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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 to 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 CAC scores in the PE group ($\rho = 0.31$, $p = 0.06$) 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.

1. 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

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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.

2. Materials and methods

2.1. 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 pre-eclampsia 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).

2.2. 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 min 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].

2.3. 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).

2.4. 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].

2.5. Statistical analysis

Clinical descriptors of the study participants were summarized with quartiles (median, 25th and 75th 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 great 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