Carotid Artery Wall Layer Dimensions during and after Pre-eclampsia

An investigation using non-invasive high-frequency ultrasound

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Abstract

Pre-eclampsia is associated with increased risk of cardiovascular disease (CVD) later in life. The ‘gold standard’ for estimating cardiovascular risk - ultrasound assessment of the common carotid artery intima-media thickness (CCA-IMT) - does not convincingly demonstrate this increased risk. The aim of this thesis was to examine whether high-frequency (22 MHz) ultrasound assessment of the individual CCA intima and media layers and calculation of the intima/media (I/M) ratio - can indicate the increased cardiovascular risk after pre-eclampsia. After validation of the method in premenopausal women with systemic lupus erythematosus (SLE) who have a recognized increased risk of CVD, women during and after normal and preeclamptic pregnancies were investigated.

Assessment of the individual artery wall layers reliably demonstrated the increased cardiovascular risk in premenopausal women with SLE, while CCA-IMT did not. The artery wall layer dimensions in women with SLE were comparable to those of postmenopausal women without SLE and were 30 years older.

Among the women with normal pregnancies negative changes to the artery wall later on in the pregnancy were seen in those with lower serum estradiol, older age, higher body mass index or higher blood pressure early in the pregnancy. About one year postpartum, both the mean intima thickness and the I/M ratio had improved, compared to values during pregnancy. These findings support the theory that normal pregnancy is a stress on the vascular system.

Women who developed pre-eclampsia (mean age 31 years) had thicker intima layers, thinner media layers and higher I/M ratios, both at diagnosis and one year postpartum, than women with normal pregnancies, indicating increased cardiovascular risk.

Women with a history of severe pre-eclampsia (mean age 44 years; mean 11 years since the last delivery) had thicker intima layers and higher I/M ratios than women with a history of normal pregnancies, indicating long-standing negative vascular effects.

Assessment of individual CCA wall layers, but not of CCA-IMT, provided clear evidence of the well-known increased cardiovascular risk in women with SLE or pre-eclampsia. The method has the potential to become an important tool in reducing cardiovascular morbidity and mortality in these women through early diagnosis and intervention.

Keywords: Systemic lupus erythematosus, normal pregnancy, pre-eclampsia, high-frequency ultrasound, common carotid artery, intima/media ratio, cardiovascular disease.
To my parents and
my daughter Tamanna
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV  Akhter T, Larsson M, Wikström A-K, Naessen T. Individual Artery Wall Layer Dimensions Indicate Increased Cardiovascular Risk in Previous Severe Preeclampsia: an investigation using non-invasive high-frequency ultrasound. Submitted

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Abbreviations

ACR American College of Rheumatology
AHA American Heart Association
AUC Area under the ROC curve
BMI Body mass index
CAD Coronary artery disease
CCA Common carotid artery
CCA-IMT Common carotid artery-intima media thickness
CI Confidence interval
CHD Coronary heart disease
CV Coefficient of variation
CVD Cardiovascular disease
DBP Diastolic blood pressure
DHEA Dehydroepiandrosterone
E2 Estradiol
EOPE Early onset pre-eclampsia
ICD International classification of diseases
I/M ratio Ratio of intima thickness to media thickness
IMT Intima media thickness
LDL Low density lipoprotein
LGA Large for gestational age
LOPE Late onset pre-eclampsia
MAP Mean arterial pressure
MHz Mega Hertz
MI Myocardial infarction
PE Pre-eclampsia
ROC Receiver operating characteristic
RR Relative risk
SBP Systolic blood pressure
SD Standard deviation
SGA Small for gestational age
SLE Systemic lupus erythematosus
SLICC Systemic Lupus International Collaborating Clinics
Introduction

Cardiovascular disease

Cardiovascular disease (CVD) is the number one cause of death globally; more people die annually from CVD than from any other cause. The World Health Organization (WHO) estimated that 17.3 million people died from CVD in 2008, representing 30% of all global deaths.¹ Low- and middle-income countries are disproportionately affected by CVD. In the year 2030, it is estimated that more than 23 million people will die from CVD, mainly from coronary heart disease (CHD) and stroke.¹ CVD affects people in their mid-life years, undermining the socioeconomic development not only of the affected individuals but also of their families and nations.

Definition and classification of cardiovascular disease

CVD comprises a group of disorders of the heart and blood vessels which includes:¹

*Atherosclerotic cardiovascular disease*
- CHD
- Cerebrovascular disease
- Peripheral arterial disease

*Non-atherosclerotic cardiovascular disease*
- Rheumatic heart disease
- Congenital heart disease
- Deep vein thrombosis and pulmonary embolism

Risk factors for cardiovascular disease

Clinical studies have identified several factors that increase the risk of CVD. These risk factors can be divided into those that are modifiable and those that are non-modifiable:²

*Non-modifiable risk factors*
- Older age
- Male sex
- Heredity
Modifiable risk factors

- Tobacco smoke
- Dyslipidemia
- High blood pressure
- Diabetes mellitus
- Obesity
- Physical inactivity
- Extensive alcohol consumption
- Stress

Cardiovascular disease in women

CVD is the largest single cause of mortality among women. More than 8.6 million women die of CVD each year around the world; these account for one third of all deaths in women. In Europe 54% of deaths in women and 43% of deaths in men are from CVD. CVD is the main cause of female deaths in Sweden. In the year 2011, 39% of all female deaths in Sweden were caused by CVD. Previous reports have focused primarily on male or shared male and female risk factors where women were under-represented. Further, differences in management and mortality rate between men and women after acute cardiac diseases have been observed. Moreover, it has been reported that women are more likely than men to have unrecognised myocardial infarctions (MIs). The American Heart Association (AHA) has published separate guidelines for CVD prevention in women. Nonetheless, there remains a need for further investigations into female-specific risk factors for CVD and the atherosclerotic process in women.

The onset of CVD occurs approximately ten years later in women than in men, which could be the result of the protective effects of female sex hormones before menopause. Because of this older age at presentation of CVD, women are more likely to also suffer from co-morbidities such as diabetes and hypertension.

Estrogen and its vascular effects in women

The atheroprotective effects of estrogen are thought to be mediated mainly by direct action on the artery wall and alterations to the serum lipid concentrations. The direct effects of estrogen on the artery wall have two components, one is rapid and non-genomic and the other is long term and genomic. Estrogen receptors have been identified in both vascular smooth muscle cells and endothelial cells; these cells are able to bind estrogen with high affinity.

In the Women’s Ischemia Syndrome Evaluation study, Merz et al. showed that hypothalamus-mediated ovarian dysfunction and estrogen deficiency in premenopausal women were associated with premature coronary
artery atherosclerosis. In a similar study, Hanke et al. reported that premenopausal women with angiographically confirmed coronary artery disease (CAD) had lower serum estradiol (E2) concentrations than control subjects.

The common carotid artery

The common carotid artery (CCA) is an elastic artery. The right CCA is a branch of the brachiocephalic artery which arises from the thoracic aorta, whereas the left CCA arises directly from the thoracic aorta. The pulsation is stronger in the upper part of the artery, which lies more superficially than the lower part.

The CCA wall is composed of three layers: the intima, the media and the adventitia, in order from the inside out. The intima is composed of the endothelium and a thin layer of underlying fibrocollagenous tissue. The media is mainly composed of elastic fibres, in contrast to the media of a muscular artery, which is composed almost entirely of smooth muscle. The adventitia is mainly composed of collagen.

Atherosclerosis

Atherosclerosis is the main underlying pathology in CVD. Atherosclerosis develops from oxidized low-density lipoprotein (LDL) molecules. LDL begins to accumulate in the arterial walls when the levels in the blood exceed the capacity of the macrophages to remove it. These lipid depositions lead to an inflammatory reaction in the arterial wall. In response to the inflammation, macrophages are attracted to the arterial wall where they absorb the oxidized LDL to form specialized foam cells. When the foam cells die, they release a large number of products, causing the formation of a lipid core. The foam cells also release growth factors particularly platelet-derived growth factors, which results in proliferation of smooth muscle cells and their migration from the media to the intima, towards the lipid core. Smooth muscle cells synthesize collagen, leading to the formation of a fibrous cap over the affected area, in an attempt to repair and stabilize the lesion. If the repair process is successful, the atherosclerotic plaque will be stable and will remain asymptomatic. If inflammation dominates over the repair mechanisms, the plaque may become active or unstable and this could lead to rupture of the fibrous cap and thrombosis.

Atherosclerosis typically begins in early adolescence. According to an autopsy study, among children aged 10-14 years who died of non-cardiovascular causes, more than half showed some evidence of atherosclerosis. In a recent autopsy study of United States armed forces with a mean
age of 26 years, the prevalence of any coronary atherosclerosis was 8.5%.\textsuperscript{25} Atherosclerotic lesions, which change the vascular anatomy, develop over decades.

**Cardiovascular disease, atherosclerosis and intima-media thickness**

The atherosclerotic process in the artery wall is an important factor in the pathogenesis of CVD. Angiographic and Doppler evaluations have been used for more than 30 years to determine the incidence and development of atherosclerosis, but the evaluations have mainly been restricted to severe atherosclerosis (stenosis).\textsuperscript{26-28} In the late 1980s, advances in ultrasound resolution techniques provided the opportunity to quantify and monitor atherosclerosis from its precursor lesion to occlusive disease using a non-invasive technique.

To assess the early progression of atherosclerosis it is necessary to visualize the artery wall itself. The high coincidence of carotid atherosclerosis with vessel pathology in other vasculature makes it an adequate window for investigating systemic atherosclerosis.\textsuperscript{29-31} The use of peripheral arteries such as the carotid arteries as a vascular endpoint for assessing the extent of atherosclerosis is widely accepted. The present ‘gold standard’ for non-invasive assessment of the development of atherosclerosis is to measure the thickness of the combined intima and media layers of the common carotid artery (CCA-IMT) using 7-10 MHz ultrasound. The AHA recommend using this method; an increase in CCA-IMT is seen as a surrogate marker for CVD.\textsuperscript{32}

Several studies have demonstrated a correlation between an increase in CCA-IMT and the occurrence of cardiovascular risk factors\textsuperscript{33-35} and increased CCA-IMT has been shown to be an independent predictor of future CVD.\textsuperscript{36-40} However, when Wald et al. performed a meta-analysis of 18 studies, they found that CCA-IMT measurement was not a sufficiently reliable screening process to discriminate between individuals with and without CHD.\textsuperscript{41} Further, in another meta-analysis, Costanzo et al. found that a decrease in CCA-IMT did not reflect a reduction in cardiovascular events.\textsuperscript{42} Similarly, Lorenz et al. and Den Ruijter et al. found no association between carotid intima-media thickness (IMT) progression and cardiovascular events.\textsuperscript{43, 44}
Separate estimates of the intima and media layers and calculation of intima/media ratio

CCA-IMT measurements have been used to assess atherosclerosis for many years. Despite the large-scale use of this measurement, interpretation of CCA-IMT ultrasound results has been questioned. For example, a validation study in cadavers by Gamble et al. showed that CCA-IMT results with 10 MHz ultrasound corresponded better to the total artery wall thickness (adventitia, media and intima) than to the intima + media thickness. Further, Adams et al. found a poor correlation between CCA-IMT results and the degree of atherosclerosis assessed by coronary angiography.

Intimal thickening is probably one of the earliest signs in the atherosclerotic process. However, it is not possible to obtain separate estimates of the intima and media layers using 7-10 MHz ultrasound. Mallery et al. and McPherson et al. measured the individual thicknesses of the intima and the media and the combined intima-media with intravascular high-frequency ultrasound (20 and 12 MHz, respectively), and showed good correlations with histological section results. Further, with increasing age and development of atherosclerosis, the intima continues to increase in thickness while the media decreases, as demonstrated by both histomorphometry and intravascular high-frequency ultrasound. Thus, assessment of the individual intima and media thicknesses could be more appropriate than assessment of the combined CCA-IMT for imaging the effects of vascular aging. This method has been validated in an animal model.

Non-invasive high-frequency ultrasound has been used to demonstrate that 70-year-old subjects with prevalent CVD had significantly thicker intima layers, thinner media layers and higher I/M ratios than subjects without CVD, whereas CCA-IMT measurements did not show any significant differences. The same method clearly demonstrated the expected effects of aging and long-term postmenopausal oestrogen therapy on the CCA wall layers. The method has also been used to demonstrate the effects of conditions such as recurrent depression and stem cell transplantation, which are suspected to be associated with an increased risk of CVD. Thus, the use of intravascular high-frequency ultrasound to measure the individual intima and media layers and calculation of the I/M ratio seem preferable to measuring the CCA-IMT, the present ‘gold-standard’ for non-invasive assessment of the artery wall status.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a systemic inflammatory disease that mainly affects women. Incidence rates of SLE vary from 1 to 10/100,000 person-years. The exact pathoetiology of SLE remains elusive. An ex-
tremely complicated and multifactorial interaction among various genetic and environmental factors is probably involved. The American College of Rheumatology (ACR) classification criteria are usually used in most SLE studies to define the disease. The criteria include nine clinical and two immunological criteria and SLE is present if a patient fulfils four or more of these eleven criteria.

CVD is reported to be a major cause of both morbidity and premature mortality in SLE patients. There is strong evidence of an increased incidence of CVD events such as CAD in women with SLE. Younger women with SLE, between the ages of 35 and 44 years, have an up to 50-fold increased risk of MI compared to women of similar age without SLE. The pathogenesis of premature CVD in patients with SLE is thought to be mediated by accelerated atherosclerosis, probably as a result of a complex interplay between traditional and non-traditional risk factors. The use of carotid ultrasound in several studies has demonstrated signs of accelerated atherosclerosis in SLE patients, with a higher prevalence of carotid plaques and coronary artery calcification than in controls.

Assessment of the CCA-IMT using 8-10 MHz frequency ultrasound has often been used to study the development of atherosclerosis in women with SLE. However, the results of these studies have been variable and difficult to interpret. A well executed review of premature CVD in patients with SLE has shown, in fact, that CCA-IMT values in SLE patients have been higher than, similar to, or even lower than those in healthy controls. A lower CCA-IMT value would imply a reduced risk of cardiovascular events, according to the classical interpretation of CCA-IMT results and this is inconsistent with the documented increased risk of CVD in this group. Thus, conventional CCA-IMT measurement has been controversial with respect to non-invasive assessment of cardiovascular risk in patients with SLE. It is postulated that estimates of the individual artery wall layers could be preferable, providing an alternative method of imaging the increased CVD risk in premenopausal women with SLE.

Normal pregnancy

Definition

Pregnancy without complication, resulting in delivery after gestational week 37 of an infant with a weight appropriate for its gestational age.
Physiological changes during normal pregnancy

A number of substantial cardiovascular changes occur during pregnancy to adapt and adjust the body to the changed requirements caused by the pregnancy. The most important changes are increases in stroke volume and heart rate that cause a rise in cardiac output, which is increased by 1 L/min by as early as 8 weeks into gestation. Further, the plasma volume increases and the peripheral vascular resistance decreases. The decrease in peripheral vascular resistance and the subsequent generalized vasodilatation result in a decrease in both the systolic and diastolic blood pressures (SBP and DBP), which reach their lowest levels in the middle of the second trimester and rise slightly again afterwards.

However, normal pregnancy is also associated with a negative cardiovascular profile, including an increase in arterial stiffness, mild systemic inflammation, altered lipid and lipoprotein profiles, and impaired glucose tolerance.

Endocrine changes during pregnancy

The placenta is a large endocrine organ, which produces huge, coordinated amounts of different hormones to establish and successfully maintain the pregnancy. Steroid hormones such as estrogen, progesterone, testosterone, and dehydroepiandrosterone (DHEA) are included in these. Mean circulating levels of E2 rise dramatically during pregnancy from pre-pregnancy levels of about 100 pg/ml (367 pmol/L) to about 15,000 pg/ml (55,050 pmol/L) at term. Mean plasma progesterone levels also rise dramatically during pregnancy, to reach about 130,000 pg/ml at term (compared to about 10,000 pg/ml in the luteal phase). Mean testosterone and DHEA levels increase during pregnancy. The main effect of estrogen is on the cardiovascular system, while progesterone prevents maternal rejection of the fetus through its anti-inflammatory and immunosuppressive effects.

Normal pregnancy effects on common carotid artery intima-media thickness and subsequent risk of cardiovascular disease

Reports of changes in the CCA-IMT during pregnancy are rare. One small study by Mersich et al. showed fairly stable mean CCA-IMT values, assessed once in each trimester. Normal pregnancy and effects on IMT later in life have been examined with varying results. Wolff et al. showed that CCA-IMT was significantly thicker in multiparous (≥ 4 births) than in primiparous women, which was also suggested by Skilton et al. In contrast, Kharazmi et al. found no significant differences in CCA-IMT between mul-
tiparous and primiparous women. In a case-control study, Blaauw et al. showed that at about 6 months postpartum the CCA-IMT was thicker in primiparous women with previous normal pregnancy than in nulliparous women. Thus, results about the effects of normal pregnancy on CCA-IMT have been controversial. The relationship between normal pregnancy and risk of later CVD has been poorly investigated. In a study of parity and risk of maternal CVD later in life, Parikh et al. showed that women who had had ≥ 5 births had an increased risk of CVD [hazard ratio 1.47; 95% confidence interval (CI) 1.37-1.57] compared to women who had had 2 childbirths. To our knowledge, there have been no previous reports on the longitudinal assessment of the individual artery wall layer dimensions during normal pregnancy, using non-invasive high-frequency ultrasound.

Pre-eclampsia

Pre-eclampsia (PE) is a pregnancy-specific syndrome which affects 3-5% of all pregnancies. Worldwide, PE is a leading cause of maternal and perinatal morbidity and mortality.

Definition and classification of pre-eclampsia

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP) PE is defined as new-onset hypertension and proteinuria after gestational week 20, with normalization of blood pressure within 3 months postpartum. Hypertension is defined as an SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg. Proteinuria is defined as leakage of protein ≥ 300 mg/24 hours or a spot urine protein/creatinine ratio ≥ 30 mg/mmol.

Risk factors for pre-eclampsia

Examples of risk factors for PE include:

- Age (older age)
- Parity (primiparity)
- Previous history of PE
- Family history of PE
- Pregnancy with more than one fetus
- Time between pregnancies (more than 10 years)
- Diabetes mellitus
- Chronic hypertension
- Renal disease
- Autoimmune disease
- Antiphospholipid syndrome
Pathophysiology of pre-eclampsia

The pathophysiology of PE is still not completely understood but it is thought to be a multisystem condition. It has long been hypothesized that PE is a two-stage syndrome. The first stage is mediated by reduced placental perfusion, possibly as a result of abnormal placentation. During normal placentation, cytotrophoblasts invade the maternal decidua and penetrate and replace the endothelium of the walls of the adjacent spiral arteries. This results in remodeling of the arterial wall, loss of the smooth muscle, and dilatation of the arteries. Preexisting maternal constitutional factors, such as hypertension, diabetes and antiphospholipid syndrome, are associated with abnormal placentation and these increase the risk of developing PE.

Poor placentation leads to placental hypoxia, which results in production of increased amounts of factors, such as inflammatory markers (e.g. tumor necrosis factor-α and interleukin-6) and anti-angiogenic factors (e.g. soluble fms-like tyrosine kinase-1 and endoglin). These factors are then released into the maternal circulation where they induce the second stage in the pathogenesis of PE: endothelial dysfunction, activation of the coagulation cascade, and vasoconstriction. These processes result in the clinical complex of PE.

Maternal constitutional factors in the pathophysiology of pre-eclampsia

In many women with PE, perfusion is not sufficiently reduced to be identified by Doppler measurements of the uterine arteries. Furthermore, many do not show typical signs of PE on pathological-anatomical examination of the placenta. Despite a four-fold greater risk that the infants of women with PE will be born small for their gestational age (SGA) compared with those from normal pregnancies, the majority of infants from pre-eclamptic pregnancies are not SGA. On the other hand, pregnancies without signs of maternal PE, but with preterm births or intrauterine growth restriction often manifest abnormal features of placentation identical to those seen in PE. Thus, it is proposed that, in some instances, the abnormal placentation interacts with maternal constitutional factors to result in the PE syndrome (Figure 1).
Pre-eclampsia and risk of cardiovascular disease

Several studies have shown that women examined 2-17 years after prior PE have higher blood pressure, increased insulin resistance, and higher levels of triglycerides. Endothelial dysfunction is the key factor in the pathogenesis of atherosclerosis and this dysfunction is common in women with previous PE. It is now a well known fact that the metabolic syndrome, which is a risk factor for atherosclerosis and subsequent CVD, is also a risk factor for developing PE. The metabolic syndrome is defined as high blood pressure, high fasting blood glucose and triglycerides, low levels of high-density lipoprotein cholesterol, and abdominal obesity. Women with this syndrome can respond to pregnancy in an abnormal way and can develop features of PE. With increasing age, women with a history of PE have an increased risk of developing manifest CVD (Figure 2).
Figure 2. Risk factors for vascular disease are identifiable during excursions into the metabolic syndrome of pregnancy. From Sattar, N. et al. BMJ 2002; 325: 157-160. Reprinted with permission from the BMJ publishing group.

A meta-analysis by Bellamy et al. included 25 prospective or retrospective cohort studies carried out between 1960 and 2006. In this analysis, women with previous PE had relative risks (RR) of 2.16 (95% CI 1.86-2.52) for ischemic heart disease and 3.70 (95% CI 2.70-5.05) for hypertension, compared to women with previous normal pregnancies, with mean follow-up times of 11.7 and 14.1 years, respectively. In addition, women who developed PE had higher all-cause mortality rates than women who did not develop PE. In another meta-analysis, McDonald et al. found similar results, indicating an increased risk of developing CVD (RR 2.33) in women with a history of PE compared to women with normal pregnancies.

Severity of pre-eclampsia and risk of cardiovascular disease

In a meta-analysis by McDonald et al., the RR of developing cardiac disease was 5.36 (95% CI 3.96-7.27) in women with previous severe PE compared to 2.00 (1.83-2.19) in women with mild PE (Figure 3).
In a study by Arnadottir et al., women with hypertensive disorders of pregnancy had a higher risk of CVD-related death than normotensive controls (RR 1.60; CI 1.29-1.98). The risk was more evident if they were suffering from PE, especially severe PE. Further, Irgens et al. showed that the long-term all-cause mortality rate was 1.2-fold (CI 1.02-1.37) higher in women with PE than in women with normotensive pregnancies. The risk was increased to 2.71-fold (CI 1.99-3.68) higher in women who had PE and delivered preterm, than in women without PE and term delivery (Figure 4). In particular, the risk of death from cardiovascular causes in women with PE and preterm deliveries was 8.12-fold higher than in women with normotensive term deliveries.
Pre-eclampsia and its effects on common carotid artery intima-media thickness

It is now well established that PE is an independent risk factor for subsequent CVD as a result of endothelial dysfunction leading to development of atherosclerosis. Chambers et al. measured flow-mediated arterial dilatation in women with previous PE about 3 years postpartum and showed that these women had impaired endothelial function compared to women with previous uncomplicated pregnancies. Similar findings have been shown in several other studies. As outlined previously, measurement of the CCA-IMT is recommended and the test is often used as a marker of subclinical atherosclerosis. Whether PE is associated with increased CCA-IMT has only rarely been investigated and the results are not convincing. For example, Blaauw et al. investigated a group of women with previous early-onset PE (EOPE), about 6 months postpartum. They found no significant differences in CCA-IMT between women with previous PE and women with previous normal pregnancies. Similarly, Haukkamaa et al. found no significant differences in CCA-IMT between women with and without previous PE.

Because of the documented increased risk of CVD in women with a history of PE and the inconsistent findings associated with CCA-IMT, it appears that there is a need for an alternative non-invasive method to assess cardiovascular risk in these women.
Aims of the study

The overall aim of the thesis was to investigate whether measurement of the individual intima and media thicknesses and calculation of the I/M ratio during and after pre-eclamptic pregnancy could provide physical evidence of the well documented increased cardiovascular risk in these women.

Specific aims of the individual studies

I. To validate the method for non-invasive ultrasound assessment of the individual CCA wall layer dimensions in young, premenopausal women with SLE with known increased risk of CVD.

II. To monitor changes in the CCA wall layer dimensions during and after normal pregnancy.

III. To compare the CCA wall layer dimensions at the time of PE diagnosis and about one year postpartum, with values in normal pregnant women of similar stage.

IV. To compare the CCA wall layer dimensions in premenopausal women with and without a history of previous severe PE.
Study populations

Figure 5. Flow-chart of the study populations.

The study populations were from both urban and rural areas of Sweden, with varying levels of education. Both nulli-/primiparous and parous women were included in the studies. Postmenopausal women and women receiving hormone replacement therapy were not eligible for inclusion in Studies I and IV. In Studies II-IV, women were not included if they had chronic hypertension, renal disease, SLE, or pre-gestational or gestational diabetes, or if they were pregnant with more than one fetus at the index pregnancy.

Study I
Forty-seven premenopausal women, fulfilling four or more of the ACR classification criteria for SLE were included. Consecutive patients were invited to participate at the time of outpatient visits at the Rheumatology Depart-
ment at University Hospital, Uppsala, Sweden. Two groups of women without SLE served as controls: 20 premenopausal women and 17 postmenopausal women were recruited from a previous study.\textsuperscript{53}

Study II

The women were recruited during their first routine visit at two of the Uppsala County’s antenatal clinics (Figure 6). Fifty-seven women with a normal pregnancy and expected normal pregnancy outcome remained in the third trimester evaluation. At the time of the postpartum examination, three women were pregnant again and one woman had moved away from Sweden. Thus, 53 women remained in the final evaluation.

\begin{itemize}
  \item 112 women were asked to participate
  \item Miscarriages, 9 \quad Did not want to participate, 34
  \item 1st (baseline) examination \((n=69)\)
  \item Miscarriages, 3 \quad Dropouts, 3
  \item 2nd examination \((n=63)\)
  \item Pregnancy complications, 6
  \item 3rd examination \((n=57)\)
  \item Were pregnant again, 3 \quad Moved away from Sweden, 1
  \item Postpartum examination \((n=53)\)
\end{itemize}

\textit{Figure 6.} Flow chart of the study population, Study II.

Study III

\textit{Cases:} Fifty-five women with PE were recruited when they were admitted to the antenatal clinic at the Department of Obstetrics and Gynecology at University Hospital in Uppsala, Sweden. During the postpartum examination, 5
women were pregnant again and 2 did not want to participate. Thus, 48 women remained in the postpartum evaluation.

Controls: The control group comprised 64 women with healthy pregnancies. Most of the women in the control group participated in Study II and were recruited during their routine visit at two of Uppsala County’s antenatal clinics. Eight women were recruited during a visit to the emergency outpatient department at University Hospital, Uppsala. In the postpartum examination, four women were pregnant again, one did not want to participate and one had moved out of Sweden. Thus, 58 women remained in the postpartum examination.

Study IV

Cases: Women between 40 and 50 years of age with one or more pregnancies complicated by severe PE were included in this study. We used the Register of diagnosis at University Hospital, Uppsala, Sweden, to identify women with a history of previous severe PE. The Swedish versions of the International Classification of Diseases (ICD) -9 and -10 were used to identify the women (ICD-9: 642F and ICD-10: 014.1A and 014.1B). Before inclusion, the birth records were reviewed for confirmation of the diagnosis. We found 255 women with a diagnosis of severe PE in the Register. Forty-two women remained in the study after exclusions of those whose delivery records could not be tracked, whose diagnosis could not be confirmed, who were referred from other regions, who had chronic diseases or twin/triplet pregnancies, who did not answer or declined participation in the study, or who had moved out of the country, etc. (Figure 7).
Figure 7. Flow chart for the recruitment process of cases in Study IV.

Controls: Women between 40 and 50 years of age who had had solely one or more normal pregnancies were recruited. Women living in Uppsala County, Sweden, many of them working at the Uppsala University Hospital, were recruited by personal invitation.
Methods

Assessments and data collection

Gestational age was defined as completed weeks of gestation based on the second trimester routine ultrasound dating, at pregnancy weeks 16-18. Height and weight were measured and body mass index (BMI) was calculated in kg/m². Data were collected about age, reproductive history, smoking habits, medical history and medications. Blood pressure was measured after about 15 minutes’ rest, in the supine position, on the right upper arm, with automated (Study I) or manual (Studies II-IV) blood pressure equipment (Umedico, with a calf-size appropriate for the arm circumference). Mean arterial pressure (MAP) was calculated as DBP + ⅓ (SBP - DBP) (Studies III & IV).

The delivery records were checked and data were collected about possible pregnancy-related complications, gestational week at delivery, and the birth weight of the baby. A healthy pregnancy was defined as a normotensive pregnancy with delivery of a baby with normal birth weight [within ± 2 standard deviations (SD) of the mean birth weight for gestational age],¹¹² in gestational week 37 or later. Pre-eclampsia was defined as new-onset hypertension (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, observed on at least two separate measurements ≥ 6 hours apart), combined with proteinuria (≥ 2 on a dipstick or a 24-hour urine sample showing leakage of ≥ 300 mg albumin/24 hours), both after gestational week 20. PE was diagnosed as severe when blood pressure was pronounced (SBP ≥ 160 mmHg and/or DBP ≥ 110 mmHg) and/or the proteinuria was massive (≥ 5 g/24 hours). PE was categorized into EOPE, when it was diagnosed before gestational week 34 and late onset PE (LOPE), when it was diagnosed in gestational week 34 or later. Preterm birth was defined as birth before gestational week 37. SGA and large for gestational age (LGA) babies were defined as those with birth weights ≥ 2 SDs below or above the reference population’s mean birth weight for the gestational age.¹¹²

Study I

The investigation of SLE patients included an interview and a physical examination by a rheumatologist. SLE disease activity was determined using

¹¹²
the modified SLE Disease Activity Index.\textsuperscript{113} Cumulative disease damage was measured using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index.\textsuperscript{114} A venous blood sample was collected during the examination for routine chemical analyses and assessment of complement levels.

Study II
Participants were examined three times during pregnancy, in the first (between gestational weeks 11 and 13), the second (between gestational weeks 21 and 24) and the third (between gestational weeks 34 and 37) trimesters and once more, about one year postpartum, when the women had ended lactation and started to menstruate. A venous blood sample was collected from each subject, at each examination. After collection, the blood samples were kept at room temperature for about half an hour before being centrifuged for 10 minutes at 2000g. The serum samples were then separated and stored at –70ºC until the level of E2 was analyzed, using a Cobas E instrument (Roche Diagnostics, Mannheim, Germany). The total coefficient of variation (CV) of the instrument for analysis of E2 was 2.6% at 13,000 pmol/L.

Study III
The participants were examined twice. The first examination was performed when the women were diagnosed with PE. The mean pregnancy duration at inclusion for controls was similar to that for the PE group. The second examination was performed about one year after delivery, when the women had ended lactation and started to menstruate.

Study IV
The participants were examined once, at inclusion. At the examination the women were interviewed about the date of their last delivery, their own history of CVD events and CVD-related risk factors, and any regular medication. Angina pectoris, MI, stroke and other vascular events were categorized as cardiovascular events and CVD-related risk factors included hypertension, diabetes mellitus, and hyperlipidemia.
Ultrasound assessment of the common carotid artery

The CCA wall layers were imaged using high-resolution ultrasound equipment fitted with a broadband probe at 22 MHz center frequency (Osteoson®, Minhorst Company, Meudt, Germany). The method has been extensively described in previous reports. In brief, the artery wall layers were examined with the women sitting upright and looking straight ahead after they had rested for about 15 min. The transducer was applied at the point of maximal pulsation of the left CCA, in front of the sternocleidomastoid muscle. The depth of penetration was up to 20 mm. The three-layer image of the pulsating near wall showed two echo-dense zones (the adventitia and the intima) with an echo-lucent area (the media) in between followed by the echo-lucent artery lumen (Figure 8). About twenty point estimates of the artery wall, not adjusted to the cardiac cycle, were saved on a PC by one researcher. The individual artery wall layer dimensions were measured off-line for all participants by another researcher, who was blinded with regard to the study group and the time of assessment. The means of about 10 technically acceptable measurements were calculated and used in the analysis. In our laboratory, the calculated intra-reader CV was about 4.3% for intima thickness and about 4.1% for media thickness. The inter-reader variability was about 5.4% for intima thickness and about 3.2% for media thickness.
Figure 8. Ultrasonographic image of the common carotid artery near wall, obtained by non-invasive high-frequency (22 MHz) ultrasound. C, cutis; SC, subcutis; A, adventitia; M, media and I, intima. From Akhter, T. et al. AJP-Heart Circ Physiol 2013; 304: H229-H234. Reprinted with permission from the RightLink publishing group.

Ethical considerations

The local Ethics Committee of the Medical Faculty of Uppsala University approved all studies, and informed written consent was obtained from each subject included in the studies.

Statistical methods

The results are presented as mean (standard deviation) or number (%). Differences in proportions between groups were tested by Chi-square tests. For continuous variables, between-group differences were tested using the Mann-Whitney U-test and within-group differences using the Wilcoxon Signed Rank test. Between-group differences for the main outcomes were presented as mean differences (95% CI), in addition to the non-parametric test (Studies III & IV). Between-group differences in artery wall layer dimensions were also adjusted for differences in BMI and MAP, using the
non-parametric Willett’s residual method. Correlations were analyzed using the Spearman Rank Correlation test. Receiver operating characteristic (ROC) curve analysis was undertaken to illustrate and compare the discriminatory capacities of estimation of individual artery wall layer dimensions, combined CCA-IMT and MAP to correctly predict prevalent PE (Study III) or to correctly discriminate between women with regard to previous severe PE (Study IV).

The level of significance was set at a p value ≤ 0.05. All statistical analyses were performed using Statistica version 9, Statsoft Inc. (Study I) or the SPSS, version 20.0 (SPSS Inc. PASW statistics, Chicago, IL, USA) (Studies II-IV).
Results

Study I

At the time of investigation, SLE disease was latent for all patients. Some of the traditional cardiovascular risk factors differed significantly between SLE patients and controls (Table 1). Five women in the SLE group had had CVD events (angina pectoris, MI or stroke) whereas none in the control group had.

Table 1. Traditional cardiovascular risk factors in women with SLE and controls.

<table>
<thead>
<tr>
<th></th>
<th>Women with SLE (n=47)</th>
<th>Premenopausal controls (n=20)</th>
<th>Postmenopausal controls (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>38 (8)</td>
<td>40 (4)</td>
<td>69 (6)‡</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25 (6)</td>
<td>24 (4)</td>
<td>25 (3)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>5 (11)</td>
<td>4 (20)</td>
<td>4 (24)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>120 (11)</td>
<td>110 (11) *</td>
<td>134 (16)†</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>79 (10)</td>
<td>68 (9) *</td>
<td>77 (11)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>20 (43)</td>
<td>0 (0)‡</td>
<td>9 (53)</td>
</tr>
<tr>
<td>Use of lipid-lowering drugs, n (%)</td>
<td>3 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Mean (standard deviation) or number (%)

*p < 0.001 and †p < 0.01 compared to SLE patients (non-parametric test).

Artery wall layer dimensions

In the premenopausal women with SLE, the mean carotid intima was thicker (0.19 vs 0.12 mm), the media was thinner (0.45 vs 0.68 mm), and the I/M ratio was higher (0.45 vs 0.20) than in the age-matched healthy premenopausal control women (all p < 0.0001) (Figure 9). Further, in the women with SLE versus postmenopausal women who were 30 years older, the intima (0.19 vs 0.14 mm, p < 0.0001) and the media (0.45 vs 0.34 mm, p < 0.001) were thicker, while the I/M ratio was similar (0.45 vs 0.45 mm). The total CCA-IMT was lower in SLE patients than in age-matched controls (0.64 vs 0.80 mm, p < 0.01) but higher than in the postmenopausal women (0.64 vs 0.48 mm, p < 0.0001). SLE patients with a history of either CVD events or hypertension had significantly thinner media than patients without such a history, p < 0.05. Patients treated with azathioprine or mycophenolate mofetil had a thinner media than patients not treated with these drugs, p < 0.05.
Figure 9. Carotid artery near-wall dimensions assessed using 22 MHz ultrasound in premenopausal women with SLE compared to pre- and postmenopausal women without SLE. Mean ± SD. **** p < 0.0001 and *** p < 0.001 compared to SLE.

Correlation analysis between artery wall layer dimensions and SLE disease activity index

In the SLE patients, higher SLICC/ACR damage index, higher cumulative dose of prednisone, and higher C3d/C3 ratio were associated with a thinner carotid media, all p < 0.05. There were no significant correlations between age, disease duration or antimalarial medication and intima or media thickness or I/M ratio.

Study II

The mean age of participants in Study II was about 30 years, 50% were nulliparous and very few were current smokers (4%). As expected, BMI increased during pregnancy and decreased again postpartum. SBP was stable during pregnancy and decreased postpartum (p < 0.05), whereas DBP showed a decrease in the second trimester assessment (p < 0.05), but was otherwise stable.

Artery wall layer dimensions

The mean values for intima and media layers and I/M ratio remained fairly stable during pregnancy. However, by about one year postpartum, the mean intima thickness and the I/M ratio had decreased (improved) compared to all trimesters, all p < 0.001 (Table 2). The combined CCA-IMT was also fairly stable during pregnancy and had decreased by one year after delivery. There were no significant differences in artery wall layer dimensions between...
smokers and non-smokers or between nulliparous and parous women, at any of the assessment points.

Table 2. Artery wall layer dimensions in each trimester during pregnancy (n=57) and about one year postpartum (n=53).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Trimester</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td>Gestational length (wk) or time postpartum (mo)</td>
<td>13 (1)</td>
<td>23 (1)</td>
</tr>
<tr>
<td>Intima thickness (mm)</td>
<td>0.11 (0.02)</td>
<td>0.11 (0.02)</td>
</tr>
<tr>
<td>Media thickness (mm)</td>
<td>0.57 (0.14)</td>
<td>0.54 (0.15)</td>
</tr>
<tr>
<td>Intima/media ratio</td>
<td>0.20 (0.05)</td>
<td>0.21 (0.06)</td>
</tr>
<tr>
<td>Intima media thickness (mm)</td>
<td>0.68 (0.15)</td>
<td>0.65 (0.16)</td>
</tr>
</tbody>
</table>

Mean (standard deviation)
* p < 0.001 compared to 1st, 2nd and 3rd trimester; † p < 0.01 compared to 1st trimester; and ‡ p < 0.05 compared to 3rd trimester (non-parametric test).

Correlations between first trimester characteristics and artery wall layer dimensions in different trimesters

Lower levels of serum E2, older age, and higher BMI and BP in the first trimester were often associated with a numerically thicker carotid intima layer, a thinner media layer and a higher I/M ratio, especially in the second trimester. Thus, in the second trimester, the I/M ratio was significantly lower in women with higher serum E2 levels, and higher in women who were older, or had higher BMI, SBP or DBP (all p < 0.05). In the third trimester, the associations were similar but less apparent than those in the second trimester.

Correlations between base line characteristics and changes in the artery wall layer dimensions

With regard to changes in the artery wall dimensions from the first to the second trimesters, first trimester higher serum E2 levels were associated with a reduced I/M ratio, whereas a higher BMI, SBP and DBP were all associated with an increased I/M ratio (Table 3). Further, older age was associated with an increased intima thickness (p < 0.05). Higher serum E2 levels were associated with an increased media thickness (p < 0.05), whereas higher BMI, SBP and DBP were associated with a reduced media thickness (all p < 0.05) (Table 3). Similar, but less apparent associations were found with regard to changes from the first to the third trimesters (data not shown).
Table 3. Association between maternal characteristics in the first trimester and changes in the carotid artery wall layer dimensions from the first to second trimesters.

<table>
<thead>
<tr>
<th>First Trimester Characteristics</th>
<th>Changes From the First to Second Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intima thickness</td>
</tr>
<tr>
<td>Serum estradiol</td>
<td>-0.09</td>
</tr>
<tr>
<td>Age</td>
<td>0.31*</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.15</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.12</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Values are Spearman rank correlation coefficients.  * p < 0.05; † p < 0.01; ‡ p < 0.001

Study III

Women with PE did not differ significantly from women with normal pregnancies regarding mean maternal age, smoking habits or gestational duration at inclusion, but they were more often nulliparous and had a higher BMI, SBP, DBP and MAP in the first trimester, at inclusion and about one year postpartum (Table 4). Among women with PE, 42% had EOPE, 69% had severe PE and 85% were on anti-hypertensive medication. Gestational duration at birth was on average 4 weeks shorter in the PE group than in controls (p < 0.0001). Infants born to pre-eclamptic mothers had a significantly lower mean birth weight than infants born to mothers with normal pregnancies, both before and after adjustment for gestational duration (p < 0.001).

Table 4. Clinical characteristics of the study population in Study III.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Pre-eclampsia</th>
<th>Normal Pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First trimester</td>
<td>At inclusion</td>
</tr>
<tr>
<td>Gestational duration (wk)</td>
<td>35 (4)</td>
<td>33 (7)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28 (6)*</td>
<td>33 (7)†</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>124 (11)*</td>
<td>146 (12)*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78 (7)*</td>
<td>91 (11)*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>94 (8)*</td>
<td>109 (10)*</td>
</tr>
</tbody>
</table>

Means (standard deviation). BP, blood pressure; MAP, mean arterial pressure.  * p < 0.0001 and † p < 0.001 compared to corresponding values in normal pregnancies (non-parametric test).

Artery wall layer dimensions

At inclusion, women with PE had a thicker CCA intima (p < 0.0001), a thinner media (p < 0.001) and a higher I/M ratio (p < 0.0001) than controls. The differences in CCA intima thickness and I/M ratio remained significant between study groups after adjustment for first trimester BMI and MAP (both p < 0.0001) (Table 5). At the postpartum examination, about one year after
delivery, the intima thickness and I/M ratio had decreased (improved) in both PE patients and controls (both p < 0.0001) (Table 5), but the differences between study groups remained highly significant (both p < 0.0001) (Table 5). In contrast, there were no significant differences in CCA-IMT between PE patients and controls, either during pregnancy or at the postpartum examination.

Table 5. Common carotid artery wall layer dimensions in women with pre-eclampsia, at the time of diagnosis and about one year postpartum and in women with normal pregnancies.

<table>
<thead>
<tr>
<th>Layer dimensions</th>
<th>Pregnancy</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>Normal</td>
</tr>
<tr>
<td>Intima (mm)</td>
<td>0.18 (0.03) *†</td>
<td>0.11 (0.02)</td>
</tr>
<tr>
<td>Media (mm)</td>
<td>0.47 (0.12)§</td>
<td>0.55 (0.14)</td>
</tr>
<tr>
<td>I/M ratio</td>
<td>0.41 (0.14) *†</td>
<td>0.20 (0.05)</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.65 (0.13)</td>
<td>0.66 (0.15)</td>
</tr>
</tbody>
</table>

Mean (standard deviation) or percentage differences. CCA, common carotid artery; I/M, intima/media; IMT, intima-media thickness; PE, pre-eclampsia

† p < 0.05; § p < 0.001 and * p < 0.0001 (non-parametric test) compared to normal pregnancy.

† p < 0.0001 compared to normal pregnancy, after non-parametric adjustment for body mass index and mean arterial pressure in the first trimester and postpartum, respectively.

‡ p < 0.0001, compared to corresponding values during pregnancy.

In women with PE, none of the artery wall layer dimensions differed significantly with parity, EOPE, LOPE, severity of PE or antihypertensive therapy.

**ROC curve analysis to discriminate between women with and without prevalent PE**

At inclusion, the area under the ROC curve (AUC) value for the CCA intima thickness was 0.98 and that for the I/M ratio was 0.94, thus correctly differentiating women with regard to prevalent PE at diagnosis (Figure 10A). One year postpartum, the corresponding AUC values were 0.95 and 0.90, respectively (Figure 10B). In contrast, estimates of the combined CCA-IMT were not useful for differentiating women with regard to prevalent PE, with AUC values of 0.49 during pregnancy and 0.46 about one year postpartum. AUC values for MAP were 0.97 and 0.83, respectively (Figures 10A and B).
Figure 10. Receiver operating characteristic (ROC) curves illustrating area under the curve (AUC) values for individual artery wall layer dimensions, combined intima-media thickness of the common carotid artery and MAP with regard to pre-eclampsia, A) at diagnosis, and B) about one year postpartum. I/M, intima/media; IMT, intima-media thickness; MAP, mean arterial pressure.

Study IV

Women with a history of previous severe PE did not differ significantly regarding age, smoking habits, time since last delivery, or BMI from the controls, but they were more often primiparous and had significantly higher SBP, DBP and MAP. Women with a history of previous PE were more often suffering from prevalent hypertension and using antihypertensive medication than controls. Further, there were two women in the previous PE group who had had CVD events (Table 6).

Table 6. Clinical characteristics of women with previous severe pre-eclampsia (n=42) and women with previous normal pregnancies (n=44).

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>Pre-eclampsia</th>
<th>Normal Pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>44 (3)</td>
<td>44 (3)</td>
</tr>
<tr>
<td>Primiparous, n (%)</td>
<td>11 (26) *</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>3 (7)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Time since last delivery (y)</td>
<td>11 (5)</td>
<td>11 (5)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 (5)</td>
<td>25 (4)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128 (15) ‡</td>
<td>119 (12)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82 (8) ‡</td>
<td>76 (8)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>97 (10) ‡</td>
<td>91 (8)</td>
</tr>
<tr>
<td>Antihypertensive therapy, n (%)</td>
<td>8 (19) *</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Cardiovascular disease events, n (%)</td>
<td>2 (5)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Mean (standard deviation) or number (%)
* p < 0.05 and ‡ p < 0.01 compared to normal pregnancies (non-parametric test).

Among the women with previous severe PE at the index pregnancy, 64% had an EOPE, 86% were delivered preterm and 14% had infants born SGA.

Artery wall layer dimensions

Women with previous severe PE had significantly thicker CCA intima layers and higher I/M ratios (both p < 0.0001) and non-significantly thinner media layers (p = 0.08) than women with previous normal pregnancies. After adjustment for BMI and MAP, the differences in intima thickness and I/M ratio were still highly significant (both p < 0.0001). However CCA-IMT did not differ significantly between women with and without previous severe PE (Table 7).
Table 7. Common carotid artery wall layer dimensions in women with previous severe pre-eclampsia (n=42) and in women with previous normal pregnancies (n=44).

<table>
<thead>
<tr>
<th>CCA wall layers</th>
<th>Pre-eclampsia</th>
<th>Normal Pregnancies</th>
<th>Mean diff (95% CI)</th>
<th>% diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima (mm)</td>
<td>0.13 (0.02) *,†</td>
<td>0.08 (0.01)</td>
<td>0.05 (0.04, 0.06)</td>
<td>+63</td>
</tr>
<tr>
<td>Media (mm)</td>
<td>0.50 (0.11)</td>
<td>0.54 (0.11)</td>
<td>-0.04 (-0.09, 0.01)</td>
<td>-7</td>
</tr>
<tr>
<td>I/M ratio</td>
<td>0.27 (0.07) *,†</td>
<td>0.15 (0.03)</td>
<td>0.12 (0.10, 0.14)</td>
<td>+80</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.63 (0.12)</td>
<td>0.61 (0.12)</td>
<td>0.01 (-0.04, 0.06)</td>
<td>+3</td>
</tr>
</tbody>
</table>

Mean (standard deviation). CCA, common carotid artery; I/M, intima/media; IMT, intima media thickness; CI, confidence interval.

* p < 0.0001 (non-parametric test)
† p < 0.0001, after non-parametric adjustment for body mass index and mean arterial pressure.

Within the group of women with previous severe PE, those with a history of either CVD events or prevalent hypertension had thicker CCA intima layers and higher I/M ratios than women without such a history (all p < 0.05). Primiparous women had higher I/M ratios than women who were parous (p < 0.05). No significant differences in artery wall layer dimensions were found with regard to smoking habits or types of PE (EOPE or LOPE; repeated or not repeated PE; preterm or term PE; PE with or without SGA birth).

**ROC curve analysis to discriminate between women with and without previous severe PE**

In the ROC curve analysis, both the carotid intima layer and the calculated I/M ratio strongly discriminated between women with and without previous severe PE; the AUC value was 0.98 for intima thickness and 0.93 for the I/M ratio. In contrast, estimates of the combined CCA-IMT were not useful for discriminating between women with and without previous PE, with an AUC value of 0.52. The AUC value for MAP was 0.69 (Figure 11).
Figure 11. Receiver operating characteristic (ROC) curves illustrating area under the curve (AUC) values for different artery wall layers, combined intima media thickness and MAP, to discriminate between women with and without previous severe pre-eclampsia. I/M, intima/media; IMT, intima-media thickness; MAP, mean arterial pressure.
Discussion

Cardiovascular disease in women, especially after pregnancy complications

Cardiovascular disease is the main cause of mortality in women, all over the world. Globally, more women die from CVD than from cancer, malaria, tuberculosis and HIV combined.\(^3\) According to the AHA, mortality from CVD has declined in the last 25 years, but significantly less for women than for men. Further, the global population is increasing in number and age, resulting in a marked increase in the number of women at risk for CVD.

PE is associated with an increased risk of CVD later in life.\(^{106,107}\) Pregnancy resulting in preterm delivery and birth of SGA infants are also associated with increased risk of CVD morbidity and mortality in later life.\(^{116,117}\) It has been demonstrated that pregnancies resulting in preterm birth\(^{94}\) and SGA infants\(^{95}\) but without maternal signs of PE show features of abnormal placentation that are identical to those seen in PE. Preterm birth can be caused by inflammation and infection,\(^{118}\) and the process of atherosclerosis is also largely inflammatory in its origin.\(^{22}\) A possible mechanism for intrauterine growth retardation is maternal hemodynamic maladaptation, to which maternal constitutional factors such as obesity or diabetes could contribute.\(^{119}\) Thus, several pregnancy complications could have potential negative effects on cardiovascular risk. Cardiovascular studies in women are therefore warranted, especially in women who are at higher risk after pregnancy complications, such as after PE. Early diagnosis of women at risk of CVD could help to determine early intervention to reduce the cardiovascular morbidity and mortality.

Cardiovascular disease and carotid artery wall imaging

Measurement of CCA-IMT by 7-10 MHz ultrasound has been used for a long time for non-invasive assessment of vascular health in patients with CVD, or as an indicator of progression or regression of CVD. Although the CCA-IMT is considered the 'gold standard' for assessment of vascular status,\(^{32}\) the interpretation of CCA-IMT measurements has been questioned. There are no significant and consistent differences in CCA-IMT measurements between individuals with or without CVD, according to a meta-
analysis,\textsuperscript{41} and there are no significant associations between progression of CCA-IMT measurements and risk of CVD events.\textsuperscript{43,44} A possible reason for this could be that the artery wall layers develop in divergent directions, the intima thickness increasing and the media decreasing, with aging and development of atherosclerosis. This theory is supported by the findings of Gussenhoven et al., who used high-frequency (30-40 MHz) ultrasound to demonstrate that a decrease in the media thickness was inversely related to an increase in the atherosclerotic lesion.\textsuperscript{50} Further, in a study on cadavers, Kadar et al. showed that the I/M ratio increases and type III-IV atherosclerotic lesions in the coronary arteries and aorta become worse with increasing age.\textsuperscript{49} In a validation study on cadavers, Gamble et al. also showed that CCA-IMT assessed with 10 MHz ultrasound corresponds better to the total artery wall thickness (adventitia, media and intima) than to the intima + media thickness.\textsuperscript{45}

Studies have shown that intimal thickening is probably one of the earliest signs of the process of atherosclerosis, developing as a result of inflammation and recruitment of inflammatory cells into the endothelium.\textsuperscript{22,23} The compensatory medial thinning occurs partly because of the migration of smooth muscle cells from the media to the intima layer\textsuperscript{22} and partly because of inflammation in the media.\textsuperscript{120} Thus, medial thinning is more apparent in the later stages of atherosclerosis.

The use of 22 MHz ultrasound to show the three artery wall layers separately provides opportunities for calculating the I/M ratio, and examining and following up on the divergent development of intima and media thicknesses in atherosclerosis. The results of studies estimating the thickness of the individual artery wall layers using this high-frequency non-invasive method suggest that it is preferable to conventional measurement of CCA-IMT for assessing atherosclerosis and the risk of CVD. Subjects with CVD have been shown by this method to have a thicker intima, a thinner media and a higher I/M ratio (a sign of negative vascular effect) than subjects without CVD, whereas conventional CCA-IMT does not differentiate between these two groups.\textsuperscript{52} Lower estrogen levels after menopause is also associated with negative vascular effects. The effects of aging and administration of long-term postmenopausal estrogen on the individual CCA wall layers have been investigated using high-frequency ultrasound measurement.\textsuperscript{53} The artery wall layer dimensions in long-term estrogen users were significantly better than in age-matched non-users and were similar to values in premenopausal women who were about 30 years younger.\textsuperscript{53} In contrast, CCA-IMT measurement did not show any differences between the groups.\textsuperscript{53}
Carotid artery wall imaging in premenopausal women with systemic lupus erythematosus

A bimodal mortality pattern has been suggested in patients with SLE, with an early peak due to active SLE disease and a later peak due to cardiac disease; however, this has not been confirmed in a more recent study by Nossent et al. The risk of death due to SLE-specific disease activity has been reduced by modern treatments, but the risk of CVD-related mortality has not. The well known increased risk of CVD in women with SLE has been studied using CCA-IMT, but with varying results. A well-executed study by Roman et al., published in the NEJM showed that the CCA-IMT value was lower in women with SLE than in women without SLE. Classical interpretation of CCA-IMT results suggests that a lower value means a healthier artery wall, which is inconsistent with the documented increased risk of CVD in women with SLE. Our results using estimation of the individual artery wall layers showed that women with SLE had significantly thicker intima layers, thinner media layers, and higher I/M ratios than women of similar age but without SLE. The artery wall layers in women with SLE were comparable to those in postmenopausal women 30 years older. Our study also showed that the CCA-IMT was lower (better) in women with SLE than in women of similar age without SLE. Thus, estimation of the individual carotid artery wall layers appears preferable to CCA-IMT for demonstrating cardiovascular risk in premenopausal women with SLE.

Carotid artery wall imaging and normal pregnancy

We found fairly stable mean values for the artery wall layer dimensions throughout normal pregnancy. Although an improvement in the vascular status might have been expected during pregnancy as a result of the successive increases in serum E2 levels, this study population comprised relatively young (mean age 30 years), fertile women who started their pregnancies from a relatively high estrogenic state without room for further improvement. Normal pregnancy is associated with a transient state of the metabolic syndrome and up-regulation of the inflammatory cascade. If these metabolic and inflammatory changes are seen as a stress on vascular function, they might counteract any improvements as a result of high E2 levels. Our findings of a thinner intima and a lower I/M ratio about one year postpartum, compared to the values during pregnancy strengthen this hypothesis. Further, we found that lower serum E2 levels, older age, or higher BMI and blood pressure (within normal ranges) at the start of pregnancy were associated with mostly negative effects on the artery wall layer dimensions during pregnancy. These effects were most apparent in the second trimester and in
the changes in artery wall layer dimensions from the first to the second trimester. Lower serum E2 levels, older age, and higher BMI and blood pressure are known to be risk factors for CVD. During a normal pregnancy, the most substantial strains on the vascular system occur during the second trimester, in parallel with a substantial increase in plasma volume.  

Carotid artery wall imaging and pre-eclampsia

Pre-eclampsia affects 3-5% of all pregnancies, which means that there are a large number of older women who have had PE. The increased risk of hypertension, cardiac disease and stroke after a pre-eclamptic pregnancy means that there is an equally large number of women who are at increased risk of CVD-related morbidity and mortality. 106, 107 Severe PE occurs in about 20% of all women with PE 125 and is associated with higher rates of cardiovascular morbidity and mortality than mild PE. 106, 107

Numerous studies have addressed the increased risk of CVD after PE but there are only a few studies addressing the measurement of CCA-IMT in PE. 78, 110 The findings, by some studies, that the CCA-IMT in women with previous PE was no different from that in women with previous normal pregnancies are inconsistent with the known increased risk of CVD after PE. We suggest that this can be explained by the divergent development of intima and media layers during atherosclerosis. 49, 50

Endothelial dysfunction is one of the most important factors in the development of maternal symptoms of PE. Persistent endothelial dysfunction several months to years after a pre-eclamptic pregnancy indicates that it is a long-standing process. 99, 100 PE is also associated with exaggerated inflammation. 70 The combination of endothelial dysfunction and inflammation initializes the process of atherosclerosis, which then gradually builds up and can take several years to decades to become clinically apparent. 22 The typical finding of the specific vascular lesions of PE (termed acute atherosis) in the placental bed is similar to that observed in atherosclerosis. 126, 127 Atherosclerosis plays a key role in the development of CVD. 19-21 The estimation of the individual CCA intima and media layers and calculation of the I/M ratio allowed us to show that PE, both at diagnosis and about one year postpartum, was associated with negative vascular effects that were most apparent for intima thickness and the I/M ratio. These findings could be explained by the initial thickening of the intima that occurs in the atherosclerosis process, 22, 23 and the reflection of that in the I/M ratio. This initial intimal thickening could also explain our findings that the changes from pregnancy to about one year postpartum in the intima thickness and I/M ratio were more substantial than those in the media thickness. The divergent development of intima and media layers with aging and in atherosclerosis could explain why measurement of the combined CCA-IMT has failed to show any
difference in vascular status between women with ongoing PE or one year postpartum, and women without PE.

Women with a previous history of severe PE have a greater risk of CVD later in life than women with mild PE.\textsuperscript{106, 107} Theoretically, severe PE could be associated with more severe endothelial dysfunction, which should lead to accelerated atherosclerosis. The mean differences in intima thickness and I/M ratio between premenopausal women with and without previous severe PE (mean age 44 years) were greater than those in younger women (mean age 31 years) with and without PE, analyzed one year postpartum. These findings could indicate that the artery wall layers of women with previous severe PE are affected more negatively with aging than those of women of similar age with previous normal pregnancies. Further, among women with previous severe PE, those with either a history of cardiovascular events or currently taking antihypertensive therapy had thicker intima layers and higher I/M ratios than those without such a history, indicating a more adverse cardiovascular profile.

PE and CVD share several common metabolic abnormalities as risk factors\textsuperscript{96, 97} and several mediators of endothelial dysfunction are up-regulated in both PE and CVD.\textsuperscript{99, 100} However, it remains unclear whether PE is a formal risk factor for CVD or whether it just identifies women at increased risk of CVD later in life.\textsuperscript{128} One study from Norway concluded that the association between PE and cardiovascular risk in later life could be largely due to shared pre-pregnancy risk factors rather than a direct influence of PE.\textsuperscript{129} Our studies of the artery wall status in women with PE were not designed to answer the question of whether the differences in artery wall layer dimensions between PE and normal pregnancy are an effect of PE only, or whether the damage to the artery walls already existed before pregnancy in the women who developed PE. This is a question that needs to be addressed in further studies.

Methodological consideration

Estimation of the individual artery wall layers by non-invasive, 22 MHz ultrasound enabled us to identify all three layers of the artery wall and to calculate the I/M ratio. The I/M ratio amplifies the divergent development of the intima and media layers in the process of atherosclerosis.

To understand the changes in the artery wall layer dimensions in women with PE, it is important to understand what happens during normal pregnancy. During pregnancy, huge physiological cardiovascular changes occur along with numerous hormonal and metabolic adaptations and adjustments. Longitudinal assessment of artery wall layer dimensions is necessary to demonstrate possible vascular changes during normal pregnancy. Further, the vascular effects of normal pregnancy can be better understood with an
additional postpartum assessment. We measured CCA wall layer dimensions once in each trimester during normal pregnancy, and one year postpartum (Study II). At the postpartum examination, almost all women had ended lactation and regained menstruation, which is a sign of returned ovarian function with restoration of normal serum E2 levels. Further, results from our Study II were used in Study III for comparison with PE, and could be used in future studies as reference material for comparison with data from women with pregnancy complications.

A limitation of Studies II and III was that we did not have pre-pregnancy data on the artery wall status, in light of the seemingly improved artery wall status one year postpartum in both studies. However, we were able to compare data from these studies with our previous results from non-pregnant women, using the same method for artery wall assessment.

A strength in our studies was that one researcher made all the ultrasound examinations and another researcher, who was blinded to group or time of examination, analyzed all the ultrasound pictures offline. We also examined our populations both during and after pregnancy, which gave us the opportunity to analyze changes from pregnancy to the postpartum period (Studies II and III).

Further, we only included women with previous severe PE in Study IV, since this has been shown to be associated with a more adverse cardiovascular risk later in life, and we validated the diagnosis before inclusion. On the other hand, inclusion of only women with severe PE could have limited the generalizability of the results to all women with previous PE. The relatively small size of the cohort could also have contributed to the lack of significant differences in artery wall layer dimensions between EOPE and LOPE, repeated- or non-repeated PE, preterm or term PE, and PE with or without SGA birth.

Further studies, preferably including pre-pregnancy, pregnancy and postpartum evaluations of artery wall status, are highly warranted for confirmation of our results.
Conclusions

The results of our studies using estimation of the individual CCA intima and media thicknesses and calculation of the I/M ratio indicated that

- there is an increased cardiovascular risk in premenopausal women with SLE
- normal pregnancy could be a stress on the cardiovascular system
- there is an increased cardiovascular risk in women with PE (mean age 31 years) both at diagnosis and about one year postpartum
- there is an increased cardiovascular risk in women with a history of severe PE (mean age 44 years; 11 years since last delivery).

These results are in agreement with the documented increased risk of CVD in women with PE or SLE, whereas measurement of the CCA-IMT, the current ‘gold standard’ method, did not demonstrate any changes indicative of increased cardiovascular risk in these women.

We suggest that measurements of individual CCA wall layer thicknesses could become a clinically useful tool for stratifying women with PE with regard to cardiovascular risk, enabling early intervention and potential reduction in their long-term cardiovascular risk.
Future plans

The possibility of providing physical evidence indicating an association between PE and the future risk of CVD, by assessing the thickness of the individual artery wall layers seems promising. Better understanding of this association could help us to identify women at increased risk of CVD, give these women an opportunity to modify their lifestyle and risk factors, and improve early intervention and follow up, with the aim of reducing their subsequent cardiovascular morbidity and mortality. We therefore plan to assess various cardiovascular biomarkers (e.g. pentraxin-3, asymmetric dimethylarginine, B-type natriuretic peptide, and troponin I) in women with PE, especially those with ongoing PE. We plan to investigate how the levels of these biomarkers correlate with the artery wall layer dimensions. Relaxin, a protein hormone that is produced in large amounts during pregnancy, is a potential vasodilator. Women with PE should have lower relaxin levels than those with normal pregnancies. Confirmation of this hypothesis and correlation of the results with the artery wall layer dimension results could improve our insight into the pathophysiology of PE.

Diabetes mellitus in general,\textsuperscript{130} and pregnancy complications such as gestational diabetes,\textsuperscript{131} preterm delivery,\textsuperscript{116} and pregnancy with SGA infants,\textsuperscript{117} are associated with an increased risk of cardiovascular morbidity and mortality. It would be very interesting to investigate how pregnant women with these conditions differ from women with normal pregnancies, with respect to artery wall status and cardiovascular biomarkers.

It is not only women with a history of PE who are at increased risk of CVD later in life; their offspring are also at increased risk of CVD. Increased blood pressure\textsuperscript{132} and increased risk of stroke\textsuperscript{133} in later life have been observed in the offspring of pre-eclamptic mothers. Improving our understanding of the vascular status of the offspring of pre-eclamptic mothers could provide an opportunity to reduce their cardiovascular morbidity and mortality through modification of life styles and cardiovascular risk factors.

Preeklampsi (”havandeskapsförgiftning”) är en graviditetsspecifik sjukdom som drabbar omkring 3-5% av alla gravida kvinnor. Diagnosen preeklumpsi baseras på att blodtrycket har stigit över en viss nivå (140/90 mmHg) samt av förekomsten av ett nytillkommet läckage av äggvita i urinen (>0,3g/L), båda efter graviditetsvecka 20. Det finns olika svårighetsgrad av sjukdemon
- från mild/måttlig till svår. Preeklampsi är associerad med en ökad grad av inflammation i kroppen och med en funktionsnedsättning hos kärlets innersta lager (endotelet) vilket ger upphov till en s.k. endoteldysfunktion. Studier har visat att kvinnor som haft preeklampsi och speciellt de kvinnor som haft svår preeklampsi, har en ökad risk för hjärtkärlsjukdomar senare i livet. Denna förhöjda risk har inte kunnat påvisas genom mätning av karotis-IMT när kvinnor med tidigare preeklampsi har undersöks.

Målet med avhandlingsarbetet var att undersöka eventuella kärlförändringar under och efter normal graviditet liksom vid preeklampsi med vår metod baserad på separata mätningar av karotisartärens intima- och medialager och beräkning av I/M ratio.

Utöver detta, ville vi först utvärdera (validera) metoden vid undersökning av premenopausala kvinnor med systemisk lupus erythematosus (SLE) vilka har en sedan tidigare välkänt ökad risk för hjärtkärlsjukdomar.

**Studie I**

Syftet med denna studie var att undersöka om vår föreslagna metod kan påvisa den kända förhöjda risken för hjärtkärlsjukdomar hos premenopausala kvinnor med SLE. SLE är en systemisk inflammatorisk sjukdom och är förenad med en dokumenterat ökad morbiditet och mortalitet i hjärtkärlsjukdomar senare i livet. Studier baserade på konventionell karotis-IMT-mätning har visat varierande resultat, ibland har man till och med fått data som indikerat ”friskare” kärl hos personer med SLE. Premenopausala kvinnor med SLE (n=47) undersökte och resultaten jämfördes med två kontrollgrupper: den en bestående av friska premenopausala kvinnor i motsvarande ålder (n=20), den andra av postmenopausala kvinnor (n=17) som var omkring 30 år äldre än SLE-gruppen. Vi fann att kvinnor med SLE hade tjockare intima, tunnare media och en högre I/M ratio än ålderslåta kvinnor utan SLE och att värdena var jämförbara med dem hos 30 år äldre postmenopausala kvinnor. Vår föreslagna metod kunde alltså påvisa den dokumenterade förhöjda risken för hjärtkärlsjukdomar hos premenopausala kvinnor med SLE, vilket inte kunde visas med hjälp av karotis-IMT-mätningar.

**Studie II**

Syftet med denna studie var att undersöka eventuella förändringar i karotisartärens vägglagar under normal graviditet och fram till cirka ett år efter förlossning. Kvinnor med normal graviditet undersökte tre gånger under graviditeten (n=57) och cirka ett år efter förlossning (n=53). Under graviditeten fann vi väsentligen stabila medelvärden, både för intima och media samt
för I/M ration. Ett år efter förlossning hade både intimatjockleken och I/M ration minskat (d.v.s. förbättrats). Vi fann också att de kvinnor som i början av graviditeten hade ett lägre serum-östadiol, var äldre, hade högre bodymass-index eller ett förhöjt blodtryck oftast uppvisade "negativa" effekter på artärväggslagren senare i graviditeten, mest tydligt under den s.k. andra trimestern.
Karotis-IMT visade emellertid ofta motsatta resultat.

Studie III
Syftet med denna studie var att undersöka om kvinnor som diagnostiserades med preeklampsi har en negativ påverkan på artärväggslagren jämfört med normalgravida kvinnor. Kvinnor med preeklampsi (n=55) och med normal graviditet (n=64) undersöktes vid tidpunkten för diagnos under graviditeten och cirka ett år efter förlossningen. Vi fann att kvinnor med preeklampsi hade en tjockare intima, en tunnare media och en högre I/M ratio jämfört med kvinnor med en normal graviditet. Cirka ett år efter förlossningen hade både intiman och I/M ration blivit bättre i båda grupper, men det fanns en kvarstående, signifikant skillnad i artärväggslagren mellan grupperna. Dessa fynd talar för en ökad kardiovaskulär risk hos kvinnor med preeklampsi.

Med karotis-IMT fann vi ingen skillnad mellan grupperna, varken under graviditet eller ett år efter förlossning.

Studie IV
Syftet med denna studie var att undersöka om kvinnor som tidigare genomgått en eller flera graviditeter med svår preeklampsi (n=42) har en negativt påverkad artärvägg jämfört med kvinnor med en eller flera tidigare normala graviditeter (n=44). Kvinnorna undersöktes i medeltal 11 år efter föregående graviditet och vi fann att kvinnor med tidigare svår preeklampsi hade signifikant tjockare intima och en högre I/M ratio jämfört med kvinnor med tidigare enbart normal graviditet. Detta bekräftar den ökade risken för hjärtkärlsjukdomar hos kvinnor med preeklampsi.
Karotis-IMT visade återigen ingen skillnad mellan grupperna.

Sammanfattningsvis tyder våra studier på att separata ultraljudsmätningar av karotisartärens intima- och medialager och beräkning av I/M ration är en bättre metod än karotis-IMT för att påvisa den väldokumenterade förhöjda risken för framtida hjärtkärlsjukdom och hjärtkärlsdöd hos kvinnor med SLE respektive hos kvinnor med tidigare preeklampsi. Eventuellt kan metoden användas på dessa patientgrupper för att följa aterosklerosutveckling och som ett underlag för ställningstagande till interventioner vilka förhoppningsvis skulle kunna minska risken för kommande hjärtkärlsjukdomar.
Acknowledgements

I wish to express my sincere gratitude and deepest appreciation to all those who supported and encouraged me all the way. In particular I wish to express my gratitude to:

**All the women** who participated willingly in this research, it would have been simply impossible without YOU.

**Tord Naessen**, Professor and my supervisor, for believing in me and for introducing me to clinical research. Thank you for having such patience and for sharing your enormous knowledge and experience along the way. Your enthusiasm encouraged me when things did not work in research. I look forward to future work under your guidance.

**Anna-Karin Wikström**, Associate Professor, my co-supervisor, for sharing your wealth of knowledge with me about research and especially about pre-eclampsia. Your stimulating feedbacks have always encouraged me. I’m so impressed by your never-ending energy. I look forward to working with you in the future.

**Marita Larsson**, Research Nurse, for being an important part of our research group. You are such an impressive, well-organized person. Thank you for all your help.

**Anders Larsson**, Professor, at the Department of Clinical Chemistry, for co-authorship and for the help with bio-chemical analysis.

**Lars Rönnblom**, Professor and **Dag Leonard**, PhD-student at the Department of Rheumatology, for co-authorship.

**Lars Berglund**, Associate Professor, for statistical support and advice.

**Jan Gustafsson**, Professor and head of the Department of Women’s and Children’s Health, Uppsala University, for giving me the opportunity to perform my PhD-studies.
Ove Axelsson, Professor Emeritus in our department. I’m proud to have you as my examiner in my research study. Thank you for all of your stimulating comments and support.

Inger Sundström Poromaa, Professor, for your enthusiasm and stimulating feedback.

Matt Olovsson, Professor, for a fantastic year with you when we worked together with medicine students/KLASS. I learned a lot from you at this time.

Ulf Högberg, Professor, you are such a good person. Proud to have you in our department.

Masoumeh Rezapour, Bo sultan, Gunilla Hallberg and Elisabeth Darj, my chiefs in clinical practice, for giving me working conditions that have enabled me to finish my thesis.

All my colleagues in the Department of Obstetrics & Gynaecology, for all support and appreciation.

Eva Bergman, for all talk and support about research, Alkistis Skalkidou, for being helpful and sharing your statistical knowledge and Olle Eriksson, for your help with proofreading of “Swedish summary”.

All staff at Svartbäckens- and Samariterhemmets MVC, for all the help with the recruitment of normal pregnant women.

Hans Lindgren, Kerstin Stålberg, Martin Selinus and Barbro Westerberg, for all the logistical help.

My late father, Dr. Ahad, my inspiration, who would have been most proud and most glad for this thesis today, if only he was alive today to share my joy and achievement. May GOD bless you in paradise. My mother, Ruby, I’m very glad that you have experienced the process of this book with me. Last time you were so concerned about me and how I would manage “everything”.

My sisters, Sobnom & Naju and My brothers, Daniel & Reza, and all in my family in law, for your endless love, support and encouragement throughout the years.

My family: My husband Mahfuz, for all the support and encouragement and for also “being a mam” to Tamanna when I was not there. Last but not least,
my daughter, Tamanna, my treasure, you have fulfilled my life. You missed me so much when I was away from you and worked on this thesis.

The studies in the thesis were supported by grants from the Erik, Karin and Gösta Selander Foundation, the Thuréus Foundation, Research Council in Uppsala from Uppsala County Council, and ALF funding from Uppsala University Hospital.
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