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## Homocysteine Levels and Disease Duration Independently Correlate With Coronary Artery Calcification in Patients With Systemic Lupus Erythematosus

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**Objective.** To compare the incidence and extent of coronary artery calcification (CAC) as measured by electron beam computed tomography (EBCT) in patients with systemic lupus erythematosus (SLE) and controls, and to identify variables associated with CAC in patients with SLE.

**Methods.** Female patients with SLE and matched controls were recruited; EBCT of the coronary arteries was performed, and laboratory values (including the homocysteine concentration, the lipid level, the highsensitivity C-reactive protein [hsCRP] concentration, the glomerular filtration rate [GFR], and the level of soluble CD154 [sCD154]) were determined. For patients, Systemic Lupus International Collaborative Clinics Damage Index and the Systemic Lupus Erythematosus Disease Activity Index scores were recorded. Tests of association between the CAC score and the above-mentioned variables were performed.

**Results.** The incidence of CAC was higher in patients with SLE than in controls ( $P = 0.009$ ), and patients had a higher mean raw CAC (rCAC) score (87.9 versus 9.6 in controls;  $P = 0.02$ ). In particular, more CAC-positive patients than CAC-positive controls had rCAC scores above the 75th percentile ( $P = 0.003$ ). Among both patients and controls, those with CAC were 10 years older than those without CAC. In addition to age, a significant determinant of positive CAC status in both groups was the number of cardiovascular risk factors. In patients with SLE, CAC was associated with a higher homocysteine concentration, a lower GFR, and longer disease duration. In controls, the total cholesterol level correlated positively with CAC. When multivariate logistic regression methods were applied to candidate explanatory variables, homocysteine concentration, age, and disease duration (but not the levels of sCD154 or hsCRP) contributed significantly to CAC status. The methylenetetrahydrofolate reductase C677T genotype did not predict hyperhomocysteinemia or CAC status.

**Conclusion.** Among patients with SLE, the homocysteine concentration, the GFR, age, and disease duration were associated with CAC. CAC occurred more frequently and was more extensive in patients with SLE than in controls, suggesting that EBCT could be used to detect premature atherosclerosis in the former group. An elevated homocysteine concentration might identify patients with SLE who are likely to have premature atherosclerosis and who would benefit from evaluation of CAC by EBCT.

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## INTRODUCTION

Patients with systemic lupus erythematosus (SLE) have a 4–10-fold greater risk of atherosclerotic cardiovascular disease (ASCVD) compared with the general population (1). Although traditional Framingham risk factors may be inadequate for assessing ASCVD risk in patients with SLE (2), the high incidence of cardiovascular morbidity and mortality in these patients underscores the need for objective and accurate means by which to identify those with subclinical disease and to apply targeted interventions. Electron beam computed tomography (EBCT) is used to detect coronary artery calcification (CAC) within atheromatous plaques. CAC scoring by EBCT correlates with the total histopathologic and arteriographic burden and predicts future cardiovascular events (3). CAC scoring by EBCT has been used in many large population-based studies to evaluate the prognostic utility of this approach in the primary prevention setting (4,5). The pathogenesis of ASCVD in SLE is likely multifactorial, involving traditional risk factors, early menopause, treatment side effects, and immunologic/ inflammatory components. Although elevated homocysteine levels have been associated with stroke and thrombosis in patients with SLE, reports linking hyperhomocysteinemia with ASCVD are lacking (6). In the general population, however, hyperhomocysteinemia is independently associated with an increased risk of ASCVD (7), and the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism is associated with hyperhomocysteinemia in healthy individuals with lowfolate status (8). The aim of this study was to determine the incidence and extent of CAC, an early manifestation of ASCVD, in patients with SLE compared with controls, and to identify variables that are associated with CAC in this population. We postulated that patients with SLE are more likely than controls to have CAC, and that EBCT could identify subclinical ASCVD in women with SLE.

## PATIENTS AND METHODS

**SLE and control samples.** Consecutive nonpregnant women older than age 18 years who fulfilled at least 4 of the American College of Rheumatology revised criteria for the classification of SLE (9) were invited to participate. Age- and race-matched nonpregnant female controls were recruited from the University of Pennsylvania clinics. The study was approved by the University of Pennsylvania Institutional Review Board, and informed consent was obtained from each participant.

**Data collection.** A medical history was obtained for all study participants. All participants underwent a physical examination, electrocardiography, and EBCT, and had a fasting blood sample drawn. SLE disease activity and organ damage were assessed using the Systemic Lupus Erythematosus Disease Activity Index (10) and the Systemic Lupus International Collaborative Clinics Damage Index (11).

**Clinical assessments. Laboratory analysis.** Fasting blood samples were tested to determine the lipid profile, the antiphospholipid antibody profile (dilute Russell's viper venom test, anticardiolipin antibodies by enzyme-linked immunosorbent assay [ELISA], antibodies to  $\beta_2$ -glycoprotein I by ELISA), the levels of C3, C4, and anti-double-stranded DNA antibody (by ELISA), the Westergren erythrocyte sedimentation rate, the level of high-sensitivity C-reactive protein (hsCRP), the complete blood count, the level of creatinine, and the total plasma homocysteine concentration.

**Measurement of soluble CD154 (sCD154).** Plasma was tested in duplicate for sCD154 levels, by ELISA (Chemicon, Temecula, CA).

**Measurement of homocysteine.** Whole blood (5 ml) was drawn into EDTA, placed on ice, and centrifuged at 2,500 rpm for 5 minutes at room temperature. Plasma homocysteine concentrations were determined by fluorescence polarization immunoassay (AxSYM Homocysteine; Abbott Laboratories, Abbott Park, IL).

**Genetics analysis.** DNA was isolated using Generation Capture Column Kits (Genra Systems, Minneapolis, MN).

*MTHFR* C677T genotypes were analyzed using a heteroduplex generator method, as previously described (12).

**Glomerular filtration rate (GFR).** To assess possible renal insufficiency, the GFR for each participant was calculated using the Modification of Diet in Renal Disease equation (13).

**Risk factors.** The following cardiovascular risk factors were assessed: diabetes, hypertension (systolic blood pressure [BP]  $\geq$  140 mm Hg and/or diastolic BP  $\geq$  90 mm Hg on 2 occasions), postmenopausal status (i.e.,  $\geq$  1 year since last menstrual period), history of ASCVD (i.e., previous myocardial infarction [MI], coronary artery bypass surgery, or angiographically proven stenosis), a significant family history of heart disease (i.e., MI, sudden cardiac death, or a revascularization procedure in a first-degree male relative younger than age 55 years and/or a first-degree female relative younger than age 65 years), and current-smoker status. Each category was assigned a value of 1 point, and the summed value (range 0–6) was determined for each participant (14). Hypercholesterolemia was analyzed as a separate risk factor and was not included in the summed value.

**EBCT.** Patients with SLE and controls had electrodes placed for gated data acquisition and were positioned supine in a GE Imatron C150 electron beam CT scanner (General Electric Medical Systems, Milwaukee, WI) for 2 image acquisitions. Electrocardiographic triggering of scans ensured that images were obtained during diastole. Serial, contiguous, 3-mm-thick transverse images were obtained (during breathholding), commencing at the root of the aorta cephalad to the coronary sinuses and proceeding caudally through the entire coronary tree (Aquarius software; Teracon). Data were quantitatively scored in a blinded manner by a registered radiology technologist. The total CAC burden was based on the area of calcification, the average density, and the number of plaques. Scores were calculated using the method described by Agatston et al (15). Total CAC was quantified by summing across the 4 major epicardial arteries, resulting in a raw CAC [rCAC] score. These scores were compared with rCAC scores for historical (age- and sex- matched) individuals that had been preprogrammed as control data to provide the following EBCT percentile categories (EBCT-PCs):  $\leq$  25th, 25th–50th, 50th–75th, 75th–90th, or  $\geq$  90th (16). EBCT-PCs may be preferable to rCAC scores, because they are easier to interpret.

**Statistical analyses. Descriptive analyses.** For continuous variables (e.g., age), the means and standard deviations were computed, stratified by group (SLE versus control), race, EBCT-PC (e.g.,  $\geq$  75th percentile or not), and rCAC score (e.g.,  $\geq$  0 or not). For discrete variables, (e.g., smoking), the stratified frequencies and percentages were computed. **Inferential analysis.** Spearman's correlation coefficients for the rCAC score and all continuous variables of interest were computed, overall and stratified by group. Additionally, correlation coefficients were computed for EBCT-PCs above or below the candidate values of 75% and 90%. This exploratory component was used to assess agreement of analyses involving rCAC scores compared with those involving the more traditional EBCT-PCs. Associations between EBCT-PC classification and categorical explanatory variables were assessed by chi-square analyses. Variables showing significant association with rCAC score ( $\geq$  0) status were included as candidate explanatory variables in a stepwise

**Table 1.** Characteristics of patients with systemic lupus erythematosus and age- and race-matched controls\*  
Characteristic Patients

\*\*\*Insert Figure 1

multiple logistic regression with CAC status as the outcome. Clinically plausible models were explored to identify variables predictive of CAC status. All statistical analyses were produced using SAS software (SAS Institute, Cary, NC), with a Type I error rate of 0.05 for 2-sided tests of significance.

## RESULTS

**Sample demographics.** The study group comprised 152 women with SLE (mean  $\pm$  SD age 43.3  $\pm$  11.3 years, mean  $\pm$  SD disease duration 11.1  $\pm$  8.5 years). Eighty-one (53%) of the patients were African American, 58 (38%) were white, 7 (5%) were Asian, and 5 (3%) were Hispanic; the racial distribution among matched controls was similar (Table 1). **CAC.** Direct associations between the rCAC score and EBCT-PCs were evident for both the SLE and control groups; however, the magnitude of the association was greater for patients with SLE. The percentages of rCAC scores  $\geq$  0 in patients with SLE and controls were 29.6% and 16.2%, respectively ( $P = 0.009$ ). Twenty-four patients (15.8%) had rCAC scores above the 90th EBCT-PC, compared with 10 controls (7.0%) ( $P = 0.02$ ), and 38 patients (25.0%) had scores above the 75th EBCT-PC, compared with 16 controls (11%) ( $P = 0.003$ ). The rCAC scores for patients and controls in the age categories 26–35 years, 36–45 years, 46–55 years, and 56–65 years (comprising 87.8% of the study subjects) are shown in Figure 1. Participants with a rCAC score  $\geq$  0 were older than those with a rCAC score of 0, among both patients (51.0 versus 40.1 years;  $P = 0.0001$ ) and controls (51.6 versus 42.0 years;  $P = 0.002$ ). In each of the 4 age categories, patients had a greater CAC burden than did controls.

### Predictors of CAC among patients with SLE and controls.

Analysis of SLE patient data (Table 2) revealed that age-adjusted rCAC scores  $\geq$  0 were significantly associated with the number of ASCVD risk factors, disease duration, the homocysteine concentration,

and (inversely) the GFR. Among controls, age-adjusted rCAC scores  $\geq 0$  were significantly associated with the levels of total cholesterol and low-density lipoprotein, and with the body mass index (Table 3). **Regression analyses.** Due to the expected correlation among candidate explanatory variables, ordinal logistic regression with rCAC as the outcome variable was performed on various pairings with the explanatory variables ASCVD risk, smoking status, the GFR, history of hypertension, disease duration, age, and homocysteine. For each model, a test of second-order interaction was performed; if the results were not significant, the analysis was repeated without the interaction term. In single-risk-variable logistic regression models, significant determinants of a positive rCAC score (other than age) for both patients with SLE and controls were the number of ASCVD risk factors (for SLE,  $P = 0.003$  [age-adjusted  $P = 0.07$ ]; for controls,  $P = 0.02$  [age-adjusted  $P = 0.46$ ]), postmenopausal status (for SLE,  $P = 0.0002$  [age-adjusted  $P = 0.53$ ]; for controls,  $P = 0.09$  [age-adjusted  $P = 0.39$ ]), and pack-years of smoking (for SLE,  $P = 0.02$  [age-adjusted  $P = 0.62$ ]; for controls,  $P = 0.06$  [age-adjusted  $P = 0.35$ ]). Among patients, the GFR ( $P = 0.0015$  [age-adjusted  $P = 0.043$ ]), the homocysteine concentration ( $P = 0.001$  [age-adjusted  $P = 0.002$ ]), and SLE disease duration ( $P = 0.0001$  [age-adjusted  $P = 0.005$ ]) were all significantly associated with a positive rCAC score. Application of stepwise multivariate logistic regression to the multiple candidate explanatory variables identified age ( $P = 0.0001$ ,  $r = 0.43$ ), homocysteine concentration ( $P = 0.002$ ,  $r = 0.24$ ), and disease duration ( $P = 0.01$ ,  $r = 0.19$ ) as significant predictors of a rCAC score  $\geq 0$ . For this model,  $R^2 = 0.28$ . In multivariate analysis among controls, age ( $P = 0.0002$ ,  $r = 0.38$ ) and cholesterol level ( $P = 0.006$ ,  $r = 0.25$ ) were significant predictors of a rCAC score  $\geq 0$ .

**Soluble CD154.** Paradoxically, sCD154 concentrations in the 125 patients with SLE for whom such data were available were lower than those in their matched controls ( $P = 0.0001$ ). Associations between sCD154 concentrations and rCAC scores for patients and controls were not statistically significant.

\*\*\*Insert Table 2 & 3

**Homocysteine and GFR.** To reduce the effects of skew in their respective distributions, homocysteine concentrations  $\geq 13$  micromoles/liter and GFRs in the lowest quartile (i.e.,  $\leq 71$  ml/minute) were classified as abnormal. A nominal logistic regression of CAC status (positive versus negative) using these 2 categorical variables produced a significant odds ratio of 3.6 ( $P = 0.0002$ ) for homocysteine concentrations  $\geq 3$  moles/liter, and a nonsignificant odds ratio of 1.8 ( $P = 0.18$ ) for the GFR. Results of a test for second-order interaction between these 2 factors were not significant ( $P = 0.79$ ), indicating that the relationship between hyperhomocysteinemia and CAC positivity is not dependent on a reduced GFR.

**Medications.** Patients with SLE who were receiving beta-blockers were more likely to have positive rCAC scores than were those who were not receiving these drugs ( $P = 0.0079$ ). In contrast, prednisone, hydroxychloroquine, azathioprine, mycophenolate mofetil, methotrexate, cyclophosphamide, statins, cyclooxygenase 2 inhibitors, angiotensin-converting enzyme inhibitors, aspirin, hormone replacement therapy, and oral contraceptives were not associated with CAC or with a rCAC score  $\geq 0$  in patients with SLE (Table 2).

**The MTHFR C677T polymorphism, homocysteine, and CAC.** Mean rCAC scores did not differ significantly between *MTHFR* C677T genotypes (TT/CT versus CC) in either patients with SLE ( $P = 0.49$ ) or controls ( $P = 0.33$ ). Furthermore, no significant association between the *MTHFR* C677T genotype and the homocysteine concentration was observed. When analysis was restricted to the younger half of participants in each group, or when African Americans and whites were considered separately, the lack of association between genotype and phenotype (i.e., CAC or homocysteine) persisted.

## DISCUSSION

We have shown that female patients with SLE have a statistically significantly higher incidence of CAC, as quantified by EBCT, compared with age- and racematched female controls. Although many traditional risk factors were significantly associated with CAC, in a multivariate analysis only age, hyperhomocysteinemia, and disease duration were associated with an elevated rCAC score in patients. Hyperhomocysteinemia has been observed in other SLE cohorts and has been identified as a risk factor for atherothrombotic events in SLE (17,18). This study is the first to identify hyperhomocysteinemia as a statistically significant risk marker for CAC, as assessed by EBCT, although a nonsignificant trend in another SLE cohort was previously reported (19). Additionally, a nonsignificant trend was observed using

carotid ultrasonography (20). These results are consistent with a growing body of literature on the relationship between hyperhomocysteinemia and atherothrombotic disease in the general population. An interaction between hyperhomocysteinemia and inflammation may predispose members of vulnerable populations to accelerated ASCVD. Because folic acid can lower homocysteine concentrations, SLE patients with hyperhomocysteinemia may be able to lower their risk of ASCVD and consequent excess morbidity and mortality by using vitamin supplements that contain folic acid. In healthy persons and in patients with ASCVD but without overt inflammatory disease, hyperhomocysteinemia is underpinned, at least partly, by the *MTHFR* C677T genotype (21). Surprisingly, we observed no association between the *MTHFR* genotype and the homocysteine concentration in either patients with SLE or controls. Published reports that have linked the *MTHFR* 677TT genotype and hyperhomocysteinemia have often been biased toward older male subjects. The lack of association between the *MTHFR* genotype and hyperhomocysteinemia in the controls reported here may reflect their relatively young age, female sex, African American race, or other demographic feature. The lack of association between the *MTHFR* genotype and hyperhomocysteinemia or CAC in patients with SLE suggests that other mechanisms, not directly involving differential *MTHFR* activity, generate an SLE-related phenotype characterized by high homocysteine levels and premature ASCVD. Nonetheless, in our study, hyperhomocysteinemia was clearly associated with higher rCAC scores in patients with SLE and may be one of the few laboratory markers that correlate with ASCVD in such patients. In contrast to homocysteine, neither hsCRP nor sCD154, each of which has received attention as a predictive marker for patients who have an enhanced risk of cardiovascular events (22,23), was associated with CAC in our SLE patients. This is not entirely surprising, because most patients with SLE have some systemic inflammation; i.e., a serologic marker of inflammation that varies over time may not identify unique subsets of patients with SLE who are at risk for ASCVD. More than half of our SLE population was African American. Before undertaking this study, it was unclear how race might affect the analysis of ASCVD detected by EBCT in a racially diverse population, because the software of the EBCT scanners compares each rCAC score with an historical age- and sex-matched, but not-race matched, control group that is predominantly white (16). The Coronary Artery Risk Development in Young Adults study (4) previously demonstrated that race does not significantly affect the prevalence of CAC in either men or women, even after adjustment for traditional risk factors. However, in another study (24), CAC was almost twice as prevalent in whites as in African Americans. In the current study, the incidence and extent of CAC were similar for African American and white participants in both the SLE and control groups, validating the use of EBCT as an effective screening tool in patients of both races. In summary, we have shown that EBCT can be used to detect CAC, an objective indicator of the early stages of ASCVD, in a racially diverse population of patients with SLE. The only SLE-related variable significantly associated with CAC was disease duration. Although the incidence and extent of CAC are known to increase with age, this alone did not explain the CAC variances in our model. However, a simple laboratory measure, an elevated homocysteine level, was also associated with CAC in both patients with SLE and controls. Therefore, our findings suggest that hyperhomocysteinemia is a potentially useful marker for CAC and subclinical ASCVD. If our results are confirmed by other investigators, hyperhomocysteinemic patients with SLE might benefit from EBCT screening. Clinical management of those with CAC could then incorporate strategies, perhaps including aggressive folic acid supplementation, designed to limit cardiovascular comorbidity.

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