

## Progression of Carotid Intima-Media Thickness and Plaque in Women With Systemic Lupus Erythematosus

Trina Thompson,<sup>1</sup> Kim Sutton-Tyrrell,<sup>1</sup> Rachel P. Wildman,<sup>2</sup> Amy Kao,<sup>1</sup> Shirley G. Fitzgerald,<sup>1</sup> Betsy Shook,<sup>1</sup> Russell P. Tracy,<sup>3</sup> Lewis H. Kuller,<sup>1</sup> Sarah Brockwell,<sup>1</sup> and Susan Manzi<sup>1</sup>

**Objective.** Women with systemic lupus erythematosus (SLE) are at high risk of cardiovascular disease (CVD). The goals of this study were to determine the extent of atherosclerotic progression among women with SLE compared with a group of healthy controls and to determine whether factors attributed to SLE or its treatment were associated with atherosclerotic progression independent of traditional CVD risk factors.

**Methods.** A longitudinal study of women with SLE from the Pittsburgh Lupus Registry was conducted. Women 18 years of age and older ( $n = 217$ ) underwent carotid ultrasound at baseline and at followup, an average of 4.19 years later. Clinical, serologic, and SLE-related factors, and disease treatment were evaluated. Outcomes were changes in carotid intima-media thickness (IMT) and plaque. Progression of CVD in a sample of women without lupus was used for comparison.

**Results.** The patients' mean  $\pm$  SD age at baseline was  $45.1 \pm 10.3$  years, and the mean  $\pm$  SD IMT progression rate was  $0.011 \pm 0.03$  mm per year. After controlling for traditional CVD risk factors, higher serum creatinine levels were associated with IMT progression ( $P = 0.0006$ ). Plaque prevalence was 31% at

baseline and 40% at followup; plaque progression occurred in 27% of the patients. Higher serum C3 levels and immunosuppressant use at baseline were related to plaque progression ( $P = 0.04$  and  $P = 0.02$ , respectively) independent of traditional CVD risk factors. The plaque progression rate was higher than, and the IMT progression rate was similar to, those in the control group.

**Conclusion.** SLE patients have accelerated plaque progression compared with controls. SLE-related risk factors are associated with the progression of IMT and plaque after controlling for traditional CVD risk factors. Carotid B-mode ultrasound may serve as a surrogate end point in SLE intervention trials and clinically to track SLE management.

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease that primarily affects women. It manifests with frequent exacerbations of inflammatory flares that ultimately may cause organ failure. Treatment consists of antiinflammatory and immunosuppressive agents. Patients with SLE are at higher risk of cardiovascular disease (CVD) compared with women of similar ages (1–5). Individuals with SLE who are younger than 45 years of age were found to have a 50-fold higher risk of myocardial infarction (MI) compared with women of similar age in the Framingham Study (6). Cross-sectional and retrospective studies have shown that traditional risk factors, including hypertension, obesity, diabetes mellitus, smoking, hyperlipidemia, hyperhomocysteinemia, and sedentary lifestyle, play a role in this accelerated atherosclerosis (3,7,8). However, the diagnosis of SLE remains a strong risk factor for CVD, even after controlling for traditional risk factors. The most important SLE factors contributing to premature CVD remain unknown.

Noninvasive imaging techniques have been used to explore why SLE predisposes women to excess CVD risk. Using these modalities, increased rates of carotid focal plaque (37.1% versus 15.2%) (7) and coronary

Dr. Manzi's work was supported in part by the NIH (research grants R01-AR-46588-05 and R01-AR-002213-05). Dr. Tracy's work was supported by the NIH (National Heart, Lung, and Blood Institute grant R01-HL-077449).

<sup>1</sup>Trina Thompson, DrPH, MPH, Kim Sutton-Tyrrell, DrPH, Amy Kao, MD, MPH, Shirley G. Fitzgerald, PhD, Betsy Shook, MD, Lewis H. Kuller, MD, DrPH, Sarah Brockwell, PhD, Susan Manzi, MD, MPH: University of Pittsburgh, Pittsburgh, Pennsylvania; <sup>2</sup>Rachel P. Wildman, PhD: Albert Einstein College of Medicine, Bronx, New York; <sup>3</sup>Russell P. Tracy, PhD: University of Vermont, Burlington.

Dr. Shook has received consulting fees, speaking fees, and/or honoraria (less than \$10,000) from Abbott Immunology.

Address correspondence and reprint requests to Susan Manzi, MD, MPH, University of Pittsburgh Lupus Center of Excellence, S722 Biomedical Science Tower, 3500 Terrace Street, Pittsburgh, PA 15261. E-mail: sxm6@pitt.edu.

Submitted for publication February 6, 2007; accepted in revised form November 5, 2007.

calcium (30.7% versus 8.7%) (9) have been reported in women with SLE compared with controls. Other studies have also shown higher-than-expected rates of subclinical atherosclerosis in women with SLE (4,5,10,11). To date, intima-media thickness (IMT) and plaque assessment have not been used in longitudinal studies of women with SLE to evaluate rates of progression and the factors that predict change in subclinical atherosclerosis among these women would provide the option of using surrogate cardiovascular end points in clinical trials of SLE therapies. The purpose of this study was to evaluate the extent of carotid atherosclerosis progression in women with SLE and to determine the relative contribution of SLE-related risk factors.

## PATIENTS AND METHODS

**Patient population.** Patients were recruited from the Pittsburgh Lupus Registry as part of a longitudinal study of CVD in SLE funded by the National Institutes of Health. The registry includes patients who have been seen either at the University of Pittsburgh Medical Center inpatient and outpatient facilities or by practicing rheumatologists in the Pittsburgh metropolitan area. Thus, the sample represents a community-based spectrum of mild to severe SLE with minimal tertiary care center referral bias. Patients met the 1982 American College of Rheumatology revised criteria for SLE (12). The updated criteria for SLE had not been published when the study began. All women 18 years of age or older were invited to participate regardless of CVD history, which included MI, angina, stroke, transient ischemic attack, and coronary revascularization. Baseline and followup carotid duplex scans were obtained in 217 patients. At baseline, each patient also completed an interview, a physical examination, and laboratory tests, and provided written informed consent and authorization for release of medical information. The study was approved by the University of Pittsburgh's Institutional Review Board.

Another ongoing study had recruited 104 female controls with similar demographic characteristics from the same geographic area (R01-HL4664-01A2). These women comprised our control group. The controls were used only to compare progression rates of IMT and plaque. The control women had carotid scans performed and read by the same sonographers, using the same equipment as with the SLE patients. All blood assays were conducted in the same laboratory. The 104 controls were selected using a combination of voter registration tapes for 1992 for the greater Pittsburgh area and Cole's Criss-Cross Directory of households. A nonrepeating random selection process was used.

**Measurement of covariates and traditional CVD risk factors.** At the baseline clinical examination, age, ethnicity, education, smoking habits, family history of CVD, diagnosis of diabetes, and postmenopausal status were documented. This visit also included anthropometric measurements (height, weight, and waist and hip circumference), 2 consecutive seated blood pressure measurements (averaged), and a 12-hour fast-

ing blood draw. Blood samples were used to measure total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Lipid assays were performed at the Heinz Lipid Laboratory at the University of Pittsburgh Graduate School of Public Health, which is certified by the Centers for Disease Control and Prevention. The Friedewald equation was used to estimate LDL cholesterol (13).

Plasma glucose levels were determined by enzymatic assay, and plasma insulin levels were measured by radioimmunoassay. Hypertension was defined as an average systolic blood pressure of  $\geq 140$  mm Hg, an average diastolic blood pressure of  $\geq 90$  mm Hg, or the use of antihypertensive agents. Metabolic syndrome was defined, using the National Cholesterol Education Program Adult Treatment Panel III clinical guidelines (14), as the presence of  $\geq 3$  of the following components: waist circumference  $> 88$  cm, triglycerides  $\geq 150$  mg/dl, HDL cholesterol  $< 50$  mg/dl, blood pressure  $\geq 130/85$  mm Hg, and a fasting glucose level  $\geq 110$  mg/dl. The definition of hyperglycemia used was determined prior to the creation of the recent American Diabetes Association impaired glucose regulation (impaired fasting glucose/impaired glucose tolerance) criterion of  $\geq 100$  mg/dl (15).

**SLE-related disease risk factors.** SLE disease activity and cumulative organ damage were measured by the same physician (SM) using the Systemic Lupus Activity Measure (SLAM) (16) and the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI) (17). The SDI measures irreversible damage from lupus or its treatment in 12 organ systems (ocular, neuropsychiatric, renal, pulmonary, cardiovascular, peripheral vascular, gastrointestinal, musculoskeletal, skin, premature gonadal failure, diabetes, and malignancy). Because the disease end point for this study was carotid atherosclerosis, the SDI score was modified to remove values for cardiovascular and peripheral vascular disease, to avoid higher scores in patients with known cardiovascular damage. The results of the modified version are reported.

Patients also provided information on corticosteroid usage (past/current, maximum dosage, and duration), and current use of hydroxychloroquine and immunosuppressants. Immunosuppressants included cyclophosphamide, azathioprine, cyclosporine, and methotrexate. Renal disease was defined according to the ACR criteria (12). Laboratory studies included tests for lupus anticoagulant (partial thromboplastin time or dilute Russell's viper venom time with mix), serum C3 and C4 levels, anticardiolipin antibodies (IgG  $> 15$  IgG phospholipid units, IgM  $> 10$  IgM phospholipid units) (Instar, Stillwater, MN), and double-stranded DNA (dsDNA) antibodies (by *Crithidia luciliae*).

**Markers of inflammation.** Serum albumin, C-reactive protein (CRP), and fibrinogen levels were measured at baseline. A dye-binding assay was used to measure albumin, an enzyme-linked immunosorbent assay was used to determine CRP levels, and a modified clot-rate assay was used to measure fibrinogen (18). These assays were performed at the University of Vermont.

**Carotid atherosclerosis measurements.** Carotid ultrasound was performed at the University of Pittsburgh Ultrasound Research Laboratory and has been previously described (1). The common carotid artery (CCA), bifurcation, and proximal internal carotid artery (ICA) were digitized bilaterally.

**Table 1.** Baseline demographic characteristics of the female SLE patients and controls\*

	Patients (n = 217)	Controls (n = 104)	P
Age, mean $\pm$ SD years	45.1 $\pm$ 10.3	44.3 $\pm$ 6.8	0.407
White, no. (%)	194 (89)	95 (91)	0.586
Education, no. (%)			0.08
$\leq$ 12 years	78 (36)	27 (26)	–
13–16 years	98 (45)	43 (42)	–
>16 years	41 (19)	33 (32)	–
Postmenopausal, no. (%)	93 (43)	35 (34)	0.13
Hormone replacement therapy, no. (%)	45 (48)	17 (48.6)	0.99
Hypertension, no. (%)†	79 (36.4)	10 (10)	<0.001
Systolic blood pressure, mean $\pm$ SD mm Hg	120 $\pm$ 18	114 $\pm$ 15.4	0.005
Diastolic blood pressure, mean $\pm$ SD mm Hg	78 $\pm$ 10.7	74 $\pm$ 10	0.001
Total cholesterol, mean $\pm$ SD mmol/liter	193.0 $\pm$ 42.3	206.8 $\pm$ 33.7	0.002
HDL, mean $\pm$ SD mmol/liter	55.6 $\pm$ 15.9	59.6 $\pm$ 14.8	0.034
LDL, mean $\pm$ SD mmol/liter	112.2 $\pm$ 34.6	121.6 $\pm$ 29.0	0.017
Triglycerides, median (IQR) mmol/liter	103 (74–154)	110 (78–153)	0.795
Ever smoked, no. (%)	92 (42)	46 (45)	0.702
Currently smoke, no. (%)	26 (12)	16 (16)	0.361
BMI, mean $\pm$ SD kg/m <sup>2</sup>	27.5 $\pm$ 7.0	27.8 $\pm$ 6.9	0.655
Metabolic syndrome, no. (%)	46 (21)	NA	NA
C-reactive protein, median (IQR) mg/liter	2.0 (0.8–4.0)	1.5 (0.9–2.8)	0.019
Fibrinogen, mean $\pm$ SD $\mu$ mol/liter	298.9 $\pm$ 71.2	NA	NA

\* SLE = systemic lupus erythematosus; HDL = high-density lipoprotein; LDL = low-density lipoprotein; IQR = interquartile range; BMI = body mass index; NA = not available.

† Diagnosed by physician or defined as systolic blood pressure  $\geq$ 140 mm Hg or diastolic blood pressure  $\geq$ 90 mm Hg.

ally with a 5-MHz linear array transducer on a Toshiba 140 ultrasound machine (Toshiba, Tustin, CA). IMT at the baseline and followup examinations was determined by the same reader under blinded conditions, across 1-cm segments, using automated edge-detection software (Artery Measurement System, Gothenburg, Sweden) (19). Readings were spaced at least 1 month apart. Values of 8 measurements were averaged to obtain the mean average IMT. The IMT intraclass correlation coefficient (ICC) between sonographers was  $\geq$ 0.90, and between readers it was 0.87. IMT progression was assessed on a continuous scale.

Plaque was defined as a focal projection within the intima-media layer that was at least 50% greater than the adjacent IMT. The number of plaques was totaled bilaterally. The previously reported ICC for plaque measures was 0.93 (20). To assess the increase in plaque size, the plaque index was used. The CCA, carotid bulb, and proximal ICA were used to report the number and grades of plaque, an estimate of severity. The number of plaques was totaled bilaterally, and grades were also summed to create the plaque index, an estimate of overall plaque burden.

Plaque progression was defined as any increase in the number or size of plaques. When progression was based on increased plaque size, scans were reviewed and progression was verified. The change in the number of plaques ranged from –1 to 4. The 11 patients with negative change values were considered nonprogressors.

**Statistical analysis.** Descriptive statistics were reported as the mean  $\pm$  SD or median (interquartile range) for continuous variables and as percentages for categorical variables. Age-adjusted linear and logistic regression analyses were used to determine the association between baseline risk factors

**Table 2.** SLE-specific CVD risk factors at baseline

SLE duration, mean $\pm$ SD years	10.5 $\pm$ 7.5
ACR criteria at baseline	
Skin (malar or discoid rash)	115 (56.4)
Oral ulcer	119 (58)
Arthritis	198 (93)
Serositis	72 (36)
Renal	42 (20.9)
CNS (seizure/psychosis)	15 (7.5)
Hematologic	105 (50.5)
Serum creatinine, mean $\pm$ SD $\mu$ mol/liter	0.9 $\pm$ 0.7
Albumin, mean $\pm$ SD gm/liter	4.7 $\pm$ 0.4
C3, mean $\pm$ SD gm/liter	94.2 $\pm$ 25.1
C4, mean $\pm$ SD gm/liter	21.4 $\pm$ 7.8
SLAM score, mean $\pm$ SD	6.9 $\pm$ 3.7
Modified SDI score, mean $\pm$ SD	1.2 $\pm$ 1.5
Antiphospholipid antibodies	60 (27.6)
Lupus anticoagulant	45 (20.7)
Anticardiolipin	34 (15.6)
Steroid use, mean $\pm$ SD years	5.5 $\pm$ 6.8
Steroid use ever	203 (94)
Steroid use at baseline	96 (44)
Aspirin use at baseline	6 (2.8)
Warfarin drug use at baseline	26 (13)
Lipid-lowering drug use at baseline	11 (5)
Hydroxychloroquine use at baseline	97 (44.7)
Nonsteroidal antiinflammatory drug use at baseline	77 (35.5)
Immunosuppressant use at baseline	29 (13)

\* Except where indicated otherwise, values are the number (%). SLE = systemic lupus erythematosus; CVD = cardiovascular disease; ACR = American College of Rheumatology; CNS = central nervous system; SLAM = Systemic Lupus Activity Measure; SDI = Systemic Lupus International Collaborating Clinics/ACR Damage Index.

and progression in IMT and plaque measures. Univariate associations were used to select the covariates for stepwise regression. The associated variables ( $P \leq 0.10$ ) were then used to build the final regression models. The multivariate progression models were adjusted for the baseline value (IMT or plaque) and the time between scans. Progression outcomes were reported as both overall and annual changes. When multiple candidate variables were available to measure related characteristics (e.g., systolic blood pressure and pulse pressure), the most significant factor (lowest  $P$  value) in the univariate analysis was selected for inclusion in the stepwise selection procedure. If distributional assumptions of the regression procedures were not met, variable transformations were considered. Statistical analysis was performed using the SAS software package, version 9.1.3 (SAS Institute, Cary, NC).

## RESULTS

**Baseline characteristics of the patients and controls.** The 217 women with lupus included in this study were recruited from among 289 women participating in a longitudinal prospective observational study of CVD in SLE that began in 1996. The 289 women had baseline carotid scans completed between 1996 and 2000. The risk factor assessments were obtained at the time of the baseline scans. Of the 289 participants at baseline, 13 declined followup, 43 were lost to followup, 1 underwent carotid surgery and was excluded, and 15 had inadequate followup scans. Thus, 217 women were available for followup scans. These 217 women did not differ significantly from the 72 who had no followup data. The followup scans in the 217 patients were completed between 1999 and 2005. There was a mean of 4.1 years (median 3.9 years) between scans.

The 217 patients had a mean  $\pm$  SD age of  $45.1 \pm 10.3$  years and were predominantly white (89%) (Table 1). This reflects the racial composition of the Pittsburgh metropolitan area. Ninety-three women (43%) were postmenopausal at baseline. Among the postmenopausal women, 45 (48%) were taking hormone replacement therapy (HRT). Seventy-nine of the 217 patients (36%) had hypertension. The mean  $\pm$  SD total cholesterol level was  $193 \pm 42$  mmol/liter, and the LDL

cholesterol level was  $112 \pm 35$  mmol/liter. One hundred twenty-three women (57%) had elevated LDL levels ( $>100$  mmol/liter) (data not shown). Of those with elevated LDL cholesterol,  $<5\%$  were being treated with cholesterol-lowering medication. Although 42% of the women reported having smoked at some time, only 12% were current smokers. Forty-six patients (21%) met the criteria for metabolic syndrome. Twenty-seven patients (12%) had a known history of vascular disease at baseline.

The mean age, ethnicity, socioeconomic and menopausal status, use of HRT, triglyceride level, smoking history, and body mass index were similar between the patients and the controls (Table 1). Fewer controls had hypertension ( $P < 0.001$ ), but they had higher total cholesterol ( $P = 0.002$ ), LDL ( $P = 0.017$ ), and HDL levels ( $P < 0.034$ ). CRP levels were higher in women with SLE than in the controls (median 2.0 versus 1.5;  $P = 0.019$ ).

Lupus-related variables are shown in Table 2. This population of lupus patients represented a community-based sample with minimal tertiary care referral bias. Approximately two-thirds of the women with lupus were seen at the University of Pittsburgh Medical Center and one-third were seen by practicing rheumatologists in the community. As such, 21% of the women had renal disease and 13% were taking immunosuppressants. Ninety-four percent had taken prednisone at some time, with a mean  $\pm$  SD duration of  $5.5 \pm 6.8$  years. Antinuclear antibodies, anti-dsDNA or anti-Sm, and antiphospholipid antibodies were confirmed positive in  $\sim 95\%$ , 45%, and 28% of the patients, respectively (Table 2 and data not shown). The modified SDI score ranged from 0 to 7 (mean 1.2, median 1), and the SLAM score ranged from 0 to 21 (mean 6.9, median 6) (the higher the SLAM and SDI scores, the more disease activity and organ damage, respectively). These values demonstrate a broad range of disease activity and damage in this relatively young patient cohort.

**Table 3.** Ultrasound data in the female SLE patients and controls\*

	SLE patients (n = 217)	Controls (n = 104)	P
Time between scans, mean $\pm$ SD years	4.19 $\pm$ 1.95	4.97 $\pm$ 0.5	<0.001
Mean IMT at baseline, mean $\pm$ SD mm	0.632 $\pm$ 0.13	0.631 $\pm$ 0.08	0.71
Mean IMT at followup, mean $\pm$ SD mm	0.681 $\pm$ 0.14	0.672 $\pm$ 0.09	0.54
IMT yearly progression, mean $\pm$ SD mm	0.011 $\pm$ 0.03	0.008 $\pm$ 0.01	0.22
Plaque at baseline, no. (%)	67 (31)	18 (17)	0.01
Plaque at followup, no. (%)	85 (40)	21 (20)	<0.001
Plaque progression, no. (%)	58 (27)	10 (10)	<0.001

\* SLE = systemic lupus erythematosus; IMT = intima-media thickness.

**Table 4.** Age-adjusted relationship of CVD risk factors to IMT and plaque progression\*

	IMT progression		Plaque progression	
	$\beta$ (SE)	<i>P</i>	OR (95% CI)	<i>P</i>
<b>Traditional CVD risk factors</b>				
Age, univariate	0.001 (0.0005)	0.05	1.09 (1.05–1.13)	<0.001
SES, level of education	–0.0004 (0.003)	0.89	0.84 (0.71–1.00)	0.05
Postmenopausal	0.009 (0.014)	0.51	1.46 (0.63–3.36)	0.38
Hypertension	–0.007 (0.011)	0.54	1.55 (0.80–3.03)	0.20
Diastolic blood pressure	–0.001 (0.0005)	0.02	1.02 (0.99–1.05)	0.22
Total cholesterol	–0.0001 (0.0001)	0.28	1.01 (1.00–1.02)	0.10
HDL	–0.0003 (0.0003)	0.36	0.98 (0.95–1.00)	0.04
LDL	–0.0001 (0.0002)	0.42	1.01 (1.00–1.02)	0.09
Triglyceride	–0.00002 (0.00006)	0.81	1.01 (1.00–1.01)	0.02
Ever smoked	0.012 (0.010)	0.25	1.27 (0.66–2.44)	0.47
Currently smoke	0.019 (0.016)	0.24	4.00 (1.51–10.53)	<0.01
BMI	0.0009 (0.0007)	0.24	1.01 (0.97–1.06)	0.56
Metabolic syndrome	0.021 (0.013)	0.10	1.39 (0.66–2.96)	0.39
C-reactive protein	–0.0003 (0.0006)	0.61	1.01 (0.98–1.06)	0.47
Fibrinogen	0.00003 (0.00007)	0.68	1.01 (1.0–1.01)	0.05
<b>SLE-related risk factors</b>				
Duration of SLE	0.0008 (0.0007)	0.24	1.03 (0.99–1.01)	0.12
C3	–0.00003 (0.0002)	0.89	1.01 (1.00–1.03)	0.09
C4	0.0004 (0.0007)	0.51	1.03 (0.99–1.07)	0.22
Albumin	–0.0278 (0.013)	0.03	0.94 (0.40–2.21)	0.88
Serum creatinine	0.0494 (0.026)	0.06	3.31 (0.73–15.04)	0.12
SLAM score	–0.0007 (0.001)	0.61	1.10 (1.00–1.20)	0.04
SDI, modified	0.005 (0.003)	0.16	1.18 (0.95–1.46)	0.13
Positive LAC	0.007 (0.013)	0.57	1.01 (0.46–2.21)	0.99
Positive ANA	0.008 (0.018)	0.66	1.29 (0.42–3.96)	0.66
Positive anti-dsDNA	0.00004 (0.00004)	0.32	1.00 (1.00–1.01)	0.17
Years of steroid use	0.0014 (0.0008)	0.07	1.01 (0.96–1.06)	0.73
Anticardiolipin	0.005 (0.015)	0.75	0.99 (0.40–2.47)	0.98
Steroid use ever	0.0216 (0.021)	0.30	1.55 (0.37–6.53)	0.55
Steroid use at baseline	–0.0004 (0.010)	0.97	1.23 (0.64–2.38)	0.53
Aspirin use at baseline	0.020 (0.018)	0.27	1.05 (0.32–3.47)	0.93
Warfarin use at baseline	0.0308 (0.016)	0.47	2.05 (0.32–13.31)	0.45
NSAID use at baseline	–0.013 (0.011)	0.24	1.07 (0.54–2.11)	0.85
Hydroxychloroquine use at baseline	–0.006 (0.010)	0.57	0.73 (0.38–1.42)	0.36
Cholesterol-lowering medication use at baseline	0.069 (0.023)	0.003	3.77 (0.96–14.86)	0.06
Immunosuppressant use at baseline	–0.028 (0.015)	0.07	3.57 (1.39–9.16)	<0.01

\* CVD = cardiovascular disease; IMT = intima-media thickness; OR = odds ratio; 95% CI = 95% confidence interval; SES = socioeconomic status; HDL = high-density lipoprotein; LDL = low-density lipoprotein; BMI = body mass index; SLE = systemic lupus erythematosus; SLAM = Systemic Lupus Activity Measure; SDI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; LAC = lupus anticoagulant; ANA = antinuclear antibody; anti-dsDNA = anti-double-stranded DNA (*Critidia luciliae* titer >2); NSAID = nonsteroidal antiinflammatory drug.

### Prevalence and progression of IMT and plaque.

The mean  $\pm$  SD time between scans in the patients was  $4.19 \pm 1.95$  years, which was shorter than in the controls ( $4.97 \pm 0.5$  years) ( $P < 0.001$ ) (Table 3). The average total IMT progression rate in the patients was 0.05 mm, with a mean  $\pm$  SD annual IMT progression rate of  $0.011 \pm 0.03$  mm ( $P < 0.001$ ). The prevalence of plaque was 31% at baseline and 40% at followup. Plaque progression was seen in 27% of the women, while a decrease of 1 plaque was seen in 5% ( $n = 11$ ). The majority of patients (68%) showed no change in the number or size of plaques.

The mean  $\pm$  SD yearly IMT progression rate was  $0.008 \pm 0.01$  mm in the controls, similar to the rate of

progression in the patients ( $P = 0.22$ ). Baseline plaque prevalence was significantly higher in the patients compared with the controls (31% versus 17%;  $P = 0.01$ ). The same was true for plaque prevalence at followup (40% versus 20%;  $P < 0.001$ ). Plaque progression was significantly more frequent in the patients (27% versus 10%;  $P < 0.001$ ).

**Risk factors associated with IMT progression in SLE patients.** After age adjustment, traditional as well as SLE-related risk factors were associated with greater IMT progression (Table 4). The strongest risk factors were lower diastolic blood pressure, lower serum albumin level, higher serum creatinine level, years of steroid use, use of cholesterol-lowering medications, and not

**Table 5.** Multivariate relationships of CVD risk factors to IMT and plaque progression\*

	IMT progression†		Plaque progression†	
	$\beta$ (SE)	<i>P</i>	OR (95% CI)	<i>P</i>
Age per 5 years	0.013 (0.003)	0.0001	1.39 (1.12–1.72)	0.003
Diastolic blood pressure per 10 mm Hg	–0.013 (0.005)	0.004	–	–
Triglycerides per 25 mg/dl	–	–	1.13 (1.01–1.27)	0.04
Serum creatinine, mg/dl	0.086 (0.025)	0.0006	–	–
Serum C3 per 25 units	–	–	1.46 (1.02–2.09)	0.04
Immunosuppressant use at baseline	–	–	3.43 (1.20–9.82)	0.02

\* See Table 4 for definitions.

† Adjusted for baseline value and time between scans.

taking immunosuppressants. In multivariate analysis, controlling for traditional CVD risk factors, elevated serum creatinine was significantly related to IMT progression ( $P = 0.0006$ ) (Table 5).

**Risk factors for plaque progression.** After controlling for age, several traditional and SLE-related factors were associated with carotid plaque progression (Table 4). These included lower socioeconomic status, lower level, higher LDL level, higher triglyceride level, current smoking, higher serum fibrinogen level, higher serum C3 level, SLAM score, use of cholesterol-lowering medications, and immunosuppressant use at baseline. Controlling for traditional CVD risk factors, higher serum C3 level and immunosuppressant use at baseline were significantly related to plaque progression (Table 5).

## DISCUSSION

This study shows for the first time that CVD progression can be measured using B-mode carotid ultrasound in women with lupus. In the lupus patients, the frequency of plaque progression was higher (27% versus 10%) and the degree of IMT progression was similar (0.011 mm/year versus 0.008 mm/year) compared with those in a control group. While IMT difference was not statistically significant, the 0.003 mm/year difference between patients and controls is annualized and thus accumulates over time. A larger sample or a longer duration of followup may have resulted in significant differences. Since IMT is known to be predictive of subsequent CVD outcomes in other high-risk populations (8,21,22), it seems unlikely that it would not play some role in SLE, especially if there is intervention targeting traditional risk factors.

IMT progression was measurable in this group of patients, and SLE-associated risk factors were related to

IMT progression. Higher serum creatinine was an independent predictor of IMT progression. This finding suggests 2 things: the vasculature is a target of the generalized organ damage that occurs with SLE, and the renal damage caused by SLE either contributes to or is a marker of CVD progression. The health of the kidneys and that of the vasculature are intimately intertwined, and the kidneys are particularly susceptible to the consequences of vascular damage (23). Thus, monitoring kidney function likely provides useful information on the vascular effects of SLE. This idea is supported by cross-sectional data on women with SLE, which have shown that impaired renal function is related to coronary artery calcification (11) and carotid atherosclerosis (24).

Of interest was the negative association between diastolic blood pressure and IMT progression. Low diastolic blood pressure is a marker of arterial stiffening, and arterial stiffening is associated with atherosclerosis progression (25). Thus, this is a possible explanation for this observation.

The 31% prevalence of carotid plaque found here is similar to that in other populations of women with SLE (17–40%) (1,5,7,8,24,26). These rates are higher than in our control group (17%;  $P = 0.01$ ) and also in other populations of healthy women, where frequencies have been reported to be 11–25% (7,8,27). The 31% prevalence is even higher than the 19.3% prevalence reported in an older population of women (ages 59–71 years) in the Aging Vascular Study (EVA) (28). Twenty-seven percent of our patients had plaque progression, which is also higher than in the control group (10%), despite the fact that the patients had lower LDL levels and a shorter time between scans. The 27% progression rate is also higher than that of an older population in the EVA (18.3%) (28). Thus, it appears

that women with SLE develop lesions earlier and these lesions may progress faster than among age-similar and older women.

Both prevalence and progression of plaque were associated with SLE-related factors after controlling for age. It is likely that the heightened inflammation process in SLE contributes to higher CVD risk. Elevated serum C3 levels were found to be related to plaque progression ( $P = 0.04$ ). We have previously reported that high serum C3 levels are related to increased aortic stiffness in women with SLE (26,29). Serum C3 levels have also been found to be associated with coronary calcification (11). These observations are particularly surprising because decreased levels of C3 (a precursor of complement activation) are traditionally associated with lupus pathogenesis.

There are 2 possible explanations for our observation that increased serum levels of C3 are associated with carotid plaque progression and vascular stiffness in lupus. First, it may reflect an acute-phase response. We believe this is unlikely since other acute-phase proteins, such as CRP and fibrinogen, were not independently associated with plaque or vascular stiffness in the same patients. Second, activation of the complement system may actually contribute to increased vascular disease and progression of disease; however, compensatory increases in C3 and C4 synthesis mask consumption, a situation that cannot be resolved with standard assays for C3 and C4.

It is recognized that inflammation is involved in all stages of atherosclerosis development, and complement is the final common pathway in all physiologic and pathophysiologic inflammatory processes (30–33). One direct mechanism includes increased endothelium permeability, which leads to plasma protein influx into the arterial wall (34). Indirectly, serum C3 may be involved through stiffening of the vasculature, which later creates an environment in which plaques are more likely to form. SLE-related inflammatory abnormalities may also function synergistically with traditional risk factors, making them particularly harmful.

Immunosuppressant use at baseline was associated with plaque progression. This finding is inconsistent with cross-sectional data indicating that cyclophosphamide nonuse was associated with plaque among women with SLE (7). It is important to point out that the objective of our study was to determine the risk factors measured at one point in time (baseline visit) that predict the progression of vascular disease over the 4-year followup period. Cumulative disease activity and changing therapies over the course of the 4 years were

not ascertained in this study. Therefore, the role that sustained disease activity and long-term immunosuppression plays in CVD and SLE remains unclear and requires further investigation. This limitation applies to changes in all risk factor profiles over the course of followup. It is important to point out that the women with lupus in this study were primarily white and represented a mix of tertiary care center referral patients and those seen by practicing rheumatologists. Our results may not be applicable to all lupus populations.

In conclusion, both carotid IMT and plaque progression can be measured using B-mode ultrasound in women with SLE. Plaque progression appears to be greater in women with SLE than in healthy women, and monitoring the progression of plaque may be more useful than monitoring IMT in women with SLE. It is important to note that the controls chosen for this study were similar to the patients with regard to demographic features and mix of traditional CVD risk factors, but they were otherwise healthy with no underlying inflammatory disease or exposure to corticosteroids. Rates of progression of both IMT and plaque are related to both SLE-associated and traditional risk factors, and these may work together to increase CVD risk. This study provides data to support the use of carotid B-mode ultrasound as a surrogate end point in future clinical trials that examine the efficacy of CVD prevention strategies in SLE.

## ACKNOWLEDGMENTS

The authors thank Dr. Evelyn Talbot for use of the comparison group data. The authors would also like to thank the study coordinator, Penny Shaw, RN, and the staff of the Ultrasound Research Laboratory at the University of Pittsburgh for their dedication and their expertise with the ultrasound data.

## AUTHOR CONTRIBUTIONS

Dr. Manzi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study design.** Sutton-Tyrrell, Fitzgerald, Manzi.

**Acquisition of data.** Thompson, Sutton-Tyrrell, Kao, Fitzgerald, Shook, Tracy, Manzi.

**Analysis and interpretation of data.** Thompson, Sutton-Tyrrell, Wildman, Kao, Shook, Tracy, Kuller, Manzi.

**Manuscript preparation.** Thompson, Sutton-Tyrrell, Kao, Fitzgerald, Tracy, Brockwell, Manzi.

**Statistical analysis.** Thompson, Sutton-Tyrrell, Wildman, Kao, Brockwell, Manzi.

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