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Pregnancy history and blood-borne microvesicles in middle aged women with and without coronary artery calcification

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Abstract

Background and aims—Having a history of preeclampsia increases the risk for future coronary artery calcification (CAC). This study evaluated the association of blood-borne, cell-derived microvesicles (MV) with CAC in middle-aged women.

Methods—Twelve pre-selected, antigen-specific MV were measured by digital flow cytometry in the blood of age- and parity-matched women (median age 60 years) without a history of cardiovascular events, but with either a history of preeclampsia (PE, n=39) or normotensive pregnancy (NP, n=40). CAC was determined by computed tomography.

Results—CAC scores ranged from 0-47 and 0-602 Agatston Units in the NP and PE groups, respectively. Waist circumference and insulin resistance were greatest in PE women with CAC. MV positive for tissue factor or stem/progenitor cell antigen (CD117) differed between NP and PE groups. In univariate analysis, those positive for tissue factor, ICAM-1, stem cells, and adipocytes (P16-set) antigens associated with CAC in the PE group. Principal components (PC) analysis reduced the MV variables to three independent dimensions. PC1 showed a modest correlation with

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Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

CAC scores in the PE group (ρ=0.31, p=0.06) and associated with CAC in a multivariable model on pooled groups that included all 3 PC variables when adjusted for pregnancy status (p=0.03). The association was lost when corrected for body mass index or waist circumference.

Conclusions—In women with a history of PE and elevated metabolic risk profile, a group of specific antigen-positive MV associated with CAC. These MV may reflect cellular processes associated with CAC. Their diagnostic potential for CAC remains to be determined.

Keywords

Extracellular vesicles; glucose; insulin; hypertension; microparticles

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in women. However, CVD risk calculators, such as the Framingham Risk Score, typically underestimate risk for adverse cardiovascular events in women. Therefore, there is a need to identify additional factors for cardiovascular risk prediction in women (1, 2).

Hypertensive pregnancy disorders, such as preeclampsia, are risk factors for future CVD. Screening guidelines recommend that pregnancy history be included for cardiovascular risk assessments in women (3-5). Our group reported that postmenopausal women with histories of preeclampsia had higher coronary arterial calcification (CAC) scores compared to age- and parity-matched women with histories of normotensive pregnancies (6). However, mechanisms contributing to accelerated development of cardiovascular disease in women with a history of preeclampsia remain to be defined. The only conventional cardiovascular risk factors that differed between women with and without a history of preeclampsia were body mass index (BMI) and current diagnosis of hypertension. Therefore, it is important to characterize the intravascular cellular processes activated by conventional risk factors to better identify and treat subclinical disease.

Cells activated by physical forces (blood pressure), inflammatory factors (cytokines and chemokines), metabolic factors (glucose, insulin, lipids), and sex hormones shed plasma membrane bound extracellular microvesicles (MV, 0.04 μm to1μm) into the blood. For example, in post-menopausal women characterized by metabolic syndrome, systolic blood pressure and waist circumference, reflecting metabolic central adiposity, independently associated with circulating monocyte- and endothelium-derived MV (7).

Circulating MV carry bioactive molecules, such as RNAs, proteins, receptors, and metabolites from parent cells to other cells initiating cellular signaling events (8-10). The circulating pool of MV, including their counts, expression of surface proteins and receptors, and content of bioactive molecules will depend upon their cellular origins and the stimuli that initiate their formation. In advanced cardiovascular disease, numbers of MV were 200 fold higher in atherosclerotic plaque than in plasma(11, 12) and in a study of women being screened for inclusion in the Kronos Early Estrogen Prevention Study based on CAC scores of < 50 Agatston Units (AU), the numbers of pro-thrombotic, platelet- and endothelium-derived MV were significantly elevated in the plasma of women with CAC scores > 50 AU,

compared to those with scores < 50 AU (13). Therefore, MV may represent the intermediate step connecting conventional risk factors and development of vascular disease. The present study was designed to: 1) confirm these previous findings of the association of endothelium-derived and procoagulant MV with CAC(13), and 2) identify other blood-borne MV that might associate with CAC in women at risk for CVD based on their pregnancy histories. Such information would inform future studies of the general population to perhaps develop cost-effective screening tests to identify women with subclinical CAC.

MATERIALS AND METHODS

Study participants

This study was approved by the Institutional Review Board at Mayo Clinic and Olmsted Medical Center in Rochester, MN. All participants gave written informed consent. Eighty post-menopausal women with and without confirmed histories of preeclampsia were recruited from the Rochester Epidemiology Project (14-16) to investigate the association between preeclamptic pregnancy and subclinical CVD. Women with histories of normotensive pregnancy (NP) were matched for parity and age at index birth to women with histories of preeclampsia (PE) and invited to participate in a clinical visit that included the measurement of CAC. All pregnancy histories and current covariates obtained at the time of the CAC measurements [i.e., body mass index (BMI), waist and hip circumference, systolic and diastolic blood pressures, blood chemistries, smoking status, education, marital status, and current medications] were confirmed by review of the medical records (6). All women, except one, underwent measurement of CAC by electron beam computed tomography, as previously described (13). CAC scores are reported as Agatston Units (AU).

Blood collection

Fasting early morning blood was collected from an antecubital vein using a 21 gauge needle and placed into tubes containing the appropriate anticoagulants for specific tests as indicated below. All samples were maintained at 33°C and testing of each sample was performed within 30 minutes of blood collection to provide consistency and to avoid inadvertent platelet activation. All measurements were obtained using standardized methodology previous published by our group (13, 17).

Blood clinical chemistries

Conventional cardiovascular risk factors as well as cytokines associated with chronic inflammation were measured by the Mayo Clinic Clinical Laboratories, Rochester, MN. These included: total cholesterol, low (LDL) and high (HDL) density lipoproteins, triglycerides, fasting blood glucose, hemoglobin A1C, insulin, tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and high sensitive-C reactive protein (hs-CRP).

Isolation, identification, and characterization of blood-borne MV

Detailed standardized methods of MV isolation from protease inhibitor anti-coagulated blood by differential centrifugation, and identification and characterization by digital flow cytometry were as previously published (17). The concentrations of blood-borne MV are expressed as MV/μL plasma. A set of 12 MV were tested based the rationale of that chronic

inflammation and/or oxidative stress promotes a pro-coagulant condition resulting from activation and interactions among the blood elements, endothelium and vascular smooth muscle that subsequently initiate processes resulting in vascular calcification. The set of 12 MV included those positive for pro-coagulant surface phosphatidylserine (annexin-V binding), tissue factor (TF), MV positive for the anticoagulant tissue factor pathway inhibitor (TFPI), MV positive for vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), and MV derived from leukocytes (CD45), platelets (CD42a), endothelial cells (CD62e), smooth muscle cells (SM22α), stem/progenitor cells (CD 117), senescent cells (P16-set), and adipocytes (Pref-1)(18).

Statistical analysis

Clinical descriptors of the study participants were summarized with quartiles (median, 25^{th} and 75^{th} percentiles) for continuous variables, or with counts and percentages for discrete variables. Two-sample comparisons between groups of women with histories of normotensive or preeclamptic pregnancies were performed using the Wilcoxon rank sum test or Chi squared test, as appropriate. Similar methods were carried out for group-stratified comparisons between those with and without positive CAC scores. Correction for multiple comparisons was applied when testing for differences between the two groups for the 12 types of pre-selected blood-borne MV variables(19). As this number of factors was too much to simultaneously test for group differences in a multivariable model, given the sample size restrictions, and the potential inter-dependency among the variables, the list of variables was reduced into a smaller set of dimensions using data reduction techniques. Thus, an exploratory principle components (PC) analysis was used to identify variable clusters that could be represented as single scores based on linear combinations of the original MV variables, such that the first few PCs explain the majority of the total variance. For robustness, the 12 MV variables, each skewed, were transformed into rank-based, normalized measures (probits) prior to PC analysis. Spearman ρ rank correlation coefficients were used to assess the relation of each MV dimension with CAC, stratified by group. The association of MV-related PCs with CAC was also tested on the pooled set of women in a multivariable ordinal logistic model where CAC (defined as an ordinal i.e. rank-based variable) was the dependent variable, PCs were predictor variables, and the preeclampsia group was an adjusting covariate. All data analyses were carried out using SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC). An alpha level of 0.05 was used to define statistical significance.

RESULTS

Among the 79 of 80 women in whom CAC was measured, 50 (63%) women had a CAC score of zero (n=30 in the NP group; n=20 in the PE group). The remaining women had CAC scores greater than zero: 20 had scores ranging from 0.5 - 47 AU (10 each in the NP and PE groups), and 9 (all from the PE group) had scores ranging from 61 – 602 AU). The clinical characteristics of women within each group without (CAC=0) and with coronary calcification are provided in Table1. Of the conventional cardiovascular risk factors, BMI was greater in women with CAC in the NP group; whereas waist circumference was significantly greater in women with CAC in the PE group. Framingham risk scores tended to

be higher between women with CAC scores >0 in both groups (p=0.07 for each). In the PE group, fasting glucose, fasting insulin and hemoglobin A1C were greater in women with compared to without CAC. Thus, the calculated Homeostasis Model Assessment for insulin resistance (HOMA-IR) was greater in women of the PE group with compared to without CAC [median (25^{th}, 75^{th} percentiles): 3.0 (1.8, 4.7) *vs.* 1.2 (1.0, 2.1); p=0.005]. Non-specific inflammatory markers, however, including TNFα, IL-6, and hs-CRP did not differ between women with and without CAC in either group (Table 1).

Assessing differences in individual MV between women with and without a history of PE, only MV derived stem/progenitor cells (CD117) were significantly elevated in the PE group after correction for multiple comparisons (p=0.048, Table 2). MV derived from smooth muscle cells (SM22α) positively correlated with CAC scores in women of the NP group (Table 3) from the group-stratified analyses uncorrected for comparisons. The number of MV positive for ICAM-1 in the PE group correlated with CAC scores, and MV positive for tissue factor, stem cells and adipocyte antigens also showed a borderline, yet non-significant association with CAC (Table 3) with a nominal p of 0.053 - 0.073. Tests for interaction with pregnancy history revealed a nominally significant group difference in the correlation between stem/progenitor cells-derived MV and CAC (Table 3).

Using an exploratory PC analysis to account for the inter-relationships among different MV, cell-cell and cell-MV interactions, and to maximize the information contained in the collective data, the 12 types of MV variables were reduced to 3 independent dimensions that accounted for 67% of the original variability (Table 4). The same three PCs were identified for women with histories of normotensive or preeclampsia. There was a trend towards a correlation for PC1 with CAC in women with histories of preeclampsia (Spearman ρ=0.309; p=0.056; Table 4) in group-stratified analyses. Based on the loadings shown in Table 4, PC1 that represents an overall weighted average of all 12 MVs, weighted slightly higher for pro-coagulant (TF) MV, those expressing cellular adhesion molecules (VCAM-1 and ICAM-1), and MV derived from leukocytes, endothelium, senescent cells and adipocytes. A multivariable model on the combined groups was fit to relate the PC components with CAC, while adjusting for a history of preeclampsia. PC1 was nominally significantly associated with CAC using this approach (p=0.034; Table 4). After adjusting for BMI or waist circumference, the relationship between PC1 and CAC was attenuated and no longer significant (p=0.135).

DISCUSSION

There are two important findings of the present study that provide insight into processes associated with CAC in postmenopausal women. First, in women with histories of preeclampsia, both waist circumference and measures of insulin resistance (glucose, insulin, hemoglobin A1C levels and the calculated HOMA-IR) were greater in women with CAC. BMI was greater in women with CAC who had histories of normotensive pregnancies, but other measures of metabolic risk (waist and hip circumference, glucose, and hemoglobin A1C levels) did not reach statistical significance. These observations are consistent with the concept that cardio-metabolic risk factors exist on a continuum (20) and women with histories of normotensive pregnancies may be early on the continuum as evidenced by the

low levels of CAC. Because cardio-metabolic risk characterized by type 2 diabetes is greater in women than in men (21), these results suggest that this association may be even more important in women with histories of preeclampsia. Current risk calculators underestimate cardiovascular risk for women; therefore, developing a cardiovascular risk calculator that includes components of pregnancy history and insulin sensitivity (i.e., perhaps a calculated HOMA-IR score), rather than just a “yes/no” for a diabetes history (22) may improve cardiovascular risk stratification for post-menopausal women. The lack of significance in blood pressure and plasma lipids between groups and within groups by CAC status most likely reflects the use of medications to control these variables (6).

A second observation of the present study, which will require further validation, is that the MV derived from smooth muscle cells correlated with CAC only in women of the NP group. This finding may reflect the early stages of calcification in this group because all CAC scores greater than zero were < 50 AU. In the early stages of calcification, smooth muscle cells undergo trans-differentiation to express genes common to osteoblast mineralization (23-25). The relationship between formation and release of MV derived from vascular smooth muscle undergoing trans-differentiation requires further study.

There is also a potentially important finding from exploratory analysis that shows that an aggregate measure of all 12 MV was correlated with CAC scores in post-menopausal women, independent of a history of preeclampsia. An ongoing interaction among procoagulant and proinflammatory processes involving the endothelium, adipocytes and cell senescence is supported by the greater weightings of these MV to PC1. In previous studies, MV derived from platelets and endothelial cells, and pro-thrombotic MV were greater in post-menopausal women with CAC scores >50 AU(13, 26). Thrombogenic MV expressing tissue factor also correlated with CAC in participants without diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA) (27) and in plasma and plaque-derived MV of patients undergoing carotid endarterectomy (11). Also, consistent with the concept that metabolic factors reflected by BMI and waist circumference (central adiposity) contribute to development of CAC in women with a history of PE is the observation that the relationship of PC1 with CAC is no longer significant when adjusted for BMI and waist circumference. Thus, a concept that requires further investigation and validation and that represents the potential translational aspect of this work is that this panel of MV needs to be assessed in women with a history of preeclampsia and metabolic risk factors in order to identify those who might benefit from imaging for clinically significant calcification.

The mechanisms by which these populations of MV are generated and their contribution to the actual calcification process requires further study. For example, the contribution of stem cell derived MV in either contributing to or reflecting repair processes associated with calcification processes (28) needs to be clarified. However, such investigations will be hampered by the lack of a suitable animal model for preeclampsia that reflects the heterogeneity of the condition in humans. That is, the surgical, pharmacological and transgenic approaches (29) do not adequately reflect potential differences and interaction between the maternal and fetal origin of factors contributing to preeclampsia.

This study has several limitations. First, the sample size is small. The cohort also was pre-selected based on pregnancy history of preeclampsia and women with clinical CVD or prior events were excluded. Thus, the results need to be validated in larger diverse groups of asymptomatic women. Additional work also is needed to determine if post-menopausal use of hormone treatments affects production of MV or if similar relationships between MV and CAC would be observed in pre-menopausal women. Longitudinal studies are also needed to determine if the panel of MV would associate with progression of CAC, that is, to establish the temporal relationship between the presence of MV in the circulation and the development of CAC. In addition, we used PC analysis to reduce the dimensionality of MV and found that a composite measure correlated with CAC, but this finding should be considered exploratory. Finally, all but one of the women was Caucasian so the results may not apply to other ethnic/racial groups.

Despite these limitations, the study represents a community based sample in which pregnancy history and cardiovascular status was validated by the medical record and by quantitative imaging. These results provide a starting point to develop and validate a blood test to identify CAC and a possible method to examine the mechanisms of progression in asymptomatic women defined as low risk by conventional cardiovascular risk calculators.

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Highlights

A history of preeclampsia (PE) increases risk of coronary artery calcification (CAC) in postmenopausal women.

Cardiometabolic risk profile is elevated in women with a history of PE.

Microvesicles (MV) derived from vascular cells characterize women with a history of PE and CAC.

Table 1

Clinical characteristics of study participants.

Variable	History of normotensive pregnancy group			History of preeclampsia group		
	CAC=0 (n=30)	**CAC>0 (n=10)**	***p*-value**	**CAC=0 (n=20)**	**CAC>0 (n=19)**	***p*-value**
Age at study consent	59.0 (55.4, 62.5)	60.5 (58.4, 62.5)	0.169	58.1 (56.4, 62.3)	60.0 (54.9, 64.2)	0.653
BMI (kg/m^2)	**24.1 (22.4, 25.9)**	**31.3 (27.2, 36.0)**	**0.007**	26.2 (24.5, 34.2)	31.4 (29.2, 33.7)	0.122
Waist circumference (cm)	83.3 (77.5, 98.0)	92.4 (85.0, 101.0)	0.089	**92.6 (79.9, 101.7)**	**103.2 (95.5, 105.4)**	**0.011**
Hip circumference (cm)	**102.3 (96.2, 106.5)**	**113.7 (107.0, 120.5)**	**0.007**	105.0 (99.5, 114.5)	111.4 (104.2, 115.3)	0.186
Systolic blood pressure (mmHg)	130.0 (116.7, 152.7)	125.5 (114.0, 137.7)	0.502	131.3 (121.0, 139.2)	135.7 (120.0, 141.3)	0.768
Diastolic blood pressure (mmHg)	72.7 (69.7, 84.3)	78.2 (76.0, 81.0)	0.482	75.7 (68.5, 82.8)	80.3 (73.3, 85.7)	0.318
Total cholesterol (mg/dL)	203.5 (178.0, 222.0)	205.5 (191.0, 223.0)	0.901	182.5 (166.5, 215.0)	194.0 (165.0, 218.0)	0.768
LDL cholesterol (mg/dL)	123.0 (95.4, 133.6)	117.7 (100.6, 143.4)	0.563	114.2 (87.5, 129.4)	98.2 (87.6, 118.4)	0.673
HDL cholesterol (mg/dL)	64.5 (52.0, 76.0)	52.5 (47.0, 77.0)	0.542	59.0 (46.0, 70.5)	50.0 (37.0, 63.0)	0.168
Triglycerides (mg/dL)	97.0 (77.0, 121.0)	101.5 (67.0, 126.0)	0.839	98.0 (74.0, 121.0)	141.0 (91.0, 209.0)	0.075
Fasting glucose (mg/dL)	95.5 (92.0, 98.0)	94.5 (88.0, 102.0)	0.766	**94.5 (91.0, 102.0)**	**104.0 (97.0, 119.0)**	**0.028**
Hemoglobin A1C (%)	5.5 (5.2, 5.7)	5.6 (5.4, 5.9)	0.285	**5.3 (5.2, 5.5)**	**5.6 (5.3, 5.9)**	**0.032**
Insulin (μIU/mL)	4.2 (2.7, 5.7)	5.2 (4.5, 6.7)	0.075	**5.0 (4.5, 8.4)**	**11.1 (7.0, 17.0)**	**0.003**
Tumor necrosis factor alpha (pg/mL)	1.3 (0.9, 1.5)	1.3 (1.1, 1.7)	0.347	1.3 (0.9, 1.4)	1.3 (1.1, 1.4)	0.375
Interleukin-6 (pg/mL)	1.6 (1.0, 2.2)	2.2 (1.4, 2.6)	0.293	1.7 (1.0, 3.3)	2.0 (1.6, 5.4)	0.241
hs C-reactive protein (mg/dL)	0.1 (0.1, 0.1)	0.2 (0.1, 0.2)	0.209	0.2 (0.1, 0.4)	0.2 (0.2, 0.3)	0.338
Framingham 10-Year Risk (%)	2.0 (1.0, 3.0)	4.0 (2.0, 5.0)	0.071	3.0 (1.5, 4.0)	5.0 (2.0, 6.0)	0.070

Data are shown as median (25th and 75th percentile); *p*-values are from Wilcoxon rank sum test uncorrected for multiple comparisons.

Table 2

Evaluation of MV between women with a history of normotensive pregnancy and with a history of preeclampsia.

Variable (MV/μL plasma)	Normotensive group (n=40)	Preeclampsia group (n=39)	Uncorrected *p*-value	Corrected *p*-value[a]
Procoagulant MV				
Phosphatidylserine (Annexin-V binding)	1018.9 (651.9, 1241.8)	865.2 (651.1, 1450.3)	0.900	0.900
Tissue factor	**10.8 (7.2, 16.8)**	**15.7 (9.0, 31.1)**	**0.028**	0.169
Ratio of TF/TFPI	6.9 (3.9, 10.6)	8.4 (4.7, 16.5)	0.169	0.675
Cell adhesion MV				
VCAM-1	1.5 (1.0, 1.9)	1.9 (0.9, 2.6)	0.379	0.692
ICAM-1	3.6 (2.8, 6.0)	3.5 (2.6, 5.7)	0.829	0.900
Cell-derived MV				
Leukocyte (CD45)	14.4 (10.3, 16.9)	14.6 (10.1, 22.2)	0.519	0.692
Platelet (CD42a)	831.6 (480.0, 1224.1)	679.0 (557.8, 1208.1)	0.810	0.900
Endothelium (CD62E)	6.5 (4.4, 9.9)	5.6 (3.9, 8.4)	0.411	0.692
Vascular smooth muscle (SM22α)	1.0 (0.7, 2.1)	1.5 (0.7, 2.9)	0.258	0.692
Stem cells (CD117)	**4.1 (3.2, 6.1)**	**7.3 (3.9, 11.0)**	**0.004**	**0.048**
Senescent cells (P16-set)	0.9 (0.5, 1.5)	1.0 (0.6, 2.1)	0.467	0.692
Adipocytes (Pref-1)	7.8 (4.8, 9.6)	8.7 (4.9, 15.3)	0.363	0.692

Data are shown as median (25^{th} and 75^{th} percentile).

[a] *p*-values are corrected for multiple comparisons to control for false discovery rate, as described by Benjamini and Hochberg [30]. TF, tissue factor; TFPI, tissue factor pathway inhibitor; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cellular adhesion molecule-1; CD, cluster of differentiation; Pref-1, pre-adipocyte secreted factor-1.

Table 3

Correlation of microvesicles (MV) with coronary artery calcification by pregnancy history status.

	History of normotensive pregnancy group		History of preeclampsia group		Test for interaction[a]
Variable	**Spearman ρ**	***p*-value**	**Spearman ρ**	***p*-value**	***p*-value**
Procoagulant MV					
Phosphatidylserine	0.145	0.374	0.056	0.738	0.385
Tissue factor	0.199	0.220	**0.312**	**0.053**	0.647
Ratio of TF/TFPI	−.167	0.305	0.084	0.612	0.507
Cell adhesion MV					
VCAM-1	−.008	0.963	0.139	0.400	0.576
ICAM-1	0.002	0.991	**0.326**	**0.042**	0.232
Cell-derived MV					
Leukocyte (CD45)	−.118	0.471	0.206	0.210	0.265
Platelet (CD42a)	0.132	0.421	0.055	0.739	0.478
Endothelium (CD62E)	−.072	0.662	0.222	0.176	0.269
Vascular smooth muscle (SM22α)	**0.410**	**0.008**	0.036	0.827	0.156
Stem cells (CD117)	−.169	0.301	**0.290**	**0.073**	**0.049**
Senescent cells (P16-set)	0.026	0.876	0.234	0.153	0.578
Adipocyte (Pref-1)	0.105	0.521	**0.291**	**0.072**	0.250

TF, tissue factor; TFPI, tissue factor pathway inhibitor; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cellular adhesion molecule-1; CD, cluster of differentiation; Pref-1, pre-adipocyte secreted factor-1.

[a]Using ordinal logistic regression, models were constructed (separate model for each MV variable) with CAC as the dependent variable, and covariates that included pregnancy hypertension group and (probit-rank transformed) MV parameter, along with the interaction between group and MV parameter; group differences in the correlation between CAC and MV variable were determined by testing the interaction term for significance.

Table 4

Principal component (PC) analysis of MV with coronary arterial calcification (CAC) between women with a history of normotensive pregnancy and with history of preeclampsia (PE).

Microvesicles (MV) / μL plasma	PC#1	PC#2	PC#3
Procoagulant			
Phosphatidylserine	**0.17**	**0.52**	**0.40**
Tissue factor	**0.38**	*− 0.24*	**0.09**
Ratio of TF/TFPI	**0.12**	*− 0.37*	**0.42**
Cell adhesion MV			
VCAM-1	**0.35**	**0.21**	*− 0.24*
ICAM-1	**0.33**	*− 0.04*	*− 0.25*
Cell- derived MV			
Leukocytes (CD45)	**0.31**	*− 0.21*	**0.12**
Platelet (CD42a)	**0.17**	**0.46**	**0.48**
Endothelium (CD62E)	**0.34**	**0.11**	*− 0.15*
Vascular smooth muscle (SM22α)	**0.23**	**0.15**	*− 0.39*
Stem cells (CD117)	**0.20**	*− 0.45*	**0.19**
Senescent cells (P16-set)	**0.33**	**0.06**	*− 0.23*
Adipocytes (Pref-1)	**0.37**	*− 0.06*	**0.19**
Proportion	*36.7%*	*16.8%*	*13.7%*
Cumulative	*36.7%*	*53.4%*	*67.1%*
Correlation with CAC (Spearman's ρ)			
in Normotensive pregnancy	ρ=0.081 (*p*=0.619)	ρ=0.168 (*p*=0.300)	ρ=−.064 (*p*=0.694)
in Preeclamptic pregnancy	ρ=0.309 (*p*=0.056)	ρ=−.130 (*p*=0.431)	ρ=−.134 (*p*=0.416)
Multivariable Model on pooled groups[a]	***p*=0.034**	*p*=0.882	*p*=0.786

TF, tissue factor; TFPI, tissue factor pathway inhibitor; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cellular adhesion molecule-1; CD, cluster of differentiation; Pref-1, pre-adipocyte secreted factor-1.

[a] *p*-values for testing the association of each PC with CAC in the pooled group of participants were obtained in a multivariable ordinal logistic regression model of CAC scores that included all three principle components as predictor variables, and prior PE as an adjusting variable. For brevity, only factors with the highest loading values for each PC are shown (bolded results represent positive loading values, and italicized results denote negative loading values).