Alpha-Chlorofatty Acid and Coronary Artery or Aorta Calcium Scores in Women with Systemic Lupus Erythematosus. A Pilot Study

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ABSTRACT. ***Objective.*** Alpha-chlorofatty acid (α-ClFA) is one product of myeloperoxidase activity *in vivo* during atherogenesis and may be a biomarker for cardiovascular disease (CVD). We investigated if serum α-ClFA is associated with subclinical CVD as measured by coronary artery and aorta calcium scores (CAC and AC, respectively) in women with and without systemic lupus erythematosus (SLE).

Methods. This pilot project analyzed baseline data from 173 women with SLE and 186 women without SLE participating in a 5-year longitudinal investigation of the Study of Lupus Vascular and Bone Long-term Endpoints (SOLVABLE). Data collection included demographic information, CVD and SLE risk factors, and laboratory assessments. Alpha-ClFA was measured in stored serum by liquid chromatography-mass spectrometry. CAC and AC were measured by computed tomography. Outcome measures were CAC and AC present (CAC > 0 or AC > 0) versus absent (CAC = 0 or AC = 0). Associations between risk factors and CAC or AC were tested with descriptive statistics and multivariate analyses.

Results. Women with SLE had higher α-ClFA levels than women without SLE (42.0 fmol/25 μl ± 37.3 vs 34.5 fmol/25 μl ± 21.9; p = 0.020). In analyses including individual CVD risk factors, having SLE was independently associated with the presence of CAC (OR 3.42, 95% CI 1.72 to 6.78) but not AC. Alpha-ClFA was not associated with the presence of CAC or AC in patients with SLE.

Conclusion. SLE, but not serum α-ClFA, was associated with the presence of CAC in this pilot project. (J Rheumatol First Release Aug 1 2014; doi:10.3899/jrheum131361)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
CORONARY ARTERY CALCIUM
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Premature cardiovascular disease (CVD) is an important cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE). A bimodal distribution of mortality exists, with an early peak from SLE disease activity at the time of diagnosis followed by late complications including CVD[1]. Women with SLE aged 35–44 years have a 50-fold increased risk of myocardial infarction versus age-matched controls[2]. Traditional Framingham risk factors fail to fully account for these increased rates of CVD[3], and investigations now focus on defining risk factors that influence accelerated CVD development in patients with SLE.

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Myeloperoxidase (MPO) is one important mediator of atherosclerosis known to be abundant in atherosclerotic plaques[4,5]. Even though serum MPO levels were shown to be significantly elevated in SLE subjects, they were not found to be predictive of subclinical CVD[6]. In contrast, others have shown that subjects with SLE and coronary artery disease (CAD) had lower serum levels of MPO but higher serum protein oxidation products, some of which are produced through MPO-catalyzed reactions, than non-SLE subjects with CAD[7]. Together, these studies suggest that serum MPO may be metabolized more quickly in SLE patients with CAD, or that vascular wall MPO activity generates serum oxidation products[8]. Further, it is anticipated that catalytic products of enzymes, if stable in the serum, should be present in greater quantities than the serum level of the enzyme that produces it. For example, plasma chlorotyrosine and nitrotyrosine, both products of MPO-catalyzed oxidation, are elevated in patients with CVD. Thus, serum oxidation products of vascular MPO activity may be novel target risk factors for subclinical CVD[9,10,11,12].

A novel unexplored serum oxidation product of vascular MPO activity is alpha-chlorofatty acid (α-ClFA). Leukocytes containing MPO produce the reactive chlorinating species hypochlorous acid (HOCl)[13]. HOCl reacts with the vinyl ether bond of plasmalogens, a phospholipid abundant in endothelial and smooth muscle cells of the human cardiovascular system[14,15]. The direct oxidation product of this reaction, α-chlorofatty aldehyde, is further metabolized to α-ClFA[9,16]. Alpha-ClFA levels have been directly correlated with *in vivo* MPO activity and can be detected in human serum[16,17]. Further, plasma α-ClFA has been shown to be elevated in rat and mouse models of respiratory viral infection[17,18].

Elevated serum α-ClFA levels, reflecting increased vascular wall MPO activity, may be associated with the presence of subclinical CVD. This pilot study investigated associations between α-ClFA and subclinical CVD measured by coronary artery calcium (CAC) or aorta calcium (AC) in women with and without SLE.

MATERIALS AND METHODS

This investigation was a pilot study from the Study of Lupus Vascular and Bone Long-term Endpoints (SOLVABLE), a longitudinal epidemiological study assessing risk of subclinical and clinical CVD in SLE. Protocols for both SOLVABLE and this study were approved by the Institutional Review Boards at Northwestern University and the University of Illinois at Chicago. Study participants provided informed consent prior to enrollment according to the Declaration of Helsinki.

Study population. Participants with SLE were recruited from the Chicago Lupus Database (CLD), a cohort of 459 participants who met at least 4 of the 1982 or updated 1997 American College of Rheumatology (ACR) criteria for SLE. All eligible participants ≥ 18 years of age were invited to participate, and the first 185 women to respond were enrolled in SOLVABLE. Twelve SLE women had prior CVD events (myocardial infarction, percutaneous transluminal coronary angioplasty, angina, coronary artery bypass graft, cerebrovascular accident, or transient ischemic attack) confirmed on chart review, and were excluded from this pilot study. The women in SOLVABLE were similar in race/ethnicity, presence of renal disease, frequency of double-stranded DNA (dsDNA) antibody positivity, and mean levels of the lupus markers complement 3 (C3) and complement 4 (C4) compared to the remaining women in the CLD. SOLVABLE participants were older women who had a longer mean disease duration, smoked more, and used less corticosteroid therapy but more hydroxychloroquine (Appendix 1). The SOLVABLE women also used more cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil, cyclosporine, or tacrolimus, hereafter termed immunosuppressant use. The current study compares these 173 women with SLE to 186 women without SLE and includes data from participants' baseline study visit.

Data collection. Study visits included completion of a self-administered questionnaire, interview and examination by a trained physician, and collection of fasting blood and urine specimens. Serum samples were stored at –80°C. Electron beam computed tomography (EBCT) or multidetector computed tomography (MDCT) of the coronary arteries and aorta were completed for baseline measures of subclinical CVD. CAC and AC scores were interpreted at the University of Pittsburgh Cardiovascular Institute.

Traditional CVD risk factors. Information on self-reported race/ethnicity, demographics, smoking history, medication use, and menopause status were obtained from the self-administered questionnaire. In instances where menopause status was in question (e.g., hysterectomy without oophorectomy), confirmation with follicle-stimulating hormone level was performed. Waist measurements were obtained. Hypertension was defined as systolic blood pressure (BP) ≥ 140 mm Hg, diastolic BP ≥ 90 mm Hg, or use of antihypertensive medication, excluding medications used for another indication (e.g., proteinuria). The average of 2 BP measurements was used for analysis. Diabetes mellitus (DM) was defined as fasting glucose level ≥ 126 mg/dl or use of diabetes medication. Dyslipidemia was defined as total cholesterol ≥ 200 mg/dl, low-density lipoprotein (LDL) ≥ 100 mg/dl, high-density lipoprotein (HDL) ≤ 40 mg/dl, triglyceride ≥ 150, or use of lipid-lowering medication.

SLE-related factors. Information on clinical SLE manifestations and ACR criteria met was obtained from each participant and confirmed by chart review. SLE disease activity and damage were measured by trained assessors using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) and the ACR/Systemic Lupus International Collaborating Clinics Damage Index (ACR/SLICC-DI), respectively[20,21]. A modified ACR/SLICC-DI score excluding reported CVD risk factors was used. Disease duration was determined using the date the subject fulfilled the fourth ACR classification criterion for SLE[22,23]. Participants reported current hydroxychloroquine, corticosteroid, and immunosuppressant use.

Laboratory tests. Laboratory tests including fasting lipids (total cholesterol, LDL, HDL, and triglycerides) and fasting glucose were measured in the Lipid Laboratory at the University of Pittsburgh Graduate School of Public Health and Prevention. LDL level was estimated by the Friedewald equation. In instances where triglycerides were ≥ 400, LDL was measured directly. Plasma glucose levels were measured by enzymatic assay. Homocysteine was measured at the University of Pittsburgh Medical Center nutrition laboratory spectrophotometrically on the Olympus AU400 using reagents from Carolina Liquid Chemistries (Brea, CA, USA). The inflammatory markers C-reactive protein (CRP) and fibrinogen were measured at the Laboratory for Clinical Biochemistry Research at the University of Vermont. CRP was measured by immunonephelometric assay. Fibrinogen was measured by modified clot-rate assay. Albumin was measured by dye binding assay at the Lipid Laboratory at the University of Pittsburgh.

Anticardiolipin antibodies (IgG and IgM; Diasorin, Stillwater, MN, USA) and lupus anticoagulant (partial thromboplastin time or Russell's viper venom time) were measured at the Coagulation Laboratory at the University of Pittsburgh Medical Center. Anticardiolipin was considered

positive if IgG was > 10 IgG phospholipid units or IgM was > 15 IgM phospholipid units, as per laboratory standards. C3 and C4 levels were measured by nephelometry. Double-stranded DNA antibodies were measured using the *Crithidia luciliae* method and titers ≥ 1:10 were considered positive.

For α-ClFA measurement, 25 µl of serum was base-hydrolyzed in the presence of 105 fmol 2-chloro-[d_4]-hexadecanoic acid (internal standard) and total fatty acid was extracted[24]. Fatty acids were then subjected to reversed-phase high-pressure liquid chromatography using an Onyx monolithic C-18 column (50 × 2.0 mm) as solid phase and a gradient from 60% to 100% methanol (in water) containing 5 mM ammonium acetate and 0.25% acetic acid at a flow rate of 200 μl/min. 2-Chlorohexadecanoic acid (the α-ClFA measured in this study) and 2-chloro-[d_4]-hexadecanoic acid were detected using selected reaction monitoring (289–253 and 293–257, respectively), using a Thermo-Fischer Quantum Ultra triple quadruple mass spectrometer and electrospray ionization. The relative standard deviation of the entire sample set was 23%. Each group of biological samples had analyses of authentic standards to ensure reproducibility on an interassay variability. Each sample was analyzed in triplicate over 3 months and the mean value included in analyses.

Subclinical CVD outcome measures. CAC and AC for all women with SLE and for the first 140 women without SLE were measured by EBCT using the Imatron C150 Ultrafast CT scanner (General Electric Medical Systems, South San Francisco, CA, USA). For the last 46 women without SLE, CAC and AC were measured by MDCT using the Siemens Definition Dual Source CT (Siemens Medical Solutions, Malvern, PA, USA). CAC and AC were not measured in 4 and 41 women with SLE, respectively, and AC was not measured in 2 women without SLE. Aorta calcium was measured at all visualized sections of the ascending and descending thoracic aorta. Lesion calcium scores were calculated with a densitometric program available on the Imatron C150 and Siemens Definition Dual Source scanners using the Agatston method. Both CT methods were shown to be comparable in the Multi-Ethnic Study of Atherosclerosis, MESA[25]. Individual lesion calcium scores were summed to calculate total calcium score for each vascular bed. Outcome measures were the presence (CAC > 0 and AC > 0) or absence (CAC = 0 and AC = 0) of CAC or AC[26,27].

Statistical analysis. Means, standard deviations, percentiles, and ranges were used to describe patient characteristics, laboratory markers, and subclinical CVD outcome measures. In bivariate analyses, comparisons between women with and those without SLE and with and without high CAC and AC scores were made by 2-sample t-tests or Mann-Whitney tests (in non-normal distributions) for continuous variables, and by chi-square statistics for categorical variables. Multivariate logistic regression analyses were used to assess independent relationships between α-ClFA and CAC or AC (dichotomized as present vs absent). In addition to variables prespecified to have an association with CVD, all variables that had a significant bivariate relation (defined by a p value < 0.05) with the outcome were evaluated for inclusion in the model.

Multivariate analyses of all participants included α-ClFA, presence of SLE, dyslipidemia, hypertension, DM, waist circumference, age, menopause status, current tobacco use, homocysteine, fibrinogen, and albumin. Similar analyses in the SLE women incorporated these individual CVD risk factors and the SLE-specific factors SLEDAI-2K score, ACR/SLICC-DI score, C3, and C4. Finally, bivariate subgroup analyses of the women with SLE compared characteristics of SLE women with and without high CAC and AC scores.

RESULTS

Comparing the 173 women with SLE to the 186 women without SLE, the population without SLE was older by approximately 3 years, but had similar rates of menopause (Table 1). There was no difference in race/ethnic distribution between women with and those without SLE.

CVD risk factors. Rate of clinically defined hypertension was higher in the women with SLE (Table 1). There was no difference in presence of dyslipidemia, DM, tobacco use, statin use, or triglyceride level in women with and those without SLE. The women with SLE had lower total cholesterol, LDL, HDL, and fasting glucose, and were younger at menopause. Among inflammatory markers, women with SLE had lower serum albumin compared to women without SLE, while CRP and fibrinogen levels were similar.

In subgroup comparisons of women with SLE, women with CAC and AC had higher rates of menopause and increased waist circumference. Smoking rates were similar in women with and without CAC and AC (Table 2). Average homocysteine level was higher in women with CAC, but similar in women with and without AC. Statin use was higher in women with AC, but similar in CAC comparisons.

Alpha-ClFA and subclinical CVD outcomes. SLE women had a greater presence of CAC than women without SLE (34.9% vs 23.1%, respectively; p = 0.014), while presence of AC was similar. In bivariate analyses, median CAC score was significantly higher in women with than without SLE, and while median AC score was higher among women without SLE, this difference was not statistically significant (Appendix 2). Mean serum α-ClFA level was higher in the women with than without SLE (Table 1). Among participants with AC present, SLE women had higher α-ClFA levels versus women without SLE, but α-ClFA levels were similar in women with and without SLE who did not have AC. In contrast, α-ClFA levels were similar in women with and without SLE who had CAC, but higher in women with than without SLE who did not have CAC (Table 3).

SLE-specific factors. Only 19 women with SLE were not taking hydroxychloroquine, corticosteroids, or an immunosuppressant. Mean C3 and C4 levels and SLEDAI-2K scores were consistent with low disease activity. Mean ACR/SLICC-DI scores suggested overall low disease damage (Table 1). In the subgroup comparison of SLE women, those with CAC and AC had higher ACR/SLICC-DI scores, C3, C4 (for AC only), and fibrinogen levels (Table 2). In a comparison of α-ClFA levels in women with SLE who did and did not have a specific ACR criterion for SLE, there were no individual SLE manifestations associated with higher average α-ClFA levels (data not shown).

Multivariate analyses. In analyses including all participants, SLE was independently associated with CAC (OR 3.42, 95% CI 1.72 to 6.78) but not AC (Table 4). Age and increased waist circumference were associated with the presence of both CAC and AC, while dyslipidemia was also associated with CAC and menopause was associated with AC.

Similar multivariate analyses including SLE-specific variables were performed on the women with SLE (Table 5). Dyslipidemia, menopause, waist circumference, and older age were associated with CAC, while age and waist circum-

Table 1. Descriptive characteristics of women with and without systemic lupus erythematosus (SLE) at enrollment. Continuous variables are the mean ± standard deviation (SD) and categorical variables are the percentage.

Characteristic	Women with SLE, n = 173	Women without SLE, n = 186	p
Demographics			
Age, yrs, mean ± SD	42.6 ± 10.4	45.9 ± 10.4	0.006
Postmenopausal, %	33.5	33.3	0.969
Age at menopause, yrs, mean ± SD	42.5 ± 8.1	47.7 ± 7.1	< 0.001
Race/ethnicity, %			0.994
White	60.7	60.8	
Black	28.3	28.0	
Other	11.0	11.3	
Measured and clinical risk factors			
α-ClFA, fmol/25 μl, mean ± SD	42.0 ± 37.3	34.5 ± 21.9	0.020
Hypertension, %[a]	34.7	22.6	0.011
Systolic blood pressure, mm Hg, mean ± SD	117.4 ± 14.9	119.0 ± 14.9	0.304
Diastolic blood pressure, mm Hg, mean ± SD	73.5 ± 9.4	73.9 ± 9.4	0.678
Dyslipidemia, %[b]	69.9	75.3	0.258
Total cholesterol, mg/dl, mean ± SD	186.7 ± 38.7	199.7 ± 37.9	0.001
LDL cholesterol, mg/dl, mean ± SD	106.9 ± 32.0	117.3 ± 35.5	0.004
HDL cholesterol, mg/dl, mean ± SD	55.7 ± 15.1	60.2 ± 16.4	0.028
Triglyceride, mg/dl, mean ± SD	121.5 ± 68.7	113.9 ± 80.2	0.084
Diabetes mellitus, %[c]	2.9	2.7	0.907
Fasting glucose, mg/dl, mean ± SD	90.4 ± 16.6	96.5 ± 16.3	< 0.001
Waist circumference, cm, mean ± SD	86.5 ± 15.9	89.4 ± 16.8	0.065
Tobacco use (current) %	11.6	10.8	0.808
Tobacco use (history of) %	40.5	41.4	0.857
C-reactive protein, μg/ml, mean ± SD	4.3 ± 10.6	3.0 ± 4.5	0.408
Homocysteine, μmoles/l, mean ± SD	11.6 ± 5.7	8.4 ± 3.3	< 0.001
Fibrinogen, mg/dl, mean ± SD	329.3 ± 104.5	328.2 ± 89.7	0.631
Serum albumin, g/dl, mean ± SD	4.0 ± 0.4	4.3 ± 0.3	< 0.001
Anticardiolipin IgM-positive, %	18.0	19.4	0.758
Anticardiolipin IgG-positive, %	10.5	5.2	0.074
Lupus anticoagulant-positive, %	4.7	0.5	0.016
SLE-related factors			
C4, mg/dl, mean ± SD	19.4 ± 9.0	—	—
C3, mg/dl, mean ± SD	96.9 ± 27.3	—	—
Positive anti-dsDNA, %[d]	46.8	—	—
Disease duration, yrs, mean ± SD	11.9 ± 8.7	—	—
SLEDAI-2K score, mean ± SD	4.0 ± 3.6	—	—
ACR/SLICC-DI score, mean ± SD	1.6 ± 1.8	—	—
Current medication use			
Antihypertensive, %[e]	30.6	14.5	<0.001
Statin, %	6.9	4.8	0.397
Aspirin, %	15.0	5.9	0.005
Hydroxychloroquine, %	72.3	—	—
Corticosteroid, %	41.6	—	—
Immunosuppressant, %[f]	34.1	1.1	<0.001
Cyclophosphamide	1.7	—	—
Azathioprine	5.2	0.5	0.008
Methotrexate	12.1	—	—
Mycophenolate mofetil	15.6	—	—
Cyclosporine	2.9	0.5	0.110
Tacrolimus	1.7	—	—
Leflunomide	0.6	—	—
Subclinical CVD measures at enrollment			
CAC present, %[g]	34.9[h]	23.1	0.014
AC present, %	52.6[i]	50.5[j]	0.714

[a]Defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive medication use for hypertension. [b]Defined as total cholesterol > 200, LDL > 100, HDL < 40, triglyceride > 150, or use of lipid-lowering medication. [c]Defined as fasting glucose > 126 or diabetes use of medication. [d]Defined as antibody titer ≥ 1:10 by *C. luciliae* method. [e]Excludes medication use for indication other than hypertension. [f]Includes cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil, cyclosporine, or tacrolimus. [g]Present defined as CAC > 0 and AC > 0. [h]For women with SLE, n = 169 for CAC score. [i]For women with SLE, n = 133 for AC score. [j]For women without SLE, n = 184 for AC score. α-ClFA: alpha-chlorofatty acid; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CVD: cardiovascular disease; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; ACR/SLICC-DI: American College of Rheumatology/Systemic Lupus International Collaborating Clinics Damage Index; AC: aorta calcium; CAC: coronary artery calcium.

Table 2. Characteristics of women with systemic lupus erythematosus (SLE) by present versus absent CAC and AC scores[a]. Continuous variables are the mean ± standard deviation (SD) and categorical variables are the percentage.

Characteristic	CAC Present, n = 59	CAC Absent, n = 110	p	AC Present, n = 70	AC Absent, n = 63	p
Demographics						
Age, yrs, mean ± SD	48.9 ± 9.8	39.5 ± 9.2	< 0.001	46.3 ± 9.9	36.7 ± 8.0	< 0.001
Postmenopausal, %	61.0	20.0	< 0.001	51.4	11.1	< 0.001
Age at menopause, yrs, mean ± SD	43.9 ± 7.3	40.1 ± 8.8	0.178	42.7 ± 8.4	37.6 ± 6.9	0.112
Race/ethnicity, %			0.488			0.641
White	64.4	60.9		57.1	65.1	
Black	28.8	26.4		28.6	23.8	
Other	6.8	12.7		14.3	11.1	
Measured and clinical risk factors						
α-ClFA, fmol/25 μl, mean ± SD	43.4 ± 38.0	41.4 ± 37.7	0.625	40.9 ± 26.2	38.0 ± 18.0	0.935
Hypertension, %[b]	49.2	26.4	0.003	48.6	20.6	0.001
Dyslipidemia, %[c]	93.2	57.3	< 0.001	80.0	55.6	0.002
Total cholesterol, mean ± SD (mg/dl)	200.8 ± 37.9	179.4 ± 37.8	< 0.001	194.4 ± 39.0	170.1 ± 32.4	< 0.001
LDL cholesterol, mg/dl, mean ± SD	121.5 ± 31.6	99.1 ± 29.8	< 0.001	113.6 ± 33.2	95.3 ± 26.4	< 0.001
HDL cholesterol, mg/dl, mean ± SD	52.0 ± 14.8	57.6 ± 14.9	0.022	54.9 ± 16.5	55.3 ± 14.9	0.882
Triglyceride, mg/dl, mean ± SD	136.6 ± 85.0	113.9 ± 57.7	0.110	129.8 ± 77.4	98.4 ± 48.9	0.003
Diabetes mellitus, %[d]	6.8	0.9	0.051	4.3	0.0	0.246
Waist circumference, cm, mean ± SD	97.1 ± 17.9	80.5 ± 11.1	< 0.001	91.8 ± 16.9	78.7 ± 10.0	< 0.001
Tobacco use (current), %	8.5	12.7	0.404	15.7	7.9	0.169
Tobacco use, (history of) %	42.4	39.1	0.678	47.1	31.7	0.070
C-reactive protein, μg/ml, mean ± SD	6.6 ± 13.7	3.2 ± 8.9	< 0.001	5.8 ± 12.5	3.7 ± 11.5	< 0.001
Homocysteine, μmoles/l, mean ± SD	12.0 ± 3.8	11.4 ± 6.6	0.019	12.1 ± 6.3	11.1 ± 6.4	0.114
Fibrinogen, mg/dl, mean ± SD	359.5 ±100.6	312.1 ± 100.9	0.004	353.3 ± 104.5	301.4 ± 109.7	0.002
Serum albumin, g/dl, mean ± SD	4.0 ± 0.3	4.0 ± 0.5	0.855	4.0 ± 0.4	4.0 ± 0.5	0.705
Anticardiolipin IgM-positive, %	17.2	19.1	0.769	15.7	25.4	0.166
Anticardiolipin IgG-positive, %	8.6	11.9	0.512	11.4	11.1	0.954
SLE-related factors						
C4, mg/dl, mean ± SD	22.0 ± 8.8	18.1 ± 8.8	0.163	20.8 ± 8.5	17.0 ± 9.7	0.004
C3, mg/dl, mean ± SD	110.0 ± 27.5	89.8 ± 24.7	< 0.001	105.2 ± 28.6	84.7 ± 23.9	< 0.001
Positive anti-dsDNA, %[e]	40.7	51.8	0.167	50.0	55.6	0.522
SLEDAI-2K score, mean ± SD	3.7 ± 3.4	4.2 ± 3.8	0.338	4.1 ± 4.0	4.5 ± 3.6	0.298
ACR/SLICC-DI score, mean ± SD	2.0 ± 1.6	1.4 ± 1.9	0.002	2.2 ± 1.9	1.2 ± 1.9	< 0.001
Current medication use						
Statin, %	10.2	4.5	0.195	11.4	1.6	0.035
Hydroxychloroquine, %	71.2	72.7	0.831	74.3	77.8	0.638
Corticosteroid, %	42.4	40.9	0.854	42.9	41.3	0.853

[a]Present defined as CAC > 0 and AC > 0; absent defined as CAC = 0 and AC = 0. [b]Defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or antihypertensive medication use for hypertension. [c]Defined as total cholesterol > 200, LDL > 100, HDL < 40, triglyceride > 150, or use of lipid-lowering medication. [d]Defined as fasting glucose > 126 or diabetes medication use. [e]Defined as antibody titer ≥ 1:10 by *C. luciliae* method. AC: aorta calcium; CAC: coronary artery calcium; α-ClFA: alpha-chlorofatty acid; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CVD: cardiovascular disease; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000; ACR/SLICC-DI: American College of Rheumatology/Systemic Lupus International Collaborating Clinics Damage Index.

ference were associated with AC. Higher C3 level was the only SLE-specific factor independently associated with AC scores. α-ClFA was not associated with CAC or AC among women with SLE.

DISCUSSION

This pilot study investigated the association between subclinical CVD and α-ClFA, a stable metabolite produced by MPO-derived HOCl targeting the vinyl ether bond of plasmalogens, abundant on the surface of cells in the human cardiovascular system, in women with and without SLE. Serum α-ClFA reflects *in vivo* MPO activity[16,17], and studies have further established a potential physiologic role for α-ClFA in regulation of inflammation through effects on neutrophil migration *in vitro*[18] and cyclooxygenase-2 (COX-2) levels in human coronary artery endothelial cells[28]. As a stable and quantifiable metabolite in serum, α-ClFA is an attractive potential biomarker for increased local vascular wall MPO activity, particularly in groups, such as SLE patients, where serum MPO levels have not

Table 3. Serum α-chlorofatty acid levels (fmol/25 μl) by present versus absent coronary artery calcium (CAC) and aorta calcium (AC) scores; present defined as CAC > 0 and AC > 0, and absent defined as CAC = 0 and AC = 0.

Calcium Score	Women with SLE	Women without SLE	p
CAC present	43.4 ± 38.0	34.9 ± 20.6	0.130
CAC absent	41.4 ± 37.7	34.4 ± 22.3	0.016
AC present	40.9 ± 26.2	34.6 ± 21.6	0.016
AC absent	38.0 ± 18.0	34.5 ± 22.5	0.052

Table 4. Multivariate analysis for presence of coronary artery calcium (CAC) and aorta calcium (AC) in women with and without systemic lupus erythematosus (SLE). Present defined as CAC > 0 and AC > 0.

Variable	Odds Ratio	95% CI
CAC present		
SLE	3.42	1.72, 6.78
Dyslipidemia[a]	2.28	1.02, 5.11
Age	1.08	1.04, 1.14
Waist circumference	1.07	1.05, 1.09
α-ClFA	1.00	0.99, 1.01
AC present		
Menopause	2.50	1.03, 6.07
Waist circumference	1.08	1.05, 1.11
Age	1.07	1.03, 1.12
α-ClFA	1.00	0.99, 1.02

[a]Defined as total cholesterol > 200, LDL > 100, HDL < 40, triglyceride > 150, or use of lipid-lowering medication. α-ClFA: alpha-chlorofatty acid.

Table 5. Multivariate analysis for presence of coronary artery calcium (CAC) and aorta calcium (AC) in women with systemic lupus erythematosus (SLE). Present defined as CAC > 0 and AC > 0.

Variable	Odds Ratio	95% CI
CAC present		
Dyslipidemia[a]	9.50	2.08, 43.30
Menopause	5.73	1.27, 25.85
Waist circumference	1.11	1.06, 1.17
Age	1.10	1.02, 1.19
α-ClFA	1.00	0.99, 1.01
AC present		
Age	1.13	1.05, 1.22
Waist circumference	1.06	1.02, 1.11
C3	1.03	1.00, 1.06
α-ClFA	1.00	0.98, 1.03

[a]Defined as total cholesterol > 200, LDL > 100, HDL < 40, triglyceride > 150, or use of lipid-lowering medication. α-ClFA: alpha-chlorofatty acid; C3: complement 3.

been shown to correlate with the presence of subclinical CVD[6]. Importantly, baseline levels of α-ClFA were higher in women with than in those without SLE, and, among all women with CAC, SLE women had higher α-ClFA levels than women without SLE. However, multivariate analyses incorporating the presence of SLE, traditional CVD risk factors, and SLE-specific factors failed to show an independent association between α-ClFA and CAC or AC.

Limitations of study design may have affected our ability to detect significant associations between α-ClFA and the presence of CAC and AC. A small sample size and a lack of AC measurements for 43 women likely restricted the ability to detect associations between α-ClFA and AC. Post-hoc sample size calculations were not performed in this pilot study because the sample size was fixed from the parent SOLVABLE study. Further, measured levels of inflammatory markers, such as α-ClFA, reflect degree of inflammation on the day of sample collection in this cross-sectional study, while atherogenesis is a chronic inflammatory process. Elevated α-ClFA levels may be associated with early inflammatory changes in the arterial wall, such as increased endothelial COX-2 expression, as noted above, rather than the presence of CAC or AC measured in this study[18]. Alternatively, MPO has been shown to be active in plaque rupture[9], and α-ClFA could be more indicative of inflammation in an unstable plaque. A longitudinal study design may be needed to delineate significant associations between α-ClFA and atherosclerosis.

A wide range of measured CAC and AC scores were dichotomized as high based on evidence that even low CAC and AC scores are associated with CVD events[26,27]. Alpha-ClFA as a biomarker may lack the sensitivity to correlate with CAC and AC scores at the low cutoff established for our analysis. Additionally, a large number of participants had undetectable CAC and AC scores, further limiting our statistical analysis. The integrity of α-ClFA in stored serum is also unknown. The serum analyzed in this study was stored for up to 9 years prior to analysis. While α-ClFA is thought to be stable under these conditions, no confirmatory testing has been completed in samples stored for 9 years.

Despite these limitations, important relationships between α-ClFA, SLE, and CAC and AC scores were noted that may guide future investigations into the role of α-ClFA in CVD. Serum α-ClFA levels were higher in women with than without SLE, a finding that likely reflects the pro-inflammatory disease state of SLE[7,29,30]. Also, SLE women with AC had higher levels of α-ClFA than women without SLE who had AC. While α-ClFA was not independently associated with AC in SLE women in this study, an association may be detected with a larger sample size with higher rates of AC. One unexpected finding was higher α-ClFA levels in women with SLE who did not have CAC compared to women without SLE. Meanwhile, α-ClFA levels were similar between women with and without SLE who had CAC present. An explanation for this finding is not readily apparent from our analyses, but may suggest that α-ClFA is a marker of early atherogenesis before development of the

calcification detected by EBCT and MDCT. Alternatively, α-ClFA could play a role in predicting disease progression, or exert distinct regulatory effects in different vascular beds.

Our finding that SLE is independently associated with CAC is consistent with studies that have characterized increased subclinical CVD among SLE cohorts[31,32]. The SLE women in our study had increased rates of CAC versus women without SLE despite being younger.

Certain SLE-specific variables correlated with CAC and AC in women with SLE. ACR/SLICC-DI score was higher in women with SLE who had CAC and AC in univariate analyses. Interestingly, complement levels were higher in SLE women with CAC and AC (C4 only) in univariate analyses, and higher C3 was independently associated with the presence of AC. These findings may seem unexpected, since high C3 and C4 levels reflect low SLE disease activity. However, the complement cascade is thought to be active in atherogenesis[33], and studies have shown an association between higher C4 levels and atherosclerosis in the general population[34]. Among other SLE cohorts, higher C3 levels have been associated with the presence of CAC[35], carotid plaque[36], and increased aortic stiffness[37]. Traditional CVD risk factors were also associated with subclinical CVD. Correlations between dyslipidemia, menopause, increased waist circumference, and older age and the presence of CAC or AC are expected based on established general CVD risk factors.

As a stable metabolite of MPO-derived HOCl reacting with lipid targets unique to the cardiovascular system, α-ClFA may be a novel target biomarker for earlier detection of subclinical CVD. While women with SLE had higher serum levels of α-ClFA than women without SLE, no independent association between α-ClFA and CAC or AC was found in this pilot study of women with SLE. Small sample size and cross-sectional study design were important limiting factors. Further investigation of α-ClFA as a biomarker for CVD should be considered in a larger sample with early disease followed prospectively in women with SLE. Incorporating an alternative stratification of high versus low CAC and AC scores or increasing the size of the study population with abnormal CAC and AC scores may further improve detection of associations between α-ClFA and subclinical CVD. Future studies may also focus on whether α-ClFA predicts change in imaging markers of subclinical CVD in those with and without SLE.

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APPENDIX 1. Characteristics of the Study of Lupus Vascular and Bone Long-term Endpoints (SOLVABLE) cohort compared to remaining members of the Chicago Lupus Database (CLD). Continuous variables are the mean ± SD, categorical variables are the percentage.

Characteristic	SOLVABLE, n = 173	CLD, n = 459	p
Race/ethnicity, %			0.176
White	60.7	58.2	
Black	28.3	24.1	
Asian	4.6	5.3	
Hispanic	6.4	11.2	
Other	0.0	1.2	
Disease duration, yrs, mean ± SD	11.9 ± 8.7	5.7 ± 6.9	< 0.001
Age, yrs, mean ± SD	42.6 ± 10.4	35.9 ± 11.5	< 0.001
Smoking history, %	40.5	30.6	0.017
Lupus renal disease, %[a]	37.0	34.1	0.494
C4, mg/dl, mean ± SD	19.4 ± 9.0	19.8 ± 10.9	0.701
C3, mg/dl, mean ± SD	96.9 ± 27.3	96.7 ± 37.2	0.944
Positive anti-dsDNA, %[b]	46.8	50.3	0.461
Hydroxychloroquine use, %	774.6	47.5	< 0.001
Corticosteroid use, %	42.2	55.0	0.004
Immunosuppressant use, %[c]	34.1	19.1	< 0.001

[a]Defined as participants meeting American College of Rheumatology guidelines for lupus nephritis (proteinuria > 0.5 g/day or > 3+ by dipstick, and/or cellular casts, and/or renal biopsy demonstrating immune complex-mediated glomerulonephritis)[19]. [b]Defined as antibody titer ≥ 1:10 by *C. luciliae* method. [c]Includes cyclophosphamide, azathioprine, methotrexate, mycophenolate mofetil, cyclosporine, or tacrolimus. C4: complement 4; C3: complement 3.

APPENDIX 2. Bivariate analysis of coronary artery calcium (CAC) and aorta calcium (AC) scores for women with and without systemic lupus erythematosus (SLE).

	Women with SLE	Women without SLE	p
CAC score			
Mean ± SD[a]	95.8 ± 177.2	30.4 ± 71.0	0.024
25th percentile	4.2	1.9	
50th percentile	10.3	5.1	
75th Percentile	111.7	34.1	
AC score			
Mean ± SD[b]	1258.6 ± 2819.8	445.0 ± 749.2	0.767
25th percentile	9.3	24.5	
50th percentile	93.5	98.0	
75th percentile	1400.3	429.0	

[a]For CAC score, n = 59 for women with SLE and n = 43 for women without SLE. [b]For AC score, n = 70 for women with SLE and n = 93 for women without SLE.