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Full Length Article

An exploratory factor analysis of inflammatory and coagulation markers associated with femoral artery atherosclerosis in the San Diego Population Study

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ABSTRACT

Background and aims: Several biomarkers of inflammation and coagulation have been implicated in lower extremity atherosclerosis. We utilized an exploratory factor analysis (EFA) to identify distinct factors derived from circulating inflammatory and coagulation biomarkers then examined the associations of these factors with measures of lower extremity subclinical atherosclerosis, including the ankle-brachial index (ABI), common and superficial femoral intima-media thickness (IMT), and atherosclerotic plaque presence, burden, and characteristics.

Methods: The San Diego Population Study (SDPS) is a prospective, community-living, multi-ethnic cohort of 1103 men and women averaged age 70. Regression analysis was used to assess cross-sectional associations between the identified groupings of biomarkers (factors) and the ABI and femoral artery atherosclerosis measurements.

Results: Two biomarker factors emerged from the factor analysis. Factor 1 consisting of C-reactive protein (CRP), interleukin (IL)-6, and fibrinogen was significantly associated with higher odds (OR = 1.99, $p < 0.01$) of a borderline ABI value (0.91–0.99), while Factor 2 containing D-dimer and pentraxin (PTX)-3 was significantly associated with higher common femoral artery (CFA) IMT ($\beta = 0.23$, $p < 0.01$) and lower ABI ($\beta = -0.03$, $p < 0.01$).

Conclusions: Two groupings of biomarkers were identified via EFA of seven circulating biomarkers of inflammation and coagulation. These distinct groups are differentially associated with markers of lower extremity subclinical atherosclerosis. Our findings suggest that high inflammatory and coagulation burden were better markers of more severe lower-extremity disease as indicated by low ABI rather than early atherosclerotic lesion development in the femoral artery.

1. Introduction

Atherosclerosis is a chronic inflammatory disease that is the underlying disease process responsible for the majority of cardiovascular disease (CVD) morbidity and mortality [1–4]. Peripheral artery disease (PAD), one manifestation of atherosclerosis, affects an estimated 8.5 million individuals in the U.S [5]. PAD is associated with declines in functional status, increased risk of cardiovascular events, as well as cardiovascular and all-cause mortality [6–8].

Subclinical measures of atherosclerosis allow for the assessment of different stages of atherosclerosis development and for the identification of individuals at risk of cardiovascular events or complications. The ankle-brachial index (ABI), which is the clinical criterion used to diagnose PAD (ABI ≤ 0.90), is associated with reduced functional performance even in cases of a borderline ABI (0.91–0.99) suggesting that significant lower extremity atherosclerosis may occur before the ABI is considered abnormal [9]. Higher femoral artery intima-media thickness (IMT) is associated with PAD in patients with coronary heart disease [10] while plaque identified in the femoral artery is associated with reduced functional performance, and an increased risk of

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cardiovascular events [11–13]. Similarly, greater femoral plaque burden is associated with symptomatic PAD and worse functional performance [13,14].

Research investigating the inflammatory mechanisms contributing to atherosclerosis has identified several circulating biomarkers of inflammation and coagulation, including C-reactive protein (CRP), soluble intracellular adhesion molecule (sICAM)-1, interleukin (IL)-6, fibrinogen, D-dimer, Lipoprotein(Lp)-a, and pentraxin(PTX)-3, as independent predictors of cardiovascular risk [15–20]. However, less is known about the potential associations or involvement of these biomarkers in various stages of the development and progression of atherosclerotic disease in the lower extremities.

Our study had two objectives. First, we used an exploratory factor analysis (EFA) to identify the underlying structure of seven circulating markers of inflammation and coagulation. Next, we measured the associations of the derived biomarker groupings (factors) with measures of subclinical atherosclerosis: the ABI and IMT, plaque presence, burden, and characteristics in the common and superficial femoral arteries of healthy older adults.

2. Materials and methods

2.1. Participants

The San Diego Study Population (SDPS) is a prospective, multiethnic cohort study of lower extremity PAD and venous disease in men and women that has been described in detail elsewhere [21,22]. Briefly, employees of the University of California, San Diego and their significant others, all of whom resided in San Diego County, were recruited to participate in the study. Participants were selected randomly within strata of age, sex, and race/ethnicity. Women and racial/ethnic minorities (African-American, Hispanic, and Asian) were over-sampled. The present study includes 1001 SDPS participants who had both a femoral artery ultrasound and blood draw in order to measure inflammatory and coagulation biomarkers completed at a clinical exam between 2007 and 2011. All participants provided signed informed consent at baseline and follow-up, and the University of California-San Diego Institutional Review Board Committee on Investigations Involving Human Subjects approved the study.

2.2. Ultrasound measurements

Doppler ultrasound scans were conducted using an Acuson Aspen (Siemens, Inc.) to obtain images of three 10 mm segments of the right and left common and superficial femoral arteries: one at the common femoral artery (CFA) just proximal to the bifurcation, one at the bifurcation of the CFA and one at the SFA distal to the bifurcation. Five-second image clips were obtained for these segments at an angle of insonation of 90 degrees.

The measurement of femoral IMT and assessment of femoral plaque presence in the SDPS have previously been described in detail elsewhere [23]. Briefly, the posterior (far) wall of the left and right common and superficial femoral arteries was used in order to determine average common and superficial IMT using semi-automated Carotid Analyzer software from the Vascular Research Tools 5 Suite (Medical Imaging Applications LLS, Coralville, IA). Plaque presence was defined as a participant having plaque in at least one arterial segment (left SFA, right SFA, left CFA, or right CFA) and total number of plaques was defined as the sum of all plaques across the four segments. In some segments, visualization of plaque was limited due to artifact. Focal structures fitting the Mannheim consensus in these images were classified as “probable plaques”.

Plaque area and grey-scale median (GSM) were determined by one ultrasound reader using the aforementioned software. Area of each plaque was calculated by the software after tracing by the reader and outlining by the software. Total plaque area was defined as the sum of plaque area across the four arterial segments. GSM, a continuous measure of plaque echogenicity, was calculated by the software for each plaque. Normalization, which minimizes the effect of different ultrasound machine gain settings, was performed manually by the reader selecting a dark area of blood from the lumen and the brightest area of the adventitial layer. Mean GSM was calculated by averaging the GSM values for all identified plaques. The within-reader intraclass correlation coefficients (ICC) for plaque area and GSM were 0.95 and 0.99, respectively, according to a reliability study using femoral images at the University of Pittsburgh Ultrasound Research Lab. Calcification was subjectively identified by the vascular sonographers after assessing each plaque for highly echogenic areas and acoustic shadowing.

2.3. Ankle Brachial Index

With the participant in the supine position, systolic blood pressure (SBP) was measured twice in both arms, as well as both the anterior and posterior tibial and dorsalis pedis arteries using a Doppler probe (Acuson Aspen, Siemens, Inc.). The maximum of the average posterior tibial or dorsalis pedis SBP was divided by the maximum of the average left and right arm SBP to calculate ABI. The lower of the left and right leg's ABI was considered the index ABI.

2.4. Inflammatory and coagulation biomarkers

Nephelometric assays (Siemens Healthcare, Erlange, Germany) were used to measure CRP and fibrinogen from EDTA plasma, and Lp(a) in serum. Inter-assay coefficients of variation (CVs) were 4.1–5.1%, 3.2–4.7%, and 5.2–8.2% for CRP, fibrinogen, and Lp(a), respectively. sICAM-1 was measured in EDTA plasma using a non-allele specific enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) with a CV range of 10.3–11.0%. An ELISA was also used to measure PTX-3 in EDTA plasma and IL-6 in serum with CVs of 9.3–14.9% and 4.2–6.3%, respectively. Finally, D-dimer was measured in EDTA plasma using an Evolution Coagulation Analyzer (Diagnostica Stago, Parsippany, NJ) with a CV range of 2.7–24.7%.

2.5. Covariates

Age, sex, race/ethnicity, cigarette smoking habits, as well as prevalent CVD (MI, stroke, angioplasty, or revascularization) were determined via self-report. Diabetes was ascertained via self-report of the use of anti-diabetic medications or insulin and physical activity level was determined by asking participants to rate their perceived activity level compared to others their age.

Height (centimeters) and weight (kilograms) were obtained in order to calculate body mass index (BMI) as kg/m^2. Blood pressure was obtained from the right arm using a standard manual sphygmomanometer after five minutes of rest. Hypertension was defined as SBP $\geq$140 mm Hg, diastolic blood pressure (DBP) $\geq$90, or anti-hypertensive medication use. Total and high density lipoprotein (HDL) cholesterol were measured from non-fasting blood samples using a Roche Cobas 6000 analyzer (Roche Diagnostics Corporation, Indianapolis, IN). Low density lipoprotein (LDL) was calculated using the Friedewald equation [24]. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation, and a Roche Cobas 6000 analyzer (Roche Diagnostics Corporation, Indianapolis, IN) was utilized to measure serum creatinine levels via isotope dilution mass spectrometry [25].

2.6. Statistical analysis

An exploratory factor analysis was performed using maximum likelihood methods with direct oblimin rotation on Pearson correlation matrix using PROC Factor in SAS 9.3 (SAS Institute, Cary, NC). We compared the model fit and interpretability of models with one-