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Sub-clinical atherosclerosis in the common carotid artery in women with/without previous pre-eclampsia: A seven-year follow-up

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HIGHLIGHTS

- Substantially affected arteries at follow-up 7 years after preeclamptic pregnancy.
- Artery wall layer dimensions associated with CVD-risk factors and vascular inflammation.
- In line with the documented increased risk of CVD later in life in pre-eclampsia.

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ABSTRACT

Background and aims: Pre-eclampsia is associated with increased risk of cardiovascular disease and premature death. However, conventional common carotid artery intima-media thickness (CCA-IMT) measurement does not reflect this. In contrast, measurement of the individual CCA intima and media thicknesses clearly indicates increased vascular risk both at diagnosis and about one year after pre-eclampsia. This study examined whether individual CCA wall layers, risk factors for cardiovascular disease, and markers of endothelial dysfunction had normalized or remained unfavorable seven years after pre-eclampsia.

Methods: The individual CCA intima and media thicknesses were measured using 22 MHz ultrasound. Conventional cardiovascular risk factors were recorded. A thick intima, thin media and high intima/media thickness ratio (I/M) are signs of sub-clinical atherosclerosis.

Results: The median age of women with previous pre-eclampsia (cases = 23) or normal pregnancies (controls = 35) was 39/37 years. At follow-up (median about seven years), the intima remained thicker and the I/M was higher in cases than in controls [all $p < 0.0001$; $p < 0.001$ after adjustment for time to follow-up, body mass index (BMI), and mean arterial pressure (MAP)], whereas the CCA-IMT was illogically thinner. Further, BMI, MAP, hip circumference, abdominal height, serum endostatin and apolipoprotein B levels were higher in cases (all $p < 0.05$). Intima and I/M measurements were correlated with age, MAP, endostatin and apolipoprotein B, whereas no logical correlations were found for CCA-IMT.

Conclusions: The arteries in cases but not controls were still adversely affected after seven years. Measuring intima thickness and I/M appears preferable to measuring CCA-IMT for demonstrating vascular risk after pre-eclampsia.

1. Introduction

Worldwide, pre-eclampsia is a leading cause of maternal and perinatal morbidity and mortality [1]. The stresses and changes that occur during pregnancy can reveal potential risks of future chronic diseases [2] and pre-eclampsia is an independent risk factor for subsequent hypertension and cardiovascular disease (CVD) events [3,4]. CVD is the main single cause of morbidity and mortality in women [5]. The risk of CVD is higher in women with more severe pre-eclampsia [4] and is higher when the pre-eclamptic pregnancy is associated with intrauterine growth retardation (IUGR) and/or preterm birth [6]. Thus, previous pre-eclampsia would be expected to be associated with signs of sub-clinical atherosclerosis. However, assessment using conventional 7–10 MHz ultrasound of the common carotid artery intima-media thickness (CCA-IMT) does not show any significant difference in the degree of sub-clinical atherosclerosis between women with ongoing or

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previous pre-eclampsia and women with normal pregnancies [7–11]. The intima successively increases in thickness while the media decreases in thickness with increasing age and development of atherosclerosis, as demonstrated by both histomorphometry [12] and intravascular high-frequency ultrasound [13]. Thus, a thick intima, thin media and high intima/media thickness (I/M) ratios indicate a less healthy artery wall and assessment of these could be more appropriate than assessment of the combined CCA-IMT for demonstrating subclinical atherosclerosis. However, to obtain separate estimates of the intima and media layers, higher frequencies than those used for conventional ultrasound (7–10 MHz) are needed. Repeatedly, we have found that assessment of intima thickness and I/M ratio, rather than CCA-IMT, provides better evidence of the vascular effects of aging and long-term menopausal estrogen therapy [14], prevalent CVD, and risk factors for CVD [15] and systemic lupus erythematosus [16]. In contrast, assessment of CCA-IMT has not indicated any cardiovascular risk in any of these settings [14–16].

In one of our previous studies, we showed that women with pre-eclampsia had signs of sub-clinical atherosclerosis in CCA, i.e. they had thicker intima layers, thinner media layers and higher I/M ratios than women with normal pregnancies, both during pregnancy and about one year postpartum [11], whereas assessment of the CCA-IMT did not indicate any differences between the groups. To our knowledge, there is no prospective follow-up study investigating changes in the individual artery wall layer dimensions in women with previous pre-eclampsia. Based on the known increased risk of CVD in women with a history of pre-eclampsia [3,4], we hypothesized that these women would still have a thicker CCA intima layer, a thinner media layer, a higher I/M ratio, and higher values for certain serum markers of vascular inflammation and conventional cardiovascular risk factors about seven years after the index pregnancy than women with previous normal pregnancies.

2. Materials and methods

This is a follow-up study of one of our previous studies [11]. Fifty-five women diagnosed with pre-eclampsia and 64 women with normal pregnancies and pregnancy outcomes were recruited during 2007 and 2010 (termed baseline) [11]. Between the years 2008 and 2011, 48 women with a history of pre-eclampsia and 55 women with a history of normal pregnancies were re-examined about one year after the index delivery (termed postpartum) [11]. The recruitment process at baseline and postpartum have been extensively described in our previous study [11].

This follow-up study was started in March 2015 and completed in December 2016. All measurements were carried out at Uppsala University Hospital, Sweden. All women who participated in the postpartum evaluation were invited by mail to join the follow-up study. Women had to be at least three months postpartum in the case of a new pregnancy and not lactating for at least three months before they could participate.

All participants filled in a questionnaire about smoking habits, family history of cardiac disease, number of pregnancies and childbirths, and dates of the last delivery after the one-year postpartum evaluation. They also provided information about hypertension, myocardial infarction, stroke, diabetes mellitus or renal disease and any drug therapy taken after the postpartum evaluation.

Height and weight were measured and the body mass index (BMI, kg/m^2) was calculated. Blood pressure was measured after about 15 min' rest, in the supine position, on the right upper arm, using automated blood pressure equipment (Umedico, calf-size 12 × 35 cm, or otherwise as appropriate for the arm circumference). Mean arterial pressure (MAP) is a better predictor of pre-eclampsia than systolic (SBP) and diastolic blood pressure (DBP) [17] and this was calculated as DBP + ⅓ (SBP - DBP).

At follow-up, a venous blood sample was collected from each woman. The samples were kept at room temperature for about half an hour before being centrifuged for 10 min at 2000*g*. The serum samples were separated. A urine sample was also collected from each participant. Both serum and urine samples were stored at −70 °C until biochemical analysis.

2.1. High-frequency ultrasound of the artery wall

The left CCA wall layers were imaged using high-resolution ultrasound equipment fitted with a broad-band probe at 22 MHz center frequency (Collagenoson®, Minhorst Company, Meudt, Germany), as extensively described previously [14,15]. In short, 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 did not exceed 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 (Supplementary Fig. 1). Point estimates of the artery wall, not adjusted to the cardiac cycle, were obtained and about twenty point estimates were saved on a computer by one researcher (ML). The individual artery wall layer dimensions were measured off-line for all participants by another researcher (TA), who was blinded with regard to study group. Means of about 10 technically acceptable measurements were calculated and used in the analysis. In our laboratory, the coefficient of variation (CV) was 3.9% for intima thickness and 3.4% for media thickness [14].

2.2. Biochemical analyses

Serum levels of apolipoprotein A1 (9D92), apolipoprotein B (9D93), creatinine (8L24, enzymatic method), C-reactive protein (CRP) (6K26), and fructosamine (04537939190) were analyzed by Roche Diagnostics, Mannheim, Germany; levels of urine albumin (2K98) were analyzed on a BS380 instrument (Mindray, Shenzhen, China). The reagents were from Abbott Laboratories, Abbott Park, IL, USA, if not otherwise specified. Serum NT-proBNP levels were analyzed by a Roche Cobas 8000 instrument, using the e602 module (Roche Diagnostics, Mannheim, Germany) according to the specifications of the manufacturer. The instrument had a total CV of 0.9% at 107 ng/L and 1.3% at 2060 ng/L. Serum levels of endostatin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were analyzed using commercially available ELISA kits (DY1098, DY809 and DY720, R&D Systems, Minneapolis, MN). The assays had a total CV of approximately 6%. Laboratory technicians were blinded to participant assignment.

2.3. Ethical considerations

The study protocol was approved by the local Ethics Committee of the Medical Faculty of Uppsala University. Informed written consent was obtained from each woman before inclusion in the study.

2.4. Statistical analysis

The results are presented as medians and interquartile ranges. Differences in the distributions of categorical variables were tested by Chi-square tests. Between-group differences were tested using the Mann-Whitney *U* test and within-group differences were tested using the Wilcoxon signed rank test. The Spearman rank correlation test was used to test correlations for the combined group (pre-eclamptic and normal pregnancies), as justified by substantial overlapping between the groups with regard to CCA wall layer dimensions (Fig. 1). Differences in the artery wall layer dimensions between the groups at follow-

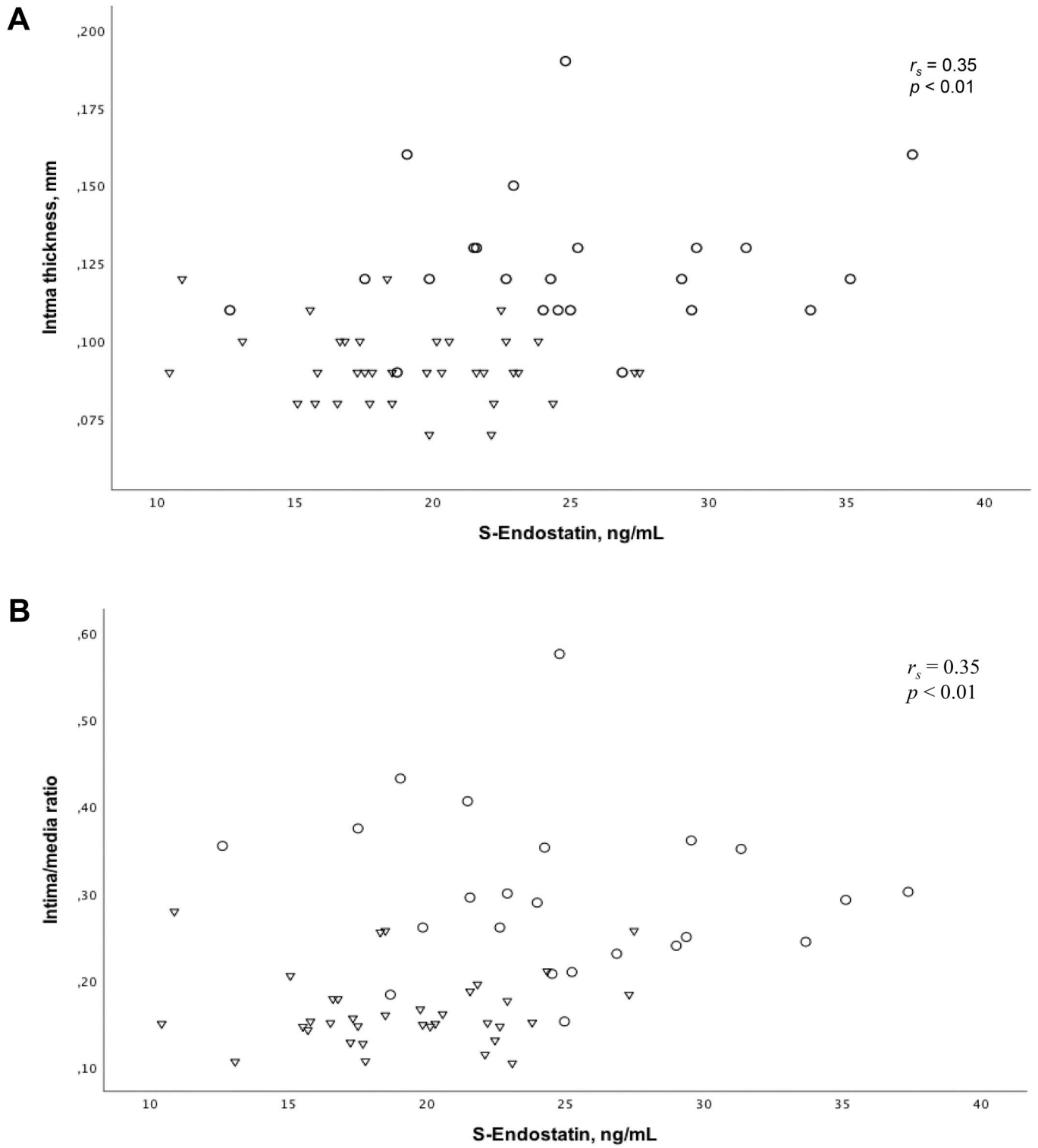


Fig. 1. Correlation between common carotid artery (CCA) layer dimensions and serum-endostatin levels in women with previous pre-eclampsia.
Correlations between CCA (A) intima thickness and (B) intima/media thickness ratio and serum levels of endostatin at follow-up after seven years in women with previous pre-eclampsia (circles) and in women with previous normal pregnancies (triangles), assessed using the Spearman Rank Correlation test. r_s = correlation coefficient.

up were adjusted for differences in the time to follow-up using simultaneous quantile regression (Bootstrap replicas 1000). The level of significance was set at a p value of < 0.05. Statistical analysis was carried out with SPSS, version 22.0 (SPSS Inc. PASW statistics) and STATA 14.0 for Windows software.

3. Results

The process of recruitment is shown in Supplementary Fig. 2. Demographic data for the study population at follow-up are shown in Table 1. The groups were similar except for the time since evaluation at postpartum, which was longer in women with previous pre-eclampsia.

Table 1
Demographic data and clinical characteristics of women with previous preeclampsia and previous normal pregnancies.

Characteristics	Preeclampsia (n = 23)		Normal pregnancies (n = 35)	
	About 1 y postpartum	At follow-up, about 7 y postpartum	About 1 y postpartum	At follow-up, about 7 y postpartum
Maternal age (y)	33 (30, 35)	39 (36, 41)	32 (30, 35)	37 (35, 40)
Primiparous	16 (70%)	6 (26%)	14 (40%)	4 (11%)
Smoking	0 (0%)	1 (4%)	1 (3%)	3 (9%)
Antihypertensive medication	0 (0%)	1 (4%)	0 (0%)	0
Time since evaluation at postpartum (mo)		72 (64, 73)*		64 (62, 65)
Time since last delivery (mo)		74 (57, 86)		75 (52, 78)
Pregnancy since evaluation at postpartum		11 (49%)		14 (40%)
Childbirth since evaluation at postpartum		10 (43%)		13 (37%)
Body mass index (kg/m^2)	27 (23, 31)†	26 (23, 31)‡	22 (20, 25)	23 (21, 26)
Systolic blood pressure (mmHg)	120 (113, 123)	115 (110, 120)‡	110 (105, 110)	110 (105, 120)
Diastolic blood pressure (mmHg)	80 (86, 96)*	80 (70, 80)†	68 (61, 74)	70 (65, 75)
Mean arterial pressure (mmHg)	90 (110, 119)*	90 (83, 93)†	82 (77, 87)	83 (80, 90)
Waist circumference (cm)		83 (75, 93)		77 (73, 84)
Hip circumference (cm)		101 (94, 107)‡		96 (92, 101)
Waist/hip ratio		0.83 (0.76, 0.87)		0.82 (0.78, 0.85)
Abdominal height (cm)		21 (19, 22)†		19 (18, 21)

Medians (first and third quartiles) or number (%).
*$p < 0.0001$, †$p < 0.01$and ‡$p < 0.05$ compared to corresponding period in women with normal pregnancy.
Data at 1 year postpartum is reused with permission from the American Heart Association.[11].

Two women in the pre-eclampsia group had recurrent pre-eclampsia in their subsequent pregnancies. One woman in the pre-eclampsia group developed chronic hypertension and type II diabetes mellitus, with mild hyperlipidemia between evaluations. Responder *vs.* non-responder analysis at follow-up showed that non-responders were younger and had higher DBP and MAP one year postpartum. However, values of BMI, SBP and artery wall layer dimensions were similar.

At follow-up, BMI, SBP, DBP and MAP values were still significantly higher in the women with previous pre-eclampsia than in the women with previous normal pregnancies, and were similar to those from about one year postpartum. Values for hip circumference and abdominal height were higher in the women with previous pre-eclampsia (Table 1).

At follow-up, women with previous pre-eclampsia still had a thicker intima layer, a thinner media layer and a higher I/M ratio than women with previous normal pregnancies (all $p < 0.0001$; all $p < 0.001$ after adjustment for time to follow-up, BMI and MAP) (Table 2 and Fig. 2).

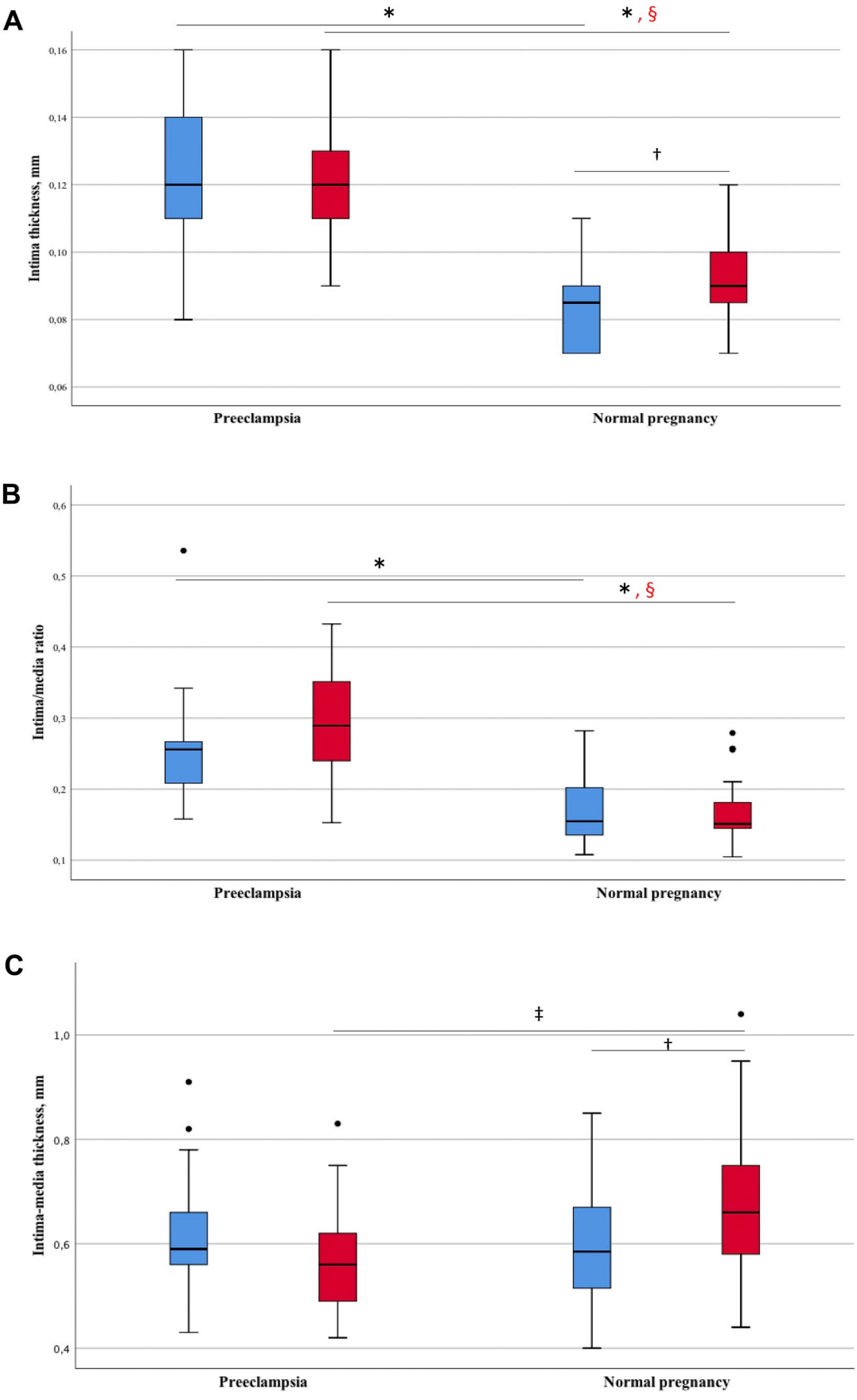
There were no substantial changes in intima thickness and I/M ratio within the study groups, but there was a significant group difference with regard to changes in media thickness involving further deterioration in women with previous pre-eclampsia and an improvement in those with previous normal pregnancies (p = 0.02) (Table 2). Serum levels of endostatin had decreased in both groups and levels of NTProBNP had decreased in the pre-eclampsia group, whereas levels of apolipoprotein A1 and apolipoprotein B had increased in both groups and levels of fructosamine had increased in the pre-eclampsia group (Table 2). Further, at follow-up, women with previous pre-eclampsia had higher levels of serum endostatin and apolipoprotein B and lower levels of VCAM-1 and ICAM-1 than women with previous normal pregnancies (Table 2).

At follow-up, intima thickness was positively associated with age, MAP and endostatin levels (r_s 0.30, 0.28 and 0.35, respectively; $p < 0.05$, < 0.05 and < 0.01, respectively) and the I/M ratio was positively associated with MAP, endostatin levels and apolipoprotein B

Table 2
Common carotid artery wall layer characteristics and cardiovascular biomarkers in women with previous pre-eclampsia and previous normal pregnancies.

Characteristics	Pre-eclampsia (n = 23)		Normal pregnancies (n = 35)	
	About 1 y postpartum (PP)	At follow-up,about 7 y PP	About 1 y PP	About 7 y PP
Intima thickness (mm)	0 0.12 (0.11, 0.14)*	0.12 (0.11, 0.13)*, ±	0.09 (0.07, 0.09)§	0.09 (0.08, 0.10)
Media thickness (mm)	0.48 (0.44, 0.57)	0.44 (0.36, 0.50)*, ±	0.51 (0.43, 0.60)§	0.56 (0.48, 0.66)
Intima/media thickness ratio	0.26 (0.20, 0.27)*	0.29 (0.24, 0.35)*, ±	0.15 (0.14, 0.20)	0.15 (0.15, 0.18)
Intima-media thickness (mm)	0.59 (0.55, 0.67)	0.55 (0.49, 0.62)†	0.59 (0.51, 0.68)§	0.66 (0.58, 0.76)
Endostatin (ng/mL)	41 (34, 57)¶	25 (21, 29)*	39 (29, 49)¶	19 (17, 22)
VCAM-1 (ng/mL)	328 (241, 407)	328 (183, 407)‡	369 (284, 448)	386 (330, 492)
ICAM-1 (ng/mL)	168 (113, 188)	141 (74, 195)†	146 (118, 205)§	203 (151, 256)
CRP (mg/L)	0.54 (0.30, 1.87)	1.2 (0.59, 2.13)	0.43 (0.18, 1.51)	0.67 (0.51, 1.46)
NT-proBNP (ng/L)	53 (35, 94) ᵘ	37 (25, 50)	59 (34, 90)	50 (26, 89)
Apolipoprotein A1 (g/L)	1.24 (1.04, 1.37)#	1.7 (1.5, 1.9)	1.29 (1.11, 1.51)¶	1.7 (1.5, 1.9)
Apolipoprotein B (g/L)	0.68 (0.58, 0.78)‡§	0.87 (0.76, 0.98)‡	0.57 (0.50, 0.74)¶	0.79 (0.65, 0.87)
Fructosamine (μmol/L)	329 (297, 374) ᵘ	370 (309, 429)	353 (299, 409)	379 (338, 402)
Serum kreatinin (μmol/L)		76 (67, 82)		75 (72, 88)
Urin albumin (mg/L)		6 (5, 15)		6 (4, 10)
Urin kreatinine (mmol/L)		7.6 (4.5, 11.5)		6.3 (2.7, 10.5)
Urin-albumin/kreatinin ratio (g/mmol)		0.9 (0.64, 1.54)		1.03 (0.71, 1.64)

Medians (first and third quartiles).
*$p < 0.0001$, ± $p < 0.001$ after adjustment for time to follow-up, blood pressure and BMI, †$p < 0.01$ and ‡$p < 0.05$ compared to corresponding period in women with normal pregnancy.
¶$p < 0.0001$, #$p < 0.001$, §$p < 0.01$ and ᵘ $p < 0.05$ compared to values at follow up within the group.
Data about common carotid artery wall layer dimension at 1 year postpartum is reused with permission from the American Heart Association.[11].



(caption on next page)

Fig. 2. Comparison of common carotid artery (CCA) wall layer dimensions in women with previous pre-eclampsia and women with normal pregnancies.
Boxplot analysis showing differences in the CCA (A) intima thickness, (B) intima/media thickness ratio, and (C) intima-media thickness (obtained by 22 MHz non-invasive ultrasound) in women with previous pre-eclampsia and in women with previous normal pregnancies, at one year postpartum (blue box) and at follow-up, about seven years postpartum (red box).
The tops and bottoms of the boxes represent the third and first quartiles. The horizontal lines within the boxes represent the median values. The bars on the sides of the boxes represent the highest and lowest values. Black-filled circles represent extreme values.
$^{*}p < 0.0001$ ($^{\S}p < 0.001$ after adjustment for time to follow-up, body mass index and mean arterial pressure). $^{\ddagger}p < 0.01$ compared to corresponding period in women with previous normal pregnancies. $^{\dagger}p < 0.01$ compared to values at follow-up within the group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Associations between common carotid artery wall layers and cardiovascular risk factors at follow-up in women with/without pre-eclampsia.

Common carotid artery wall layers	Age r_s	BMI r_s	MAP r_s	Endostatin r_s	Apolipoprotein B r_s
Intima thickness	0.30*	0.14	0.28*	0.35†	0.11
Media thickness	−0.10	−0.14	−0.27*	−0.25	−0.34†
Intima/media ratio	0.19	0.21	0.36†	0.38†	0.34†
Intima-media thickness	−0.05	−0.10	−0.23	−0.20	−0.34†

Spearman rank correlation test. r_s, correlation coefficient. $^{*}p < 0.05$; $^{\dagger}p < 0.01$.

levels (r_s 0.36, 0.38 and 0.34, respectively; all $p < 0.01$) (Table 3). In contrast, CCA-IMT was paradoxically thinner (healthier) in women with previous pre-eclampsia than in women with previous normal pregnancies (Table 2 and Fig. 2), and the CCA-IMT thickness was also paradoxically negatively associated with apolipoprotein B levels (r_s −0.34; $p < 0.01$; Table 3).

4. Discussion

About seven years after delivery, as at the one-year postpartum assessment [11], women with previous pre-eclampsia still had more adversely affected arteries, with regard to signs of sub-clinical atherosclerosis on ultrasound, than women with normal pregnancies; i.e. the intima layer was significantly thicker, the media layer was thinner, and the I/M ratio was higher. In addition, the thickness of the intima and/or the I/M ratio was correlated logically and significantly with age, MAP, and serum endostatin and apolipoprotein B levels. In contrast, the CCA-IMT was unexpectedly thinner (healthier) in women with previous pre-eclampsia and was also illogically negatively correlated with apolipoprotein B levels. These findings from vascular imaging, except for those for the CCA-IMT, are in total accordance with well documented reports of a higher risk of CVD morbidity and mortality in women with a history of pre-eclampsia [3,4].

In a recent study from 2013, McDonald et al. showed that, approximately 20 years after delivery, the cardiovascular risk profile in women with previous pre-eclampsia was often similar to that in individuals with coronary heart disease [7]. Despite these poor cardiovascular risk profiles, they found no significant difference in the CCA-IMT between women with and without previous pre-eclampsia [9]. Similarly, in a follow-up study, Blauww et al. showed that about four or five years after the index pregnancy there was no difference in the CCA-IMT between women with previous severe pre-eclampsia and those with normal pregnancies [7]. Similar results have been found in other studies investigating women with previous pre-eclampsia [8,18,19]; all are in agreement with the CCA-IMT results in our study – but are totally contradictory to the well documented increased cardiovascular risk in women with a previous history of pre-eclampsia [3,4]. Further, the results of this follow-up study are also in line with one of our previous cross-sectional studies in which women with previous severe pre-eclampsia, about 11 years after the index delivery, had more negatively affected artery wall layers than women with previous normal pregnancies [20]. However, there have been no prospective follow-up studies of the separate artery wall layer dimensions in women with previous pre-eclampsia before this.

Pre-eclampsia *per se* is an early indicator of metabolic dysfunction [21,22]. The American College of Cardiology has recently included a previous history of pre-eclampsia as a risk-enhancing factor in their "Guideline on the Management of Blood Cholesterol" [23]. The population in our study was relatively young, with a median age of 39 years for the pre-eclampsia group. Despite this young age we found that, at follow-up, women with previous pre-eclampsia were still overweight and had higher BMI, blood pressure, hip circumference, abdominal height, and levels of serum apolipoprotein B than women with normal pregnancies. Metabolic syndrome is a well-known risk factor for atherosclerosis and subsequent CVD [24]. Our findings of a positive significant correlation for intima thickness vs age and MAP, and for I/M ratio vs MAP and apolipoprotein B, indicate that the women with previous pre-eclampsia had an adverse metabolic profile that correlates with adverse arterial images about seven years after the index pregnancy. Non-fasting levels of apolipoprotein A [the protein of high density lipoprotein (HDL)] and apolipoprotein B [the protein of low density lipoprotein (LDL)] are better markers of atherogenic/metabolic status than conventional lipid status measurements and are therefore better indicators of the risk of CVD [25].

Our findings of lower levels of VCAM-1 and ICAM-1 at follow-up in women with previous pre-eclampsia is paradoxical considering the increased risk of CVD in this group. However, results in previous studies analyzing serum levels of adhesion molecules in women with previous pre-eclampsia have been contradictory - some showed higher levels than those in women with previous normal pregnancies [26] and some did not [27]. The values for adhesion molecules at follow-up were similar to those at one year postpartum. This might be because the state of acute inflammation at diagnosis of pre-eclampsia was no longer present at one year postpartum and at follow-up. This assumption is strengthened by the non-significant differences in CRP between the two evaluations and by our previous findings of non-significant differences in pentraxin-3 [28] and dimethylarginines (unpublished data) between women with and without pre-eclampsia at one year postpartum.

Endothelial dysfunction, a key factor in the pathogenesis of atherosclerosis, is common in women with previous pre-eclampsia [27,29,30]. Dimethylarginine levels are higher in pre-eclampsia than in normal pregnancy [31]. Elevated dimethylarginine levels lead to decreased nitric oxide levels, and nitric oxide is a potent vasodilator [32]. Endostatin is a C-terminal cleavage fragment of collagen XVIII and is an endogenous inhibitor of angiogenesis. At follow-up, our findings of higher levels of serum endostatin in women with pre-eclampsia and the positive correlation with intima thickness and the I/M ratio further indicate persisting endothelial dysfunction adversely affecting the

arteries, seven years after the index pregnancy.

While normal pregnancy seems to cause stress to the vascular system and artery walls, as indicated in our previous report [33], pre-eclampsia, in particular, causes stress to the metabolic and vascular status, and is an indicator of increased risk for vascular disease later in life [22]. There are numerous studies demonstrating signs of metabolic and endothelial dysfunction in women with previous pre-eclampsia [34,35]. Further, meta-analyses have unanimously indicated an increased risk of hypertension, ischemic heart disease, stroke and overall mortality many years after the index pre-eclamptic pregnancy [3,4]. One of these meta-analyses has even shown that severe pre-eclampsia and eclampsia are associated with a higher risk of cardiac disease, compared to mild or moderate pre-eclampsia [4]. Despite this well-documented increased risk of cardiovascular disease after pregnancy complicated by pre-eclampsia, to date no study using conventional CCA-IMT has been able to illustrate this increased risk. However, with increasing age and the development of atherosclerosis, the intima continues to increase in thickness while the media decreases [12,13], reducing the strength of the combined CCA-IMT. In this follow-up study, we measured the CCA wall layers separately and showed that the artery wall layer dimensions of women with previous pre-eclampsia, about seven years after the index pregnancy, were still negatively affected (sub-clinical atherosclerosis) – findings that were congruent with and associated with findings of higher BMI, higher BP, and increased metabolic and endothelial dysfunction.

The higher DBP and MAP in non-responders at one year post-partum are probably representative of regression towards the mean; i.e. they make it more difficult to show a significant difference in sub-clinical atherosclerosis at follow-up.

One major strength of the study is that both evaluations (i.e. at one year and seven years postpartum) were undertaken with the same researcher (ML) using the same equipment; ML is highly experienced in these methods of assessment. Further, all ultrasound scans were measured off-line by another researcher (TA), who was blinded with regard to the study group and the time of the examinations. The study may have been limited by the relatively small sample size, with the associated potential risk of type 2 error in the case of non-significant results. However, considering the highly significant, logical and consistent findings, the sample size seems to be less of a problem here. The lack of information about the dietary habits of the participants could also be seen as a limitation because of the well-recognized effect of diet on CVD [36]. However, in a meta-analysis, Berks et al. have shown that the risk of CVD after pre-eclampsia remains significant after adjustment for lifestyle interventions such as exercise, diet and smoking cessation [37].

In summary, using high-frequency ultrasound to measure the individual artery wall layer dimensions, we found that the arteries of women with previous pre-eclampsia remained substantially affected about seven years after the index pregnancy, compared to controls. The artery wall layer dimensions were logically and significantly associated with certain CVD risk factors and vascular inflammation markers. Our results are totally in accordance with the well documented increased risk of CVD and premature death in women with previous pre-eclampsia. In contrast, measurement of the CCA-IMT did not indicate any increased risk of CVD in these women in this study, as in previous reports. Thus, the suggested method based on assessment of intima thickness and I/M ratio, would appear to be preferable to conventional CCA-IMT measurement for reflecting the well-known increased cardiovascular risk in women with a history of pre-eclampsia.

Conflicts of interest

TN is holder of the US Patent #8556817, 'non-invasive methods for determining the cardiovascular status of an individual' using the principle of intima thickness and intima/media thickness ratio instead of conventional CCA-IMT. The other authors have nothing to disclose.

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Author contributions

TA and TN made substantial contributions to the conception and design of the study. ML carried out all ultrasound examinations. AL was responsible for all biochemical analyses. TA and TN analyzed the data. TA, AL, ML and TN made substantial contributions to interpretation of the data. TA drafted the manuscript and TN and AL critically revised the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.05.024.

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