks after conception, reaching its lowest level in the middle of the second trimester, followed by a marginal rise in SVR notes towards the end of the third trimester and at term [11, 21, 22]. Reduction in SVR correlated positively with increases in vascular-arterial compliance [23]. Figure 2 shows the cardiovascular changes in normal pregnancy.

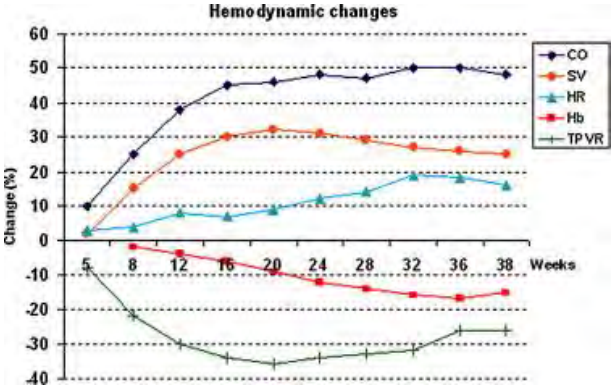


**Figure2:** Cardiac changes in normal pregnancy between gestational weeks (5-38) [22]. CO: Cardiac output, SV: stroke volume, HR: heart rate, HB: haemoglobin, TPVR: total peripheral vascular resistance. Reproduced with permission from Elsevier.

### 1.2.6 Heart changes during normal pregnancy

The enlarged pregnant uterus gradually lifts up the diaphragm. The elevated diaphragm pushes the heart laterally and enhances its rotation to the left[10].

The ECG changes during pregnancy are mostly related to the left axis deviation of the heart and due to the anatomical and physiological changes of the heart. The common findings in ECG are T-wave inversion in lead III, sinus tachycardia, ST segment depression, and premature ectopic beats [10].

The LV systolic function correlates with the SVR; a reduction in the SVR improves the LV systolic function through a reduction in the LV afterload. The ejection fraction (EF) is usually unchanged [13]. The cardiac changes observed by echocardiography are mostly related to cardiac remodeling related to the increase of the preload with slight enlargement of the cardiac chambers and the increase in the ventricular wall thickness [10, 11, 15].

Clinical cardiac examination under pregnancy could be easily misunderstood by physicians as pathological; hearing a loader first heart sound, an ejection systolic murmur, a third heart sound, and ectopic beats are common findings during normal pregnancy [10].

## 1.3 Hypertensive disease in pregnancy

The recent recommendations from the ISSHP and ACOG Task Force classification recommend the term hypertensive disorder in pregnancy, which includes chronic hypertension, gestational hypertension, white coat hypertension, masked hypertension, chronic hypertension with superimposed preeclampsia (PE), as well as preeclampsia and eclampsia [24, 25].

Different sub-classifications have been used, such as early and late-onset PE, term and preterm, and mild and severe preeclampsia. These sub-classifications are no longer in use in clinical practice because PE can be critical and mortal to the mother and the fetus at any time during pregnancy. However, these terminologies can still be useful in clinical research [24]. In Sweden, severe PE is still in use as a diagnosis for preeclamptic women with severe hypertension and /or organ dysfunction and /or placental insufficiency (IUGR).

## 1.4 Preeclampsia

### 1.4.1 Definition

Preeclampsia is one of the medical-obstetrical complications occurring during pregnancy and is associated with higher maternal mortality (18%) and fetal morbidity and mortality (40%). More than 60,000 women die yearly worldwide because of PE, with a higher prevalence in low and middle-income countries [26, 27].

PE is a hypertensive disorder that can progress to a multi-system disorder, including coagulopathy, hepatic, renal, cerebral, and cardiovascular disorder, delivery of the fetus and placenta is the only cure [24].

PE is defined as the new onset of hypertension (SPB ≥ 140 mmHg and/ or DBP ≥ 90 mmHg) on two occasions 15 minutes apart in a patient who was previously normotensive, at or after 20 gestational weeks and the presence of proteinuria is no more required for the diagnosis of PE in the presence of other advanced organ damage or placental insufficiency [24].

Severe onset PE is defined by the following features: BP≥ 160/110 on two different occasions, thrombocytes < 100,000/µL, rising liver enzymes, renal failure with elevated serum creatinine level >1.1 mg/dl or ≥ 90µL, pulmonary edema, new-onset cerebral manifestations, or utero-placental insufficiency [24].

Eclampsia is a life-threatening obstetric medical emergency, requiring acute management and is associated with high mortality and morbidity for both the mother and the fetus. Eclampsia is a newly-onset generalized convulsion, eclamptic seizures can occur during pregnancy, at labor, and in the early postpartum period. Eclampsia is thought to be related to cerebral endotheliosis which is one feature of PE, resulting in hypoperfusion and brain oedema [28].

HELLP syndrome (**H**: hemolysis, **EL**: elevated liver enzymes, and **LP**: low platelet count). HELLP syndrome does not always associated with PE, about 20 % of women with severe PE develop HELLP. HELLP syndrome can mimic other diseases like acute fatty liver, idiopathic thrombocytopenic purpura, and acute hepatitis. In the absence of rising blood pressure, delay in the diagnosis can be life threatening for the mother and the fetus [24].

### 1.4.2 Early theories and treatments

The description of what is known today (PE-eclampsia) was first observed between the late 5$^{th}$ and early 4$^{th}$ centuries BC by Hippocrates. He describes PE as heaviness in the head and grand-mal seizures and relates it to the" imbalance" of the four humors in the body (blood, phlegm, yellow bile, and black bile). The only cure was to bring the body fluids into balance by bloodletting or changing diet [29].

The term "Eclampsia" which is a Greek word that means lightning, was used for the first time by Bossier de Sauvage (1710–1795) and described the suddenly occurring seizer during pregnancy. He also separated the acute convulsion of eclampsia from epilepsy and noticed that it disappeared once the causing event was over, which is pregnancy [30].

More progress was made during the 20$^{th}$ century in understanding the pathophysiological changes occurring with PE, including pathological examination of the placenta and the other organs and analysis of different inflammatory, antioxidant, and anti-angiogenic proteins [29].

### 1.4.3 Pathophysiology

Preeclampsia is a disease of the pregnant human female. The exact pathogenesis and etiology are still unknown. However different pathophysiological mechanisms may explain the development of PE including vascular-endothelial dysfunction, immunological factors, genetics, defective trophoblastic invasion, and inflammatory factors [31, 32].

The most acceptable and widely used and described theory on pathogenesis is the two-step model [10, 33].

In normal pregnancy, the placental trophoblast cells break into the inner layer of the uterine spiral arteries, making these arteries lose their vascular smooth muscle layer and their endothelial lining. This remodeling reduces vascular resistance and increases the capacities of the spiral artery, thus enhancing the increase in the utero-placental flow. The arterial changes protract inward the myometrium [34].

Abnormal placentation (uterine-spiral artery remodeling) (stage I); In PE, especially early-onset PE (before 34 gestational weeks), spiral artery remodeling is incomplete and is limited to the superficial decidua, while the myometrium segment remains tightly coiled and narrow [35].

In maternal syndrome (stage II), the failure in remodeling of uterine spiral arteries decreases the blood flow to the placenta, thus decreasing uteroplacental flow [35].

Reduced placental perfusion and placenta damage cannot accommodate the fetal demand resulting in uteroplacental disruption which increases the shedding of vascular toxins, and pro-inflammatory factors from the fetal unit into the mother's circulation, leading to maternal vascular endothelial dysfunction and the appearance of the clinical signs and symptoms of PE [33, 35] as shown in Figure 3.

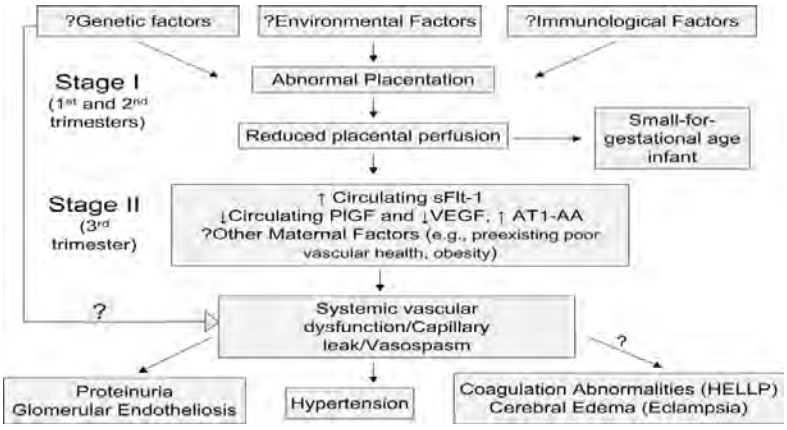


**Figure 3:** Pathogenesis of maternal syndrome of preeclampsia [33]. sFLt-1: soluble fms like tyrosine kinase, VEGF: vascular endothelial growth factor, PlGF: placental growth factor, AT1-AA: angiotensin II typ1 receptor Angonistic autoantibody, HELLP: Hemolysis, elevated liver enzymes and low platelets. Free access.

### 1.4.4 Predisposing factors

Many factors can be predisposed to the development of PE; one of them is a maternal factor; advanced or extremely young maternal age, assisted fertilization " with egg donation" and polycystic ovary are known to have an increasing risk for PE [31, 36].

Genetic factors have some consideration in the development of PE. PE has heritability for both maternal and fetal genetic risk contributions [37]. African women have a higher risk than other ethnic groups [2]. Sons and daughters born to preeclamptic women are at higher risk of fathering women with PE and being preeclamptic themselves, respectively [38, 39].

Recently, a large Finnish cohort confirmed the involvement of the sFlt-1 gene in preeclamptic women. The study showed a correlation between the Flt-1 locus and the fetal chromosome 13 [40], this can also explain the rising risk of developing PE in women carrying fetuses with trisomy 13 [41].

Multiple pregnancy and trophoblastic diseases like hydatiform mole are also associated with an increasing risk for PE, which may relate to elevated levels of sFlt-1 caused by the increasing size of the placenta [42].

Other factors are immunological; nulliparity and a change in fathering could indicate a higher likelihood of experiencing preeclampsia [43].

PE and cardiovascular diseases share several genetic and non-genetic risk factors. Obesity, chronic hypertension, diabetes mellitus, renal diseases, systemic lupus, and antiphospholipid syndrome are associated with endothelial dysfunction and may predispose to both PE and CVD [31, 32].

## 1.5 Maternal vascular changes in preeclampsia

### 1.5.1 Endothelial dysfunction in preeclamptic women

The endothelium is a large "organ", it weighs almost one kg in a 70-kg adult person. The endothelium is usually a single squamous cell layer covering the inner vascular surface and playing a profound role in controlling vascular tone and blood fluidity [44]. Additionally, having a regulatory role in hemostatic, angiogenic and inflammatory process and in controlling blood pressure [44].

Maternal vascular dysfunction is a common feature of PE and may be seen in all vascular beds, both reproductive and non-reproductive [45]. The impairment in

endothelial function is thought to play a central role in the pathogenesis of PE [7, 43].

Maternal vascular and endothelial dysfunction due to placental factors includes abnormal remodeling of the uterine artery, enhanced vasoconstriction, vascular oxidative stress, and inflammation [35, 43], which in turn leads to increased systemic vascular resistance and decreased blood perfusion to different body organs. The clinical manifestations of PE can be attributed to vascular endotheliosis represented in different organs, e.g. glomerular endotheliosis, cerebral endotheliosis, hepatic endotheliosis, and the systemic inflammatory response that results in the clinical manifestation of PE and placenta insufficiency [7].

#### *1.5.1.1 Flow-mediated vasodilatation*

A non-invasive reference method to study endothelial function in humans is flow-mediated vasodilatation (FMD) where the changes in brachial artery (BA) diameter is assessed by ultrasound.

FMD was first used to evaluate endothelial function in 1992. FMD mostly measured in the brachial artery, more rarely in the radial or femoral arteries [46]. FMD is considered safe to use in pregnant women [46].

Assessing endothelial function in the forearm usually measures by using duplex ultrasonography to measure the diameter of BA following endothelial cells response to temporary ischemia and reactive hyperemia as illustrated in Figure4 [46, 47].

Following the application and inflation of an occlusion cuff, blood flow to the lower arm temporarily stopped. Ischemia occurring in the tissue distal to the cuff causes vasodilatation of the distal vessels, leading to reduction in vascular resistance. Upon deflation of the occlusion cuff, the subsequent decrease in downstream resistance enhances blood flow to the arm -reactive hyperemia. In response to the increased shear stress, endothelium releases vasodilators such as nitric oxide, facilitating dilatation of arterial vessels. FMD is defined as the measurement of the % difference between the BA diameter measured following hyperemia and the diameter at baseline at rest before inflation of the occlusion cuff. Patients with vascular endothelial dysfunction have lower or missing responses to hypoxia or ischemia [46].

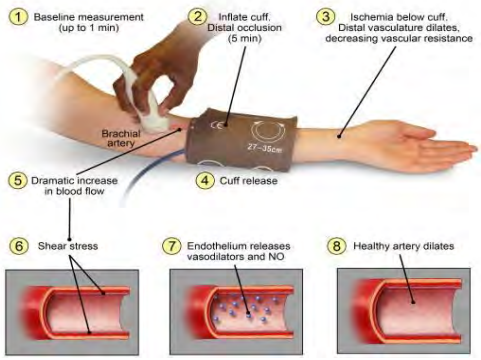


**Figure 4:** Flow mediated dilation of the brachial artery [46]. Free access

During normal pregnancy, there is increased in arterial-vascular compliance, and increased FMD as gestation progresses [48]. Vasoconstriction and endothelial dysfunction associated with PE result in impaired vascular response to ischemia and impaired FMD [49].

#### *1.5.1.2 Arterial stiffness*

Arterial stiffness is defined as the changes relating to an alteration in a vascular tone and the rigidity of the arteries. It is a pathological process in the vascular-arterial system that results from elastin degeneration and the deposition of collagen in the arteries. This process causes rigidity and thickening of the blood vessel wall [50].

Assessment of arterial stiffness and vascular function in preeclamptic women can be done by using the pulse wave analysis (PWA) methods. One of these methods is by applying applanation tonometry. Using this technique, it is possible to assess central blood pressure, measure the pulse wave volume (PWV) and the augmentation index (AIx). PWV is the speed of the pulse velocity that transmits from the beginning of the aorta to the peripheral circulation. As shown in Figure 5, recording PWV depending on two indices, first the time taken of a pulse wave between two arteries level i.e. carotid-femoral or carotid-brachial, and second the distance between the two arteries points, $PWV=\Delta L/\Delta t$ [51].

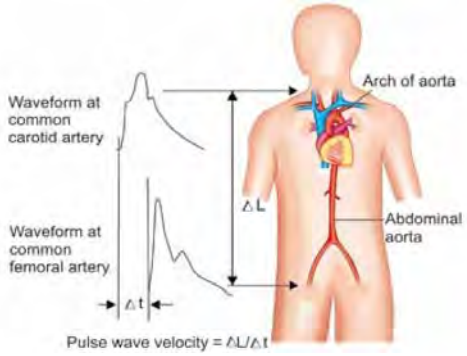


**Figure 5:** Measurement of carotid-femoral pulse wave velocity, L: Length, t: time. R. Kasliwal et al, hypertension journal 1(2):73-82,2015, free access.

AIx is a measure of arterial wave reflection and arterial stiffness [52, 53]. Every cardiac beat induces a pulse wave which goes from the heart towards the arterial vascular system. These waves reflect towards the heart when they meet points of impedance such as arterial bifurcation or area of increased stiffness. These reflected waves can interfere with the forward waves. The length of the pulse wave in the arteries is the compound of both the reflected and the forward waves as shown in Figure 6 [52].

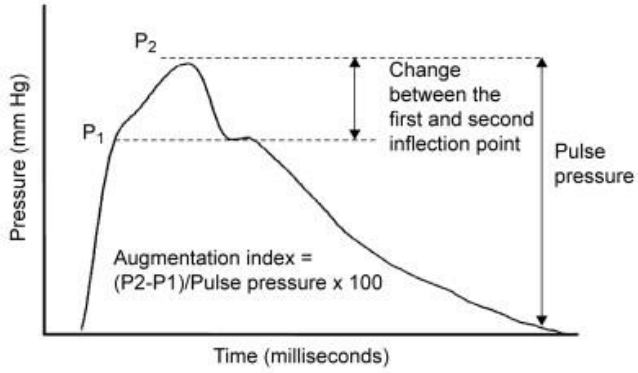


**Figure 6**: The augmentation index. The systolic peak (P1 and P2). P1: first systolic inflection point ,P2: second systolic inflection point [52]. Free access.

In normal pregnancy, AIx decreases similarly to BP, reaching its nadir in the second trimester and rising towards term [20, 52]. AIx returns to normal by six weeks postpartum [54].

By using PWV and AIx, arterial stiffness alteration in vascular adaptation could be detected as early as in the second trimester and before the development of PE [55]. Studies showed that AIx is higher in preeclamptic women in comparison to

women with gestational hypertension and/or women with normal pregnancies [53, 54]. Higher levels found in early-onset PE in comparison with late-onset [53, 56].

#### *1.5.1.3 Biomarkers and endothelial function*

##### 1.5.1.3.1 Inflammatory and vascular factors

C reactive protein (CRP) is a component of the innate system that is indicative of inflammation [57]. Measuring highly sensitive CRP (Hs-CRP) is an independent predictor of future cardiovascular events [58]. CRP is usually elevated in normal pregnancy and has been reported to be notably higher in women with PE when compared to uncomplicated normal pregnancies [49, 57].

Pentraxin 3 as an inflammatory marker is more sensitive than CRP. Pentraxin 3 is shown to be elevated in PE; the elevation is more obvious in early-onset PE [49]. Pentraxin 3 is thought to be a predictive factor for the persistence of endothelial dysfunction after delivery, and it plays a role in the prediction of CVD later in life [49].

Cathepsin B seems to play an important role in implantation, placentation, and trophoblastic differentiation. The levels of cathepsin B usually remain unchanged throughout pregnancy. However, they are positively correlated with the liver enzymes alanine and aspartate aminotransferase (ASAT, ALAT) and rise with PE [59, 60].

Homocysteine is a type of amino acid. Homocysteine is produced naturally in the body and is important for the oxidative stress process and lipid peroxidation. Homocysteine decreases during normal pregnancy. Hyperhomocysteinemia shares the same pathophysiology as PE.

In high levels, i.e., hyperhomocysteinemia, Homocysteine damages the endothelial vascular bed and causes injury to the intima-media of the arteries, activates platelets, increases leucocyte adhesion, causes vascular vasoconstriction, and increases the risk of blood clotting[61]. Homocysteine level correlates directly with the severity of PE [62].

Tissue necrosis factor-1 is another inflammatory factor; it has been suggested to be a cardioprotective factor and regulated by tissue necrosis factor-alfa (TNF-α) activation. TNF-α has a controlling effect on the growth of neoplastic or non-neoplastic cells [63].

In normal pregnancy, TNF-α has a promoting role in the implantation of the placenta and the development of the embryo. TNF-α is shown to be raised in PE and other obstetric complications like premature labor and abortion [64]. Preeclamptic women have twofold higher levels of TNF-α than women with uncomplicated pregnancy. The level persists to be higher many years after delivery [65].

Vascular endothelial growth factor (VEGF) is another endothelial factor that maintains endothelial cells and acts synergistically with placental growth factor (PlGF). The imbalance between these factors and the anti-angiogenic factors such as sFlt-1 or VEGF receptor-1 is involved in the pathogenesis of PE. The binding of sFlt-1 to VEGF and PlGF deprives the maternal vascular endothelium of angiogenic factors and causes systemic endothelial cell dysfunction[66]. The intercellular adhesion molecule-1 (ICAM-1), a cell surface glycoprotein involved in various inflammatory processes. ICAM-1 expresses on the surface of endothelial cells and plays role in leukocytes adhesion and migration throw the endothelial barrier. Elevated ICAM-1 observed in preeclamptic women may contribute to the inflammatory process and endothelial dysfunction associated with PE[67].

#### 1.5.1.3.2 Metabolic factors

There is a significant metabolic change during normal pregnancy; these changes are related mostly to the significant rise in maternal hormones progesterone and estradiol, as well as the rise in insulin and human placenta lactogen. Total cholesterol and low-density lipoprotein (LDL) increase during pregnancy [68]. However, the level of high-density lipoprotein (HDL) increases slightly in the second trimester and decreases in the third trimester, and it is generally lower in subsequent pregnancies [68]. Blood lipids have an important role during pregnancy, especially in the development of the fetus's nervous system.

Triglyceride (TG) is an ester; it is a precursor of three fatty acids, steroid hormone, and glyceride. When metabolized, TG decreases in the first trimester due to pregnancy-related nausea and vomiting. It has been shown that there is a clear and considerable correlation between the level of TG and the occurrence or events of PE. Detecting high TG levels in early pregnancy is associated with an increasing risk of developing early rather than late-onset PE [61].

During normal pregnancy, insulin production increases as early as the first trimester. Insulin sensitivity reduces gradually in the second and third trimesters. During the third trimester, physiological insulin resistance develops, resulting in

increased insulin levels and increased glucose levels[69]. Women with PE have a further reduction in insulin sensitivity and rising in insulin resistance, an abnormality that persists postpartum [49].

#### 1.5.1.3.3 Creatinine

Creatinine is a waste product produced by muscle from a breakdown of a component called creatinine. The kidney usually filters creatinine from the blood and excretes it in urine. Glomerular endotheliosis is associated with PE resulting in impaired renal function and decreased creatinine clearance and thus elevated creatinine level in the blood. Monitoring creatinine levels is commonly uses in assessing the renal function in women with PE [70].

## 1.6 Cardiovascular changes in preeclampsia and the early postpartum period:

Cardiac changes associated with PE are cardiac impairment and afterload-mediated left ventricular remodeling of the maternal heart [71, 72].

### 1.6.1 Arterial and venous system

PE is associated with a reduction in plasma volume and lower CO. The SVR increases and arterial and venous compliance decreases, resulting in elevated systemic afterload. These changes are more dominant in early and more severe PE[73].

### 1.6.2 Left ventricular structure and function:

To accommodate the rise in afterload in PE, the LV mass (LVM) increases, resulting in LV hypertrophy and cardiac remodeling.

Increased LV wall thickness has been reported up to one-year post-partum in preeclamptic women [71, 74]. Meanwhile, Tyldum et al. showed a decrease in LV wall thickness 3 months postpartum compared with the acute phase of PE [75]. Others have also described that maternal LV function, although impaired during PE, have normalized in the early postpartum period [76, 77]. One possible explanation could be a reduction in afterload due to the normalization of hypertension [75].

Systolic LV function represented by EF is mostly unchanged in the majority of women with PE, except in women with early and severe PE in whom reduced EF has been described [71, 78].

Despite the presence of cardiac remodeling and a normal or slightly impaired EF, the majority of early and severe PE patients exhibit asymptomatic heart failure [74, 79, 80] (which is defined as LV hypertrophy or LV systolic or diastolic dysfunction with preserved EF) These changes can persist 1-2 years after delivery [74].

Diastolic LV dysfunction is described by echocardiography as impaired LV filling with a lower E/A ratio and/or a higher E/é ratio. Diastolic dysfunction has been found in association with or without systolic LV dysfunction [71, 81].

The diastolic filling abnormality may also play a significant role in hypertensive crises associated with severe PE [82].

Women with advanced LV global diastolic dysfunction may also exhibit right ventricular (RV) dysfunction and impaired myocardial contractility, as well as RV hypertrophy [71, 74, 83]. These changes have been seen mostly in early-onset PE and can persist during the first year postpartum [74, 81].

Both early and late onset PE may have left and/or right ventricular impaired diastolic function with cardiac remodeling; meanwhile, about 20% of women with preterm PE, severe disease, and PE associated with IUGR (intrauterine growth restriction) undergo severe LV hypertrophy, advanced cardiac dysfunction with global biventricular dysfunction, both systolic and diastolic [81, 84, 85].

#### 1.6.3 Ventriculo-arterial coupling

Ventriculo-arterial coupling (VAC) represents the performance of the cardiovascular system. VAC explains the interaction between the energy produced by the LV pumping function and the vascular arterial load; the loss of this interaction could raise VAC. Analyses of VAC depend on the variables such as the arterial elastance Ea, and LV end-systolic elastance Ev. VAC is the ratio between the two, VAC= Ea/Ev. A level exceeding 1.3 is high, and it is associated with more energy loss and lower stroke work effectiveness [86, 87].

VAC facilitates the physiological range of blood pressure and cardiac output by promoting maximal cardiac energy, power, and functional efficiency [18, 87].

In normal pregnancy, the VAC is lower during early pregnancy due to lower arterial compliance, and it increases progressively toward term [15].

Alterations in VAC in women with PE have been reported previously [86]. VAC is more altered in severe PE (HELLP syndrome) 4 years after delivery [88].

### 1.6.4 Levels of brain natriuretic peptide

Brain natriuretic peptide (BNP) is known to be a hormone. It is produced and released as a response to stretching and extending of cardiomyocytes. In preeclamptic women, the increased level of NT-pro-BMP in the antepartum period is correlated with cardiac dysfunction [89, 90]. A rise in NT-pro-BNP may result from cardiac remodeling and impaired ventricular function associated with PE [71].

### 1.6.5 Cardiac Troponin

Troponin is a cardiac-specific protein, that appears in the blood when there is injury or damage to the cardiomyocytes. In a normal pregnancy, there is no elevation in the level of troponin. The level of troponin continues to be within the normal range, even in women with PE. However, for women with severe PE, the level of troponin could increase successively during labor, reaching its highest level during the third stage of labor[89]. Determining a higher level of troponin in these preeclamptic women suggests that other pathology or myocardial damage might already be present before the diagnosis of PE and not PE per se [71, 89, 91].

## 1.7 Long-term follow-up and consequences after preeclampsia

There is increased awareness of the increasing risk of long-term adverse health outcomes in women affected by PE [3, 7, 92].

Long-term follow-up epidemiological studies have demonstrated a relationship between PE and cardiovascular morbidity and mortality later in life. PE is associated with a 3.7-, 2.16-, and 1.8-fold relative risk for the development of hypertension, ischemic heart disease, and cerebrovascular accidents, respectively, after a mean of 10–15 years following index pregnancy [3]. Others showed a threefold increased risk of chronic hypertension and a twofold increased risk of CVD-related mortality [4, 7]. Moreover, women with a recurrent event of HDP have shorter life expectancy than women with normal pregnancies (48.9 versus 55.5 years) [93]. Women with recurrent PE have higher risk of cerebrovascular accidents (30%), ischemic heart disease (30%), and myocardial infarction (32%) [4].

Others found that women with more severe and early-onset PE have common risk factors for CVD, such as elevated fasting blood glucose, insulin, lipid level, and serum cholesterol, several years after PE, compared with women with late-onset PE or gestational hypertension [94, 95].

Since 2011, the American Heart Association (AHA) has recognized a history of pathologic pregnancy, including PE, eclampsia, PIH, and gestational diabetes, as independent risk factors for CVD [96].

It is still unclear why preeclamptic women have increased risk for CVD. There are two possible mechanisms. One explanation or mechanism is that PE itself could induce long-term metabolic, vascular-endothelial, or cardiac dysfunction that might enhance the increasing risk for the development of CVD later in life [97].

The other mechanism is that women with PE have a pre-existing CVD risk profile before pregnancy. The maternal metabolic stress manifested during pregnancy could cause these preexisting factors to manifest as PE. The metabolic stress usually disappears after pregnancy, and the women go into a pre-pregnancy state. Later in life, these pre-existing, predisposing factors are illustrated as CVD as shown in Figure 7 [92, 98, 99].

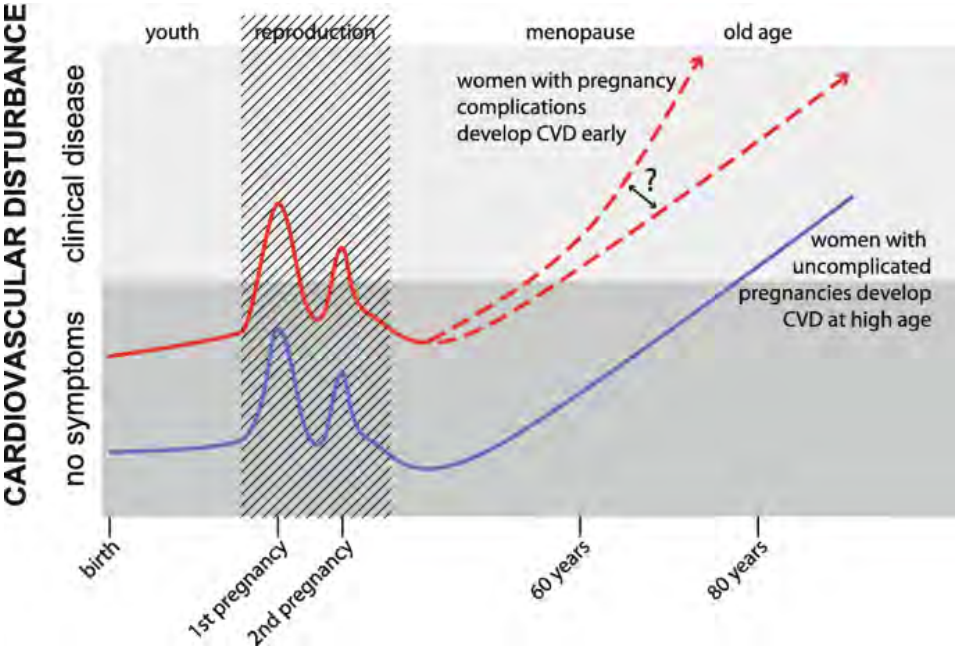


**Figure 7:** The future cardiovascular risk in women with preeclampsia[100]. CVD: cardiovascular disease. Reproduced with permission from Springer Nature.

# 2 Research aims

- Understand the mechanisms behind the increased risk for CVD in women with previous PE.
- Find early markers to identify the most vulnerable women for CVD.
- Does endothelial dysfunction present in women with PE persist over a prolonged period?
- Compare the CV risk profile in women with recent PE and those with normal pregnancy and do these potential differences persist over a prolonged time?

# 3 Materials and methods

## 3.1 Ethical Considerations Study I and II

The Ethics Committee in Stockholm approved the study, and all procedures followed were following institutional guidelines. All participants gave their written consent to participate in the study. Study I and II: Stockholm (2005/318-31/3,2008/806-32).

### 3.1.1 Study I and II:

The involved data collected from electronic medical records. Opening medical records without consent from individual patients could be perceived as a violation of personal integrity, but at that time, it was acceptable when searching for retrospective data information. To protect the women's integrity, the data and the questionnaire were pseudonymized. The data collected is protected by using identified numbers in files. The code for social security numbers is only available for responsible researchers and is locked.

The studies involved the use of ultrasound examination of the large vessels of the forearm and the neck and assessed the function of the heart. To assess the blood vessels' ability to dilate, we measured the diameter of the blood vessels at rest and during increased blood flow. To increase blood flow, a cuff is placed on the forearm. The cuff is inflated to high pressure, and the pressure is maintained for 4.5 minutes. This could cause local pain and discomfort but is completely harmless. The discomfort disappears as soon as the stasis is released. To test the vessel's ability to dilate, we used a nitroglycerine spray of 0.4 mg under the tongue and measured the vessel diameter 4 minutes later. This is harmless, but the woman might have a drop in blood pressure or dizziness that usually passes after 10 minutes.

The ultrasound examination of the carotid arteries is a routine examination and does not cause any discomfort. Cardiac function was assessed with Doppler echocardiography, which is a non-invasive routine examination that is harmless. The examinations took place in two hospitals: Karolinska University Hospital and Danderyd University Hospital, and on two separate occasions, which was uncomfortable for some women.

The examinations were done by certified experienced personnel. The women were given oral and written information about the possible side effects before signing consent for participation. If the women needed further evaluation, according to the results we found, they would be informed and referred to the primary health center.

### 3.2 Subjects in Study I and II

All women included in the Study I and II in this thesis were initially identified retrospectively from the delivery records at the Department of Obstetrics at Karolinska University Hospital over 2 years as previously described[101]. Preeclamptic women were diagnosed according to a standard recommended definition of ISSHP (International Society for Studying Hypertension in Pregnancy). However, all participants were otherwise healthy and non-smokers with no known cardiovascular risk factors, normal menstrual cycles, and no ongoing hormonal therapy or other drug treatment. All women were initially investigated 15 ± 3 months after the index pregnancy with a focus on vascular function, no cardiac assessments were done at that time [101]. Finally, 18 women with a pregnancy complicated by PE and 17 healthy women with normal uncomplicated pregnancies as a control group matched for age, parity, and date of delivery were included [101].

All participants were again invited 11.2 ± 0.6 years after the index pregnancy to undergo cardiac and vascular examinations. Two women in the PE group and one in the control group were unwilling to attend. The index pregnancy was complicated by early and severe PE in six women. All participants received a questionnaire on and history of subsequent pregnancies. Nine women in the PE group and all except one in the control group had been pregnant again. The mean parity at the time of the current examination was 1.9 ± 0.9 in the PE group and 2.2 ± 0.7 in the control group with a median value of 2 in both groups. The current examinations were performed 7.9 ± 3.3 and 6.6 ± 2.4 years after the last pregnancy in the PE group and control group, respectively (Table 1).

**Table 1: Clinical characteristics**

| Variables | Control group | Preeclampsia group | P value |
|---|---|---|---|
| Number | 16 | 15 | - |
| **Index pregnancy** | | | |
| Pregnancy length (days) | 281±6 | 245±6 | <0.001 |
| Birth weight, g | 3766±195 | 2557±208 | <0.001 |
| **11 years after delivery** | | | |
| Age, years | 41.2±3.2 | 39.4±3.6 | 0.14 |
| Body mass index, kg/m$^2$ | 23.3±3.1 | 25.8±6.1 | 0.099 |
| Body size area, m$^2$ | 1.90±0.04 | 2.01±0.04 | 0.083 |
| Parity, n | 2.5±0.7 | 1.8±0.9 | 0.18 |
| Current smoker, n | 1 | 0 | 0.5 |
| Current hypertension, n | 0 | 1 | 0.45 |
| Current diabetes mellitus, n | 0 | 0 | - |

### 3.2.1 Blood pressure measurements

Brachial blood pressure was obtained supine by an oscillometric device (OMRON 705IT, OMRON Healthcare, Kyoto, Japan) on the right arm with an appropriately sized cuff as a mean of three readings 1 min apart. Ambulatory blood pressure during 24 h (ABPM) was recorded with a Spacelabs 90207 device (Spacelab Healthcare, Issaquah, WA, USA) and a cuff of the appropriate size on the non-dominant arm. Readings were made every 20 min throughout. Default automatic editing was used, and all recordings had >80% valid measurements. The recorded values were first averaged for each hour, and values for daytime, nighttime, and 24 h were subsequently calculated from these hourly averages. We defined daytime as 1000–2000 hours, and nighttime as 0000–0600 hours. Analyses of the provided data were performed.

### 3.2.2 Pulse wave analysis

Applanation tonometry was performed using a SphygmoCor device (AtCor Pty, West Ryde, NSW, Australia). Radial artery waveforms were calibrated using brachial systolic and diastolic blood pressures; the central aortic waveform was

calculated by device software using the generalized transfer function, and central blood pressure values were derived. The augmentation index was measured through the software.

### 3.2.3 Assessment of endothelial function

*3.2.3.1 Endothelial function*

The principles of the FMD method were described above, Brachial artery flow velocity, endothelium-dependent flow-mediated vasodilatation (EDVD) was assessed by ischemia-induced reactive hyperemia in the non-dominant arm according to Corretti MC et al[102]. The measurements of the brachial artery diameter were taken non-invasive by using a Vivid 7 Dimension (GE Medical System, Horten, Norway) ultrasound device with a 9-MHz linear array transducer. All images were stored for later analyses. The mean values of three measurements of arterial diameter performed at end-diastole were calculated at rest at 30, 60, and 90 s after cuff release.

To assess endothelium-independent vasodilatation (EIVD), 0.4 mg glyceryl trinitrate was given as a sublingual spray after a washout period of at least 10 min to regain stable resting conditions. Relative changes in brachial artery diameter were calculated from rest to 4 min following drug administration.

*3.2.3.2 Analysis of pulse waveform*

Endothelial function was also assessed on an adjacent day by β-2 adrenergic agonist-induced changes in the pulse waveform [103]. By using applanation tonometry the radial artery pulse waves were recorded. The maximal systolic peak and the reflected waves were identified by the calculations of the first and second derivatives of the pulse curve. The relative height of the diastolic reflected wave (that is, the reflection index) was used as an index of endothelial function.

After a recording under resting conditions, 0.25 mg terbutaline (Bricanyl, AstraZeneca, Mölndal, Sweden) was given subcutaneously in the upper forearm, and reevaluation of the pulse wave was performed after 15 and 20 min. The maximal relative change was used. A large reduction in the reflection index indicates a good response.

### 3.2.4 Biochemical analyses

Routine blood chemistry was assessed by standard procedures. Blood was obtained from an into tubes (Becton Dickinson, Cedex, Meylan, France) with appropriate additives.

Creatinine, cystatin C, and high-sensitive C-reactive protein (Hs-CRP) were measured in plasma by enzymatic colorimetric methods on an Architect Ci8200 analyzer (Abbott, Abbot Park, IL, USA). The estimated glomerular filtration rate was calculated from cystatin C measurements. Glucose and insulin were analyzed by standard procedures was calculated by the (HOMA) as 0.167 × mmol/L fasting glucose × pmol/L fasting insulin/22.5 and by the quantitative insulin sensitivity check index (QUICKI) formula as 1/log mmol/ $l^{-}$ fasting glucose × log pmol /l fasting insulin. Fasting plasma lipoproteins, (LDL) and high-density lipoproteins (HDL) were measured.

Pentraxin 3, tissue necrosis factor (TNF) receptor 1, intercellular adhesion molecule-1 (ICAM), vascular adhesion molecule-1 (VCAM), soluble fms-like tyrosine kinase-1, placental growth factor (PlGF), cathepsin B and cathepsin S were analyzed, by enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA). These biomarkers were analyzed in plasma. Coefficients of variation were all <8%.

Amino-terminal pro-brain natriuretic peptide (NT-pro-BNP) was analyzed on a Cobas EE instrument (Roche Diagnostics).

### 3.2.5 Echocardiography

#### *3.2.5.1 Two-dimensional Doppler echocardiography*

All examinations were performed in the left antecubital position with a Vivid 7 ultrasound system (General Electric, Horten, Norway) equipped with a phased array 3.5 MHz transducer (Doppler frequency 5.0–3.5 MHz). All images were digitally stored for offline analysis using an Echo PAC workstation (version 5.1.0, General Electric, Horten, Norway). Measurements were performed on a minimum of three cardiac beats, from which mean values were calculated.

Measurements of LV dimensions were performed in end-systole (LVDes) and end-diastole (LVDed). Interventricular septum (IVS) and posterior wall thickness (PWT) were measured in diastole from M-mode recordings. LV mass was calculated as (0.8 × [1.04 × ([LVDed + IVS + PWT]$^3$ - LVDed$^3$) + 0.6 g]) and divided by body surface area to obtain LV mass index. Relative wall thickness was calculated as (IVS + PWT)/LVDed. LV volumes and EF were calculated using the modified Simpsons rule from LV area tracings in four- and two-chamber views. LV systolic function was also evaluated by the mitral annular plane

systolic excursion measured by M-mode at four sites (septal, lateral, inferior, and anterior part of the mitral annulus) from an apical view. Left and right atrial areas were measured in a four-chamber view. The RV outflow end-diastolic dimension was measured in a parasternal long-axis view. The RV inflow end-diastolic basal diameter was measured from the apical four-chamber view. Conventional pulsed wave was used for recordings of mitral inflow to evaluate LV. The peaks of early (E) and late (A) mitral flow velocities and mitral annular" e prime" velocities were measured the E/A ratio and E/e prime ratio were calculated. The deceleration time from the mitral E wave was measured.

### *3.2.5.2 Left ventricular global longitudinal strain*

Global LV longitudinal deformation i.e. global longitudinal strain (GLS) was measured by two-dimensional speckle-tracking echocardiography with strain analysis software applied to gray-scale LV images (frame rate 60–80 frames/second) from the apical long axis, four- and two-chamber views as shown in Figure 8. The GLS was calculated automatically and expressed as a percentage change from the original dimension and presented as a negative value reflecting myocardial shortening duration contraction.

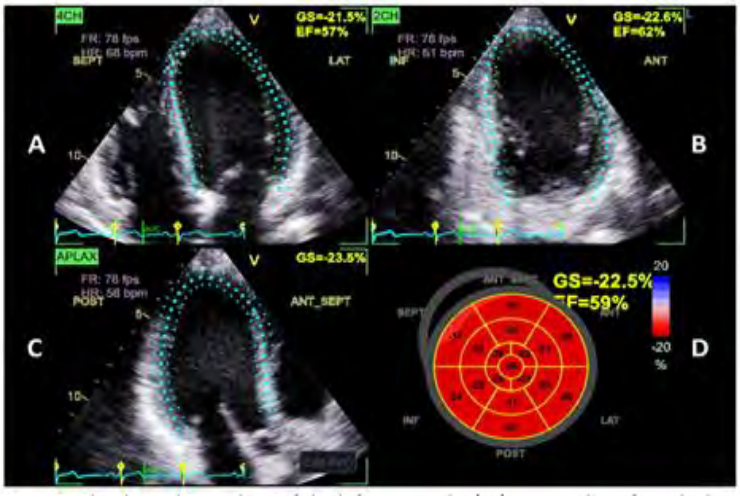


**Figure 8**: Images A, B and C show the tracking of the left ventricular (LV) myocardium for calculation of the global strain (GS) in the 4-chamber, 2-chamber and apical long axis view of the LV. Images A and B are used for the calculation of LV ejection fraction (EF). In D the bull's-eye plot shows segmental and global values of LV GS, the global GS is – 22.5% and bi-plane EF 59%.

### *3.2.5.3 Ventricular-arterial coupling*

Indices of ventricular-arterial coupling were measured as effective arterial elastance (LV end-systolic pressure/stroke volume), LV end-systolic elastance (LV end-systolic pressure/LV end-systolic volume), ventricular-arterial

coupling ratio (effective arterial elastance/LV end-systolic elastance), total (stroke volume/ [SBP - DBP]), and (mean arterial pressure/cardiac output).

### 3.2.6 Statistics

Study I. Results are presented as mean values± SD or proportions, as appropriate. Skewed variables were logarithmically transformed. Student's *t*-test or a two-way repeated measures analysis of variance was used to assess continuous data, and the $\chi^2$-test was used for variables in contingency tables. Pearson's correlation coefficient was used to measure the association between the variables. A probability (*P*) <0.05 was considered statistically significant. JMP version 10 (SAS Institute Inc., Cary, NC, USA) was used.

Study II. Results are presented as mean values ± SD or proportions, as appropriate. Student's *t-test* or the Mann-Whitney test was used to assess continuous data, and the $Chi^2$ test was used for variables in contingency tables. Pearson's correlation coefficients were obtained by analyses to assess associations for continuous data. A probability (*P*) < .05 was considered statistically significant. STATA version 10 (Stata Corp, College Station, TX, USA) and JMP version 11.2 (SAS Institute Inc., Cary, NC, USA) were used.

The sample size in our studies were based on was small. However, several studies of endothelial function show that clinically relevant differences can be demonstrated in small materials which indicates that we have sufficient number of subjects in our study. Additionally, we show a clear effect in our material regarding FMD [104].

# 4 Results

## 4.1 Ambulatory blood pressure measurement

The blood pressure profile is shown in Table 2. The preeclamptic women had slightly higher blood pressure than the control group. However, there was a significant difference in the night /day SBP and DBP ratios (0.81±0.06 vs 0.76±0.05, and 0.88±0.04 vs 0.84±0.04; both p<0.05. In women with PE, the MAP was higher during pregnancy and at one-year post-partum however, normalized 11 years post-partum.

## 4.2 Biochemical findings

The PE group had higher levels of TNF receptor 1, while other markers of inflammation were similar in the two study groups. ICAM increased in the PE group. There was a trend for impaired glucose tolerance in patients with a history of PE. Glucose tolerance was inversely related to body mass index (for example, HOMA: $r^2$=0.58, $P$<0.001; QUICKI: $r^2$=0.26, $P$<0.01). Blood lipids, brain natriuretic peptide levels, and renal function did not differ between the groups. For details se Study 1.

**Table 2: Blood pressure profile including 24-hour ambulatory blood pressure**

| Variables | Control | Preeclampsia | P |
|---|---|---|---|
| Number | 16 | 15 | - |
| **11 years after the index pregnancy** | | | |
| Heart rate, beats/min | 58 ± 9 | 59 ± 11 | >0.5 |
| Supine systolic BP, mm Hg | 111 ± 11 | 117 ± 14 | 0.18 |
| Supine diastolic BP, mm Hg | 70 ± 2 | 75 ± 2 | 0.16 |
| Mean arterial pressure, mm Hg | 92 ± 10 | 93 ± 9 | 0.23 |
| Mean 24 h systolic ambulatory BP, mm Hg | 112 ± 11 | 117 ± 11 | 0.22 |
| Mean 24 h diastolic ambulatory BP, mm Hg | 71 ± 9 | 75 ± 8 | 0.28 |
| Night/day ratio, systolic ambulatory BP | 0.76 ± 0.05 | 0.81 ± 0.06 | 0.022 |
| Night/day ratio, diastolic ambulatory BP | 0.84 ± 0.04 | 0.88 ± 0.04 | 0.021 |
| **Index pregnancy, at gestation week 12** | | | |
| Supine systolic BP, mm Hg | 114 ± 11 | 115 ± 10 | >0.5 |
| Supine diastolic BP, mm Hg | 68 ±6 | 68 ± 9 | >0.5 |
| Mean arterial pressure, mm Hg | 83 ±6 | 85 ± 10 | >0.5 |
| **Index pregnancy, at delivery** | | | |
| Supine systolic BP, mm Hg | 119 ± 13 | 169 ± 25 | <0.001 |
| Supine diastolic BP, mm Hg | 75 ± 11 | 113 ± 16 | <0.001 |
| Mean arterial pressure, mm Hg | 90 ± 11 | 128 ± 19 | <0.001 |
| **1 year after index pregnancy** | | | |
| Supine systolic BP, mm Hg | 115 ± 9 | 122 ± 8 | 0.023 |
| Supine diastolic BP, mm Hg | 76 ±7 | 81 ± 6 | 0.017 |
| Mean arterial pressure, mm Hg | 89 ±7 | 96 ± 7 | 0.027 |

*BP, blood pressure; SD, standard deviation. Mean values ± SD.*

## 4.3 Endothelial function

The ultrasound examination of the BA showed that Endothelial-dependent and -independent vasodilation, induced by post-ischemic hyperemia and glyceryl trinitrate, respectively, was similar in the two study groups. No differences were found in the ratios, post-ischemic hyperemia-/glyceryl trinitrate-induced responses were similar in the PE group and the control group. Endothelial function assessed by pulse wave analysis and terbutaline also revealed no difference between the study groups as shown Figure 9.

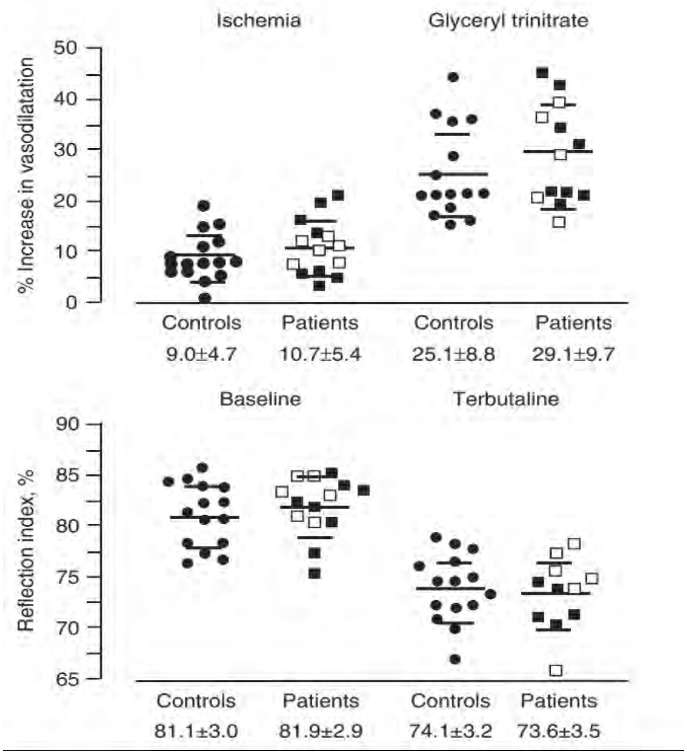


**Figure 9:** Dependent and independent Flow-mediated vasodilatation dependent and-independent vasodilatation, induced by post ischemic hyperemia and glyceryl trinitrate (top panel) and endothelial function assessed by pulse wave analysis and the reflectance index before and administration of terbutaline (bottom panel) in patients with preeclampsia (squares) and control subjects(circles) 11years after the index pregnancy. Mean values ±s.d. for 11–15 patients and 14–15control subjects. Open symbols denote six patients with severe preeclampsia delivered before 34 weeks of gestation.

## 4.4 Comparisons between measurements 1 and 11 years of follow-up

During 1 to 11 years of follow-up, FMD was unchanged in the control group, whereas endothelial function in the PE group was normalized during follow-up. Hs-CRP decreased from 1 to 11 years ($P$<0.001), similar in both groups. Systolic and diastolic blood pressures increased in both study groups during the 11 years

of follow-up. Furthermore, blood pressures appeared to be consistently higher in the PE group. Also 24h ambulatory blood pressures gave similar results, with a trend for higher systolic and diastolic values in the PE group. Body mass index remained higher in the PE group during follow-up and the PE group also displayed reduced glucose tolerance Figure 10.

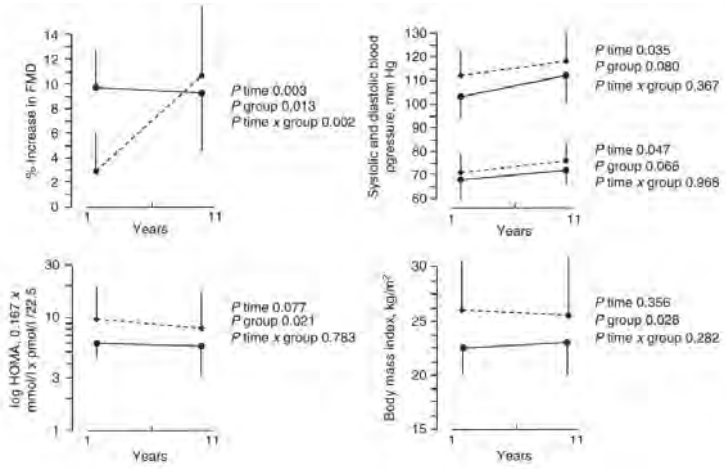


**Figure 10:** *Changes between 1 and 11 years after index pregnancy in blood pressure, endothelial function (FMD), body weight mass index and glucose tolerance (HOMA). Broken lines represent 15 patients with preeclampsia and solid lines 16 control subjects. FMD, flow-mediated dilatation; HOMA, homeostasis model assessment. Mean values and S.D. are presented.*

## 4.5 Echocardiographic variables

There were no significant differences in LV and RV dimensions, systolic function, or LV GLS. Indices of diastolic LV function left, and right atrial size did not differ between our study groups. EA/ELV was 0.53(0.45-0.6) in PE and 0.52(0.48-0.61) in controls, p=0.60 (Table3).

There was no significant correlation between NT-pro-BNP and cardiac variable LA area, LA area, RWT, EF or LVM.

**Table 3: Echocardiographic variables**

| Variables | Control | Preeclampsia | P |
|---|---|---|---|
| Number | 16 | 15 | - |
| LV end-diastolic diameter, mm | 47 ± 3 | 48 ± 4 | 0.45 |
| LV mass index, g/m2 | 83 ± 13 | 81 ± 17 | >0.5 |
| Relative wall thickness | 0.39 ± 0.04 | 0.36 ± 0.06 | 0.24 |
| **Systolic function** | | | |
| LV ejection fraction, % | 64 ± 5 | 66 ± 4 | 0.31 |
| MAPSE, mean, mm | 15 ± 1 | 14 ± 1 | 0.20 |
| LV global longitudinal strain, % | -19.2 ± 1.9 | -19.2 ± 3.0 | >0.5 |
| RV myocardial max systolic velocity, cm/s | 12.9 ± 1.8 | 13.4 ± 1.5 | >0.5 |
| **Diastolic function** | | | |
| E/A ratio | 1.9 ± 0.6 | 1.8 ± 0.6 | >0.5 |
| E-deceleration time, ms | 196 ± 26 | 193 ± 14 | >0.5 |
| E/e prime ratio mean | 6.3 ± 1.4 | 6.8 ± 1.2 | 0.39 |

*LA left atrial; LV, left ventricular; RA, right atrial; RV, right ventricular; A, peak late Doppler velocity of transmitral flow; E, peak early Doppler velocity of transmitral flow; e prime peak early diastolic myocardial tissue Doppler velocity; e' mean, the mean value of lateral, septal, anterior, and inferior e'; LV, MAPSE, mitral annular maximal systolic excursion measured by M-mode; Mean values ± SD standard deviation*

## 4.6 Arterial stiffness and ventricular-arterial coupling

Indices of arterial stiffness (that is, pulse wave velocity and augmentation index) were similar in the two study groups 11 years after the index pregnancy. Central blood pressure was similar in both groups. None of the indices of ventricular-arterial coupling differed between the two study groups (Table4).

**Table 4: Assessment of aortic stiffness and ventricular-arterial coupling by echocardiography.**

| Variables | Control | Preeclampsia | P |
|---|---|---|---|
| Number | 16 | 15 | - |
| Aortic diameter, mm | 26.1 ± 3.2 | 24.4 ± 2.0 | 0.10 |
| Central systolic BP, mm Hg | 101 ± 12 | 108 ± 15 | 0.16 |
| Central diastolic BP, mm Hg | 71 ± 10 | 76 ± 10 | 0.23 |
| Pulse wave velocity, carotid-femoral, m/s | 6.32 ± 0.46 | 6.26 ± 1.01 | >0.5 |
| Pulse wave velocity, carotid–radial, m/s | 7.91 ± 1.11 | 8.12 ± 1.06 | >0.5 |
| Pulse wave velocity ratio, carotid-femoral/carotid-radial | 0.81 ± 0.10 | 0.78 ± 0.15 | >0.5 |
| Augmentation index, % | 21.1 ± 10.3 | 25.0 ± 8.2 | 0.26 |
| Total peripheral resistance, mm Hg/mL | 33.3 ± 16.6 | 29.9 ± 5.5 | 0.50 |
| Total vascular compliance, mL/mm Hg | 2.7 ± 0.7 | 2.8 ± 0.7 | >0.5 |
| LV end-systolic elastance, mm Hg/mL | 3.39 ± 0.77 | 3.94 ± 0.97 | 0.13 |
| Effective arterial elastance, mm Hg/mL | 1.48 ± 1.15 | 1.69 ± 0.73 | >0.5 |
| Ventricular-arterial coupling ratio | 0.59 ± 0.21 | 0.51 ± 0.10 | 0.24 |

*BP, blood pressure; LV, left ventricular. Mean values ± SD.*

## 4.7 Correlations

No significant correlations between Aix and PWV and LVMI, LA area, diastolic variables, LV global strain, Night/day ratio, VAC, and E/e prime mean were found in the whole study group. No significant correlation between MAP and LVMI, LA area, VAC and E/e prime.

## 4.8 Comparison of women with a history of PE, according to severity of PE

In sub-group analyses according to the severity of PE; women neither early and severe PE (n=9) and women with early and sever PE (n=6). The women diagnosed with early and severe PE had a greater night/day ratio for ambulatory SBP and DBP. No differences in NT-pro-BNP levels were found between the groups. However, these two subgroups of PE did not differ concerning cardiac structure and function, and indices of ventricular-arterial coupling.

# 5 Discussion

## 5.1 Long-term assessment of endothelial function

In this long-term follow up study in women with history of PE 11 years earlier, we found no persisted endothelial dysfunction despite using different methods for assessing endothelial function, including FMD and PWA. When the same women were investigated one year after PE-complicated pregnancy we found impaired endothelial function [101]. Women with normal uncomplicated pregnancy in our study and normal endothelial function at one-year had still normal endothelial function at long-term follow up.

Finding impairment of endothelial function and increased arterial stiffness in early pregnancy could precede the development of HTP, including PE. Our research team has previously shown that preeclamptic women, especially those with early-onset and severe PE, have impaired endothelial function; these changes persist several months postpartum [101]. A few other small studies showed persistently impaired endothelial function up to 3 years postpartum [105].

A systemic review by Weissgerber included 37 studies assessing endothelial function using FMD [106]. Some studies showed that preeclamptic women with pre-existing risk factors had impaired FMD already before the development of PE. Preeclamptic women had lower FMD at the time of diagnosis and tended to have lower FMD three years postpartum [106]. Meanwhile, PE was not associated with impaired FMD when assessed at 10 years[106].

Our finding of normalized endothelial function many years after PE does not exclude the increased risk for CVD in the future, on contrary, these findings suggest that the pre-existing risk factors present already before pregnancy are more important than the impaired endothelial function associated with PE.

The consideration of all women with a history of previous PE as a potentially predisposed to future CVD highlights the importance of a carful pregnancy history documentation in routine clinical assessment. This practice could optimize cardiovascular risk stratification and enable early implementation of preventive strategies.

## 5.2 Blood pressure

In our long-term study, we found that the 24-hour ambulatory blood pressure at night and night/day ratios were significantly different in preeclamptic women in

comparison with the control group. Preeclamptic women had slightly higher blood pressure levels than the control group. Only a few studies evaluate 24-hour ambulatory blood pressure (systolic and diastolic BP) after PE. Short-term follow-up study by Ditisheim showed that the women with PE found to have persistent rises in day and night systolic and blood pressure 6–12 weeks postpartum [107]. Women with severe and early PE had elevated SBP, DBP and the MAP 1-2 years after delivery [74]. A long-term cohort of 2-12 years following PE by Mangos et al. showed increased nocturnal blood pressure in women with PE [108].

PE is associated with a 3.7-fold higher risk for developing hypertension [3]. A study by Lewington found that an increase in SPB >20 mm Hg and DPB >10 mmHg in previously normotensive women (115 systole and 75 diastole) is associated with 2-fold risk for death caused by CVD [109].

Hypertension can be caused by endothelial dysfunction and, at the same time can be a risk for other vascular disease, there is a correlation between hypertension and cerebrovascular events and heart infarction [110]. Preeclamptic women have an increased risk of developing what is called white-coat or masked hypertension, making 24-hour AMBP a good tool to use for the assessment of blood pressure for these women. AMBP provides better information than office-BP and predicts CVD. Additionally, nocturnal blood pressure contributes to disease prognosis and increases cardiovascular risk [111]. Of note, about 15 % of the women in general have no awareness of carrying high blood pressure [112]. Early high blood pressure detection in younger women may predict the development of PE and future CVD. This requires thorough investigation and early treatment as a preventive measure.

### 5.3 Inflammatory and biochemical markers

Various biomarkers (inflammatory, endothelial, and metabolic markers) have been measured. Regarding the inflammatory and endothelial markers, we found that women with PE have elevated levels of TNF receptors1 and a higher ICAM in the preeclamptic group.

Inflammation contributes significantly to endothelial dysfunction and the pathophysiology of PE. Elevated serum Hs-CRP provides a sensitive biomarker of chronic systemic inflammation and is an independent predictor of future CV events. Cathepsin increases inflammatory activity and the development of CVD by promoting an extracellular matrix in atherosclerotic lesion formation.

As a metabolic factor, we found that women with a previous history of PE have impaired glucose tolerance. Insulin resistance measured by homeostasis model

assessment (HOMA) is higher in women with PE. Women with PE have slightly higher BMI.

PE is associated with a reduction in insulin sensitivity and an increase in insulin resistance. Hyperinsulinemia is associated with the development of PE, thus suggesting that there is some correlation between insulin resistance and the pathogens of PE [113]. Insulin resistance is presented by elevated HOMA found in preeclamptic women 7.8 years after birth, which agrees with our results [114]

According to the WHO, the prevalence of obesity has increased in the last few decades among female populations. Obesity is associated with many obstetric complications, besides preeclampsia. BMI correlates positively with PE; thus, women with obesity have a greater risk of developing PE. The relationship between PE and obesity is not clear. Obesity and PE share many characteristics, like insulin resistance, hyperinsulinemia, changes in lipid profile, rising TNF, and decreased FMD. All these factors contribute to the development of endothelial dysfunction in both PE and obesity and can increase the risk of the future development of cardiovascular diseases [2, 115]. Losing weight before pregnancy is advisable to reduce risk for developing PE and risk for CVD later in life, this can be adjured by more exercises and getting help from dietician for diet control.

TNF receptor 1 is thought to have cardio-protective effect and it is regulated by TNF-α. During inflammation, TNF-α is produced by macrophages. TNF-α was found to increase in many inflammatory diseases. TNF-α enhances the inflammatory cascade within the arterial wall and promotes atherosclerosis-related inflammation. TNF-α levels have been linked to coronary artery disease and ischemic stroke [63].

TG is a big source of cell energy and, at normal levels, has a protective role for the internal organs. HDL has a vascular endothelial protective effect that can remove lipids from blood vessels. In women with hypertensive diseases during pregnancy, the level of lipoproteins increases, causing destruction and injury to the vascular endothelium, while the level of HDL decreases, thus decreasing the vascular protection effect. These changes impair the arterial lumen, and damage and injury to the endothelial cells lead to increases in peripheral vascular resistance, leading to a rise in blood pressure, which is the main future of PE and hypertension[116, 117]. Atherosclerosis may develop earlier in life and could progress in the presence of another risk factor. PE shares similar risk factors with atherosclerosis, suggesting measurement of lipid profile in young women with risk factor should be done

already before conception. Early detection and early treatment of hyperlipidemia can be of a value for young women to prevent development of CV events.

### 5.4 Cardiac function assessment

In our long-term follow up study of cardiac structure and function in women with or without history of PE we found no differences in any of measured echocardiographic variables between the groups. Further, we found no differences in cardiac function when analyzing PE subgroup with or without "early and severe PE ", suggesting that cardiac function may normalize over time independently of the severity of PE. The results on long-term cardiac function after PE vary considerably in the literature.

Cardiac function in our study was assessed also by speckle-tracking echocardiography (STE), which is a two-dimensional (2D) technique for evaluation of cardiac deformation expressed as GLS, which is considered to be a more sensitive measure of systolic LV function than EF. STE is accurate, safe, and widely used to assess cardiac function in pregnant women [118]. By using GLS, Clemmensen et al. observed in a long-term study 12 years after delivery, that women who had early and severe PE had normal LV geometry and systolic LV function by EF, while LV GLS was lower, suggesting that women with early and severe PE have subclinical LV dysfunction [119].

We found no alteration in the LV systolic function. EF and GLS were normal in PE, including severe PE. This finding in line with other studies [86, 120]. However, there are studies showing that women with severe preeclampsia may have persistent impairment of LV systolic function 3–7 years after delivery [74, 121]. Women with HELLP syndrome were found to have lower LV EF 4 years after delivery [16] and women with HDP had lower LV EF many years after pregnancy [122].

Regarding diastolic LV function, E/e prime ratio, is a widely used and accepted estimate of the LV filling pressure. PE was found to be associated with higher E/e prime ratio, lower E/A ratio, and impairment in myocardial relaxation. The elevated E/e prime ratio is thought to be related to pressure overload [71]. We found no differences in the indices of the LV diastolic function in preeclamptic women in comparison with women with normal pregnancies. Evans et al. also reported no differences in LV diastolic function 16 months after delivery [77].

We found normal indices of RV structure and function and no differences between patients with previous PE and control subjects. Only a few studies examined RV dimensions and function in women with PE. Melchiorre et al. revealed RV systolic

and diastolic dysfunction in women with severe and early PE in comparison with late-onset PE; these changes were associated with more severe diastolic LV dysfunction[81]. RV dysfunction and remodeling persisted for one year in women with early-onset PE [81].

PE is often associated with increased arterial stiffness, endothelial dysfunction, and LV dysfunction, causing an alteration in VAC, even though only a few studies have investigated VAC interaction. A persistent rise in VAC has been persisted 6 months to 3 years after PE [86]. One long-term study showed no changes in VAC in preeclamptic women, while only 25% of women with HELLP showed an alteration in VAC 4 years after index pregnancy [88]. We found no differences in the indices of aortic stiffness and ventricular-arterial coupling between the two study groups. Our finding may be explained by the normalizing endothelial and cardiac functions many years after PE.

The levels of NT-pro-BNP were shown to be elevated in preeclamptic women in comparison with a normal-uncomplicated pregnancy [71]. The elevation was correlated with the severity of the PE. levels of NT-pro-BNP decrease considerably 3-6 months post-partum [71]. In our long-term study, we found normal cardiac function and normal levels of NT-pro-BNP in both study groups, which in in line with other studies describing normal NT-pro-BNP many decades after PE [120]. This can be explained by the recovery of cardiac function many years after PE.

Our results suggest that PE per se does not cause persisting significant structural or functional cardiac abnormalities. However, many studies in this area are small and further research is therefore warranted.

### 5.5 Clinical application

At 11 years of follow-up, we found that preeclamptic women showed subnormal blood pressure profiles with increased diastolic blood pressure and an increased night-day ratio. This finding suggests that it is advisable to have strict follow up as well as thorough and cautious screening and control of CVD risk profiles.

Ultrasound-based FMD test is a safe, and non-invasive method which in research studies is valuable for early detection of impaired FMD even in asymptomatic or healthy subjects. Few studies have examined FMD in pregnant women between 11 and 29 gestational weeks; studies noted impaired FMD in women with high risk before the development of PE [106]. This finding considers FMD not only a valuable

test for the evaluation of CVD but may also be valuable for identification of young women with an increased risk for PE.

In Sweden, cardiovascular diseases are the leading cause of death in women (51%), which is higher than the mortality rate globally (32–34%) [123]. Literature has proven the association between PE and the heightened risk of cardiovascular diseases. Women with severe and early onset PE have increased risk, in addition, women with recurrent PE have a double risk for developing CVD and have shorter life spans [4]. Death caused by CVD occurs more often in women younger than 55, this fact highlights the importance of focusing on PE as a risk factor for CVD in women [124].

In the last decades, both the American Heart Association (AHA) and the European Society of Cardiology (ESC) have accommodated HDP, including PE, as an independent risk factor for CVD and recommended medical examination of blood pressure and assessment of metabolic factors [96].

A large proportion of obstetricians are aware of the CV risks associated with PE [125], whereas only 40% of the physicians are aware of this risk and advise on it [126]. Moreover, preeclamptic women generally have no awareness of the increased CV risk associated with PE [127]. Only a few countries have established postpartum follow-up recommendations; one of them is Sweden. The Swedish Society of Obstetrics and Gynecology (SFOG) recommends a yearly control of blood pressure at the primary health center. The German Association of Gynecology and Obstetrics (DGGG) recommends short-term medical control of blood pressure and renal function three months after delivery [128]. The Society of Obstetric Medicine Australia and New Zealand (SOMANZ) recommends 5-year control of blood pressure, metabolic factors, and lifestyle evaluation [129].

It is of great importance to integrate global guidelines with recommendations for preventive measures, educational activities, screening programs, and long-term follow-up. It is even more important that these guidelines are discussed and recommended by experienced consultants of different specialties, including obstetricians, cardiologists, and general practitioners.

Young women could develop hypertension when exposed to stress [130]. Pregnancy acts as a stress test for the women. Women with predisposing factors are more likely to develop PE. Our long-term follow-up of women with PE showed healing of the cardiac and vascular function seen during and early after PE, this finding does not exclude the increased risk for future risk of CVD. Early

management and treatment of possible risk factors is of great value for these young women. Further, long-term surveillance with close monitoring is suggested, this cannot be achieved without clear guidelines, more awareness about the disease (both the women and the medical teams) and most importantly women's medical compliance. Figure 11 shows my suggestion for a follow up protocol, it would be appropriate to monitor the preeclamptic women until their fifty decades, when they become eligible for the other established international cardiovascular risk screening and assessment protocols.

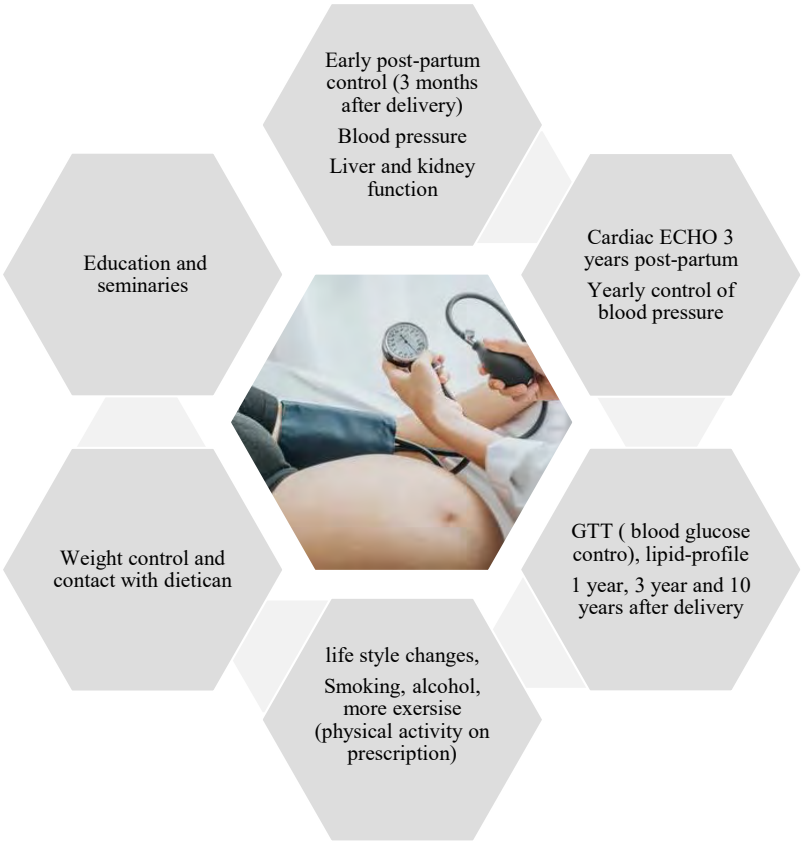


Figure 11: Suggested follow-up strategies for women with preeclampsia

# 6 Conclusions

- Endothelial dysfunctions present 1 year after pregnancy, were normalized 11 years after index pregnancy.
- Preeclamptic women have slightly higher blood pressure.
- Preeclamptic women have higher BMI, insulin resistance, and higher glucose levels which could indicate a higher risk for metabolic syndrome.
- We could not demonstrate alterations in systolic or diastolic left or right ventricular function, or in ventriculo-atrial interaction in women 11 years after a pregnancy complicated by PE, despite sensitive echocardiographic technique suggesting full recovery of cardiac changes after many years.
- Our finding of normalized cardiovascular- function many years after PE suggests that pre-existing risk factors may be more important for future cardiovascular complications than myocardial and vascular dysfunction apparent during pregnancy in women with PE.

# 7 Points of perspective

- Women who experienced PE, especially severe and early-onset, have risk factors for the cardiovascular sequelae. However, due to their young age, they are excluded from preventive and screening strategies. The delay in diagnosis could have an impact on the future health of these women.
- Many important questions remain unanswered: Should all women with risk factors be investigated and screened before conception? And should those who develop hypertensive disease in pregnancy be screened for their CVD risk early after delivery? Is it possible to reduce the risk of developing CVD by educating women who have had a history of PE regarding the risks of the disorder in future pregnancies and the future risk of vascular disease? Could Lifestyle changes prevent the development of CVD? Can Asprin make difference?
- It is important to investigate the possibility of integrating guidelines for long-term follow-up and assessment of CVD risk into clinical practice and implementing preventive measures strategies as early as after pregnancy and in the early puerperium. These guidelines may include regular control for blood pressure, blood lipids, blood glucose, and biomarkers, as well as regular assessments of cardiovascular function. Other prophylactic strategies that could be of great value, such as adjusting lifestyle, exercise habits, and diet, are also important to reduce long-term CVD morbidity and mortality in women with a history of preeclampsia.
- Finally, Preeclampsia has been a target for many researchers for more than 100 years, but PE is still a mystery disease. Studies usually exclude women with cardiovascular factors, which makes it difficult to assess these risk factors as a causal factor for PE. It can be of great value to start an international, global, and multicenter register with continuous reporting and cardiovascular assessment during pregnancy and at postpartum follow-up. The register should include both women with and without pre-existing CV risk factor.

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