1. Caraba2008

ENDOTHELIAL DYSFUNCTION IN SYSTEMIC *LUPUS ERYTHEMATOSUS*

A. CARABĂ, GERMAINE SĂVOIU**, VIORICA CRIŞAN***, CORINA GORUN**, IULIANA ZVARICI*, I. ROMOŞAN**

*Department of Internal Medicine, **Department of Pathophysiology, ***Department of Rheumatology, "Victor Babeş" University of Medicine and Pharmaceutics, Timişoara

Abstract. Systemic *lupus erythematosus* (SLE) is associated with an increased risk of atherosclerosis; endothelial dysfunction representing the first step in its pathogenesis. The aim of this study is represented by the assessment of the endothelial dysfunction in SLE and the characterization of SLE specific factors which contribute to its appearance. The study was done on 24 subjects, divided into two groups: group A (12 patients with SLE without renal involvement) and group B (12 healthy sex and age-matched controls). Total cholesterol, triglycerides, antinuclear antibodies, anti dsDNA antibodies, C_3, circulating immune complexes were determined in all patients. SLE activity was assessed using SLE Disease Activity Index (SLEDAI). Endothelial function was assessed by means of flow mediated dilation (FMD) on brachial artery, using B-mode ultrasonography. The statistical analysis was done using Pearson's test and Student's t-test. $p < 0.05$ was considered statistically significant. The group of SLE patients was formed of 12 females, with the mean age of 37.16 ± 9.69 years. The values of SLE specific tests and SLEDAI were represented by: anti dsDNA antibodies 1/682 ± 1/914, C_3 68.91 ± 11.91 mg/dL, circulating immune complexes 10.03 ± 2.85 µEq/mL, total cholesterol 208.66 ± 49.63 mg/dL, triglycerides 153.41 ± 46.26 mg/dL, SLEDAI 11.66 ± 3.70. The values of FMD were 8.85 ± 2.02% (group A) and 20.33 ± 6.19% (group B), $p < 0.001$. The statistical analysis showed a strong inverse correlation between FMD and SLEDAI, a strong correlation between FMD and C_3, respectively anti dsDNA antibodies, a moderate inverse correlation between FMD and circulating immune complexes, total cholesterol, systolic and diastolic blood pressure. Endothelial dysfunction is present in SLE patients even in the absence of traditional cardiovascular risk factors, due to disease activity.

Key words: endothelial dysfunction, systemic *lupus erythematosus*.

INTRODUCTION

Systemic *lupus erythematosus* (SLE) represents the autoimmune disease, with a wide range of clinical and biological manifestations [8]. Despite the improvement of therapeutic regimes, the morbidity and mortality associated with SLE remained at high levels. In 1976, Urowitz *et al*. [18] postulated a bimodal

Received: May 2008;
in final form June 2008.

ROMANIAN J. BIOPHYS., Vol. 18, No. 3, P. 237–244, BUCHAREST, 2008

mortality pattern in patients with this disease: in the first part of evolution, mortality is due to severe infections or to disease activity, but after 5 years of SLE course, mortality is caused by the accelerated atherosclerosis and its consequences.

During the last 3 decades, several authors studied the atherosclerosis in lupus. It was proved that atherosclerosis has a high incidence among young women with SLE. These patients have a high prevalence of coronary artery disease and an incidence of myocardial infarction up to 50 times higher than age-matched healthy subjects. This high incidence of atherosclerosis in SLE cannot be attributed only to traditional risk factors [3, 11].

In healthy subjects, endothelium is not a simple physical barrier between the blood flow and the underlying tissues. This structure has many functions, like: continuous regulation of vascular tone, leucocytes adhesion, maintenance of the balance between thrombotic and anticoagulant properties of the blood [12]. When these functions of the endothelium are affected, endothelial dysfunction appears. Endothelial dysfunction is considered the first step in the atherogenetic process; it was identified even in patients with SLE, without cardiovascular risk factors [9]. Endothelial dysfunction in SLE is produced by the clustering of traditional risk factors, adverse effects of treatment and SLE itself as an independent risk factor [1, 14]. Systemic inflammation, autoantibodies directed to double stranded DNA (dsDNA), ribonucleoproteins (nRNP), endothelial cells, phospholipids, circulating immune complexes, activated complement products, lupus nephropathy, dyslipidemia represent some factors related to SLE which contribute to appearance of endothelial dysfunction [7, 19].

A non-invasive method for the assessment of endothelial function is flow mediated vasodilation (FMD) on brachial artery, using vascular ultrasonography.

The aim of this study is represented by the assessment of endothelial dysfunction in SLE patients using vascular ultrasonography and the characterization of SLE specific factors which contribute to its appearance.

MATERIALS AND METHODS

The study was performed on two groups of subjects: group A, formed by 12 patients with SLE without renal involvement and group B, formed by 12 healthy sex and age-matched controls. The diagnosis of SLE was established based on American College of Rheumatology criteria. SLE treatment consisted of prednisone +/– azathioprine.

The procedures followed were in accordance with the ethical standards of the Hospital Ethics Commitee and with the Helsinki Declaration of 1975, as revised in 2000 (5)

Total cholesterol (Abbott photometry), triglycerides (Abbott reactive), antinuclear antibodies (immunofluorescence on Hep-2 cells), anti dsDNA antibodies (immunofluorescence on Crithidia luciliae), C_3 (Roche immunoturbidimetry), circulating immune complexes (EIA method) were determined in all patients.

The SLE activity was assessed using SLE Disease Activity Index (SLEDAI).

Endothelial function was assessed by means of flow-mediated vasodilation on brachial artery, using B-mode ultrasonography (ALOKA ProSound 4000, with linear transducer of 7.5 MHz). Before the test, the patient was relaxed in a stable room temperature between 20 – 25 ºC; the smoking was prohibited. The diameter of brachial artery was measured incident with the R wave of the electrocardiograph trace (Di). Then, ischemia was induced by inflating the pneumatic cuff to a pressure 50 mmHg above systolic one, in order to obliterate the brachial artery and induce ischemia. After 5 minutes, the cuff was deflated and the diameter was measured after 60 seconds post-deflation (Df). FMD was calculated with the formula:

$$FMD = [(Df - Di)/Di] \times 100 \quad (1)$$

All the values were presented as mean ± standard deviation. The statistical analysis was done using Pearson's test (for correlation) and Student's t-test (for comparing of FMD between the two groups). $p < 0.05$ was considered statistically significant.

RESULTS

The demographic, clinical and biological characteristics of the studied groups are shown in Table 1. Diabetes mellitus and chronic renal diseases were absent in all subjects.

Table 1

Characteristics of the studied groups

Parameter	SLE group	Control group
Males/Females	0/12	0/12
Mean age (years)	37.16 ± 9.69	35.02 ± 8.21
Mean duration of SLE evolution (years)	7.16 ± 3.66	0
Total cholesterol (mg/dL)	208.66 ± 49.63	209.25 ± 35.27
Triglycerides (mg/dL)	153.41 ± 46.26	155.71 ± 25.87
Smoking (%)	41.66%	33.33%
Blood pressure (mmHg)	137.08 ± 19.59/82.91 ± 9.87	135.94 ± 8.99/80.75 ± 9.29

The results of SLE specific tests and SLEDAI are presented in Table 2.

Table 2

SLE specific parameters

Parameter	Mean value ± SD
Anti dsDNA antibodies	1/682 ± 1/914
C_3 (mg/dL)	68.91 ± 11.91
Circulating immune complexes (μEq/mL)	10.03 ± 2.85
SLEDAI	11.66 ± 3.70

In SLE group, FMD was lower than in the control group (Table 3).

Table 3

FMD in the studied groups

	SLE group	Control group	*p*
FMD (%)	8.85 ± 2.02	20.33 ± 6.19	< 0.001

The impaired endothelial function, assessed by vascular ultrasonography on brachial artery, is presented in Figures 1, 2 (FMD = 8.57 %).

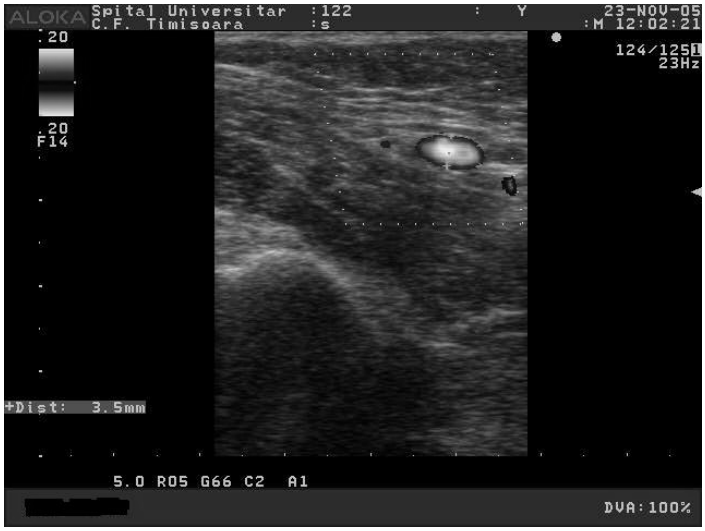

Fig. 1. Brachial artery diameter before FMD test.

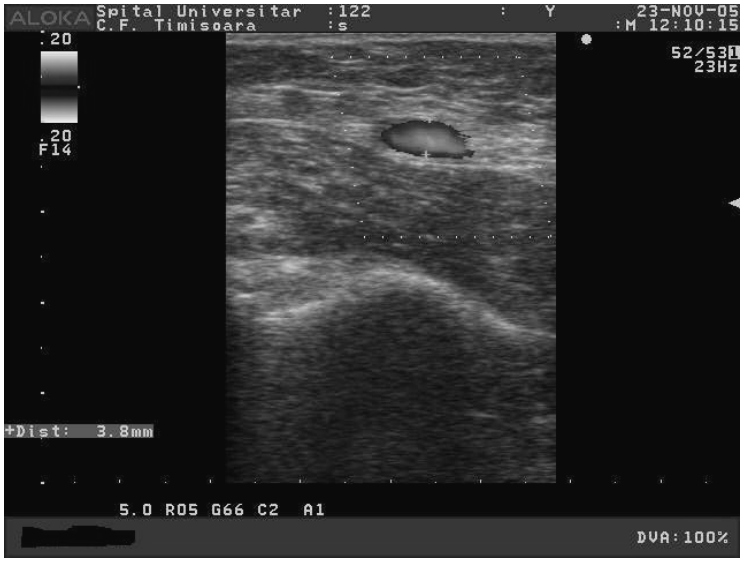

Fig. 2. Brachial artery diameter after FMD test.

The correlations between FMD and biological and immunological parameters are shown in Table 4.

The statistical analysis showed: a strong inverse correlation between FMD and SLEDAI, a strong correlation between FMD and C_3, anti dsDNA antibodies, a moderate inverse correlation between FMD and circulating immune complexes, total cholesterol, systolic and diastolic blood pressure.

Table 4

Correlations between FMD and SLE parameters

Parameter	**Correlation coefficient**
SLEDAI	$r = -0.7321$, $p < 0.001$
C_3	$r = 0.7117$, $p < 0.001$
Circulating immune complexes	$r = -0.4891$, $p < 0.01$
Anti dsDNA antibodies	$r = 0.7201$, $p < 0.001$
Total cholesterol	$r = -0.4450$, $p < 0.01$
Systolic blood pressure	$r = -0.4358$, $p < 0.01$
Diastolic blood pressure	$r = -0.4203$, $p < 0.01$

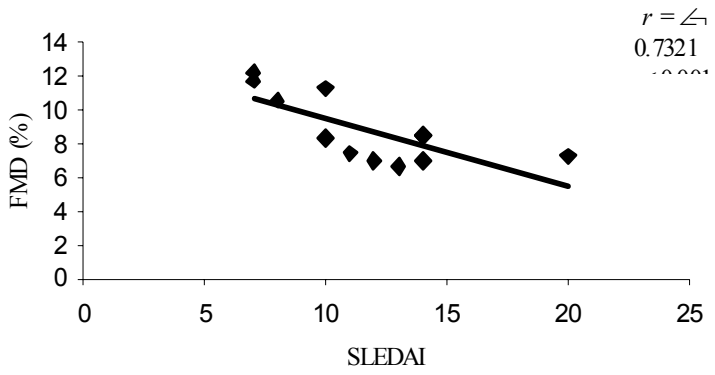

Fig. 3. Correlation between FMD and SLEDAI.

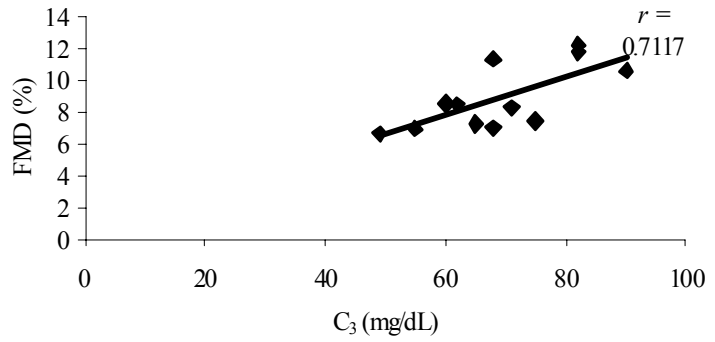

Fig. 4. Correlation between FMD and C_3.

DISCUSSIONS

The patients with SLE have a high incidence of atherosclerosis with its main consequence: coronary artery disease. Epidemiological studies have shown that SLE women aged 35 – 44 years were over 50 times more likely to develop myocardial infarction than women of similar age from general population [11]. Anatomo-pathological investigations have revealed that the SLE patients were prone to develop a premature atherosclerosis [2]. The increased risk of atherosclerosis is not exclusively related to traditional risk factors alone [9]. In the last years, SLE itself appeared like an independent risk factor for atherosclerosis, acting through autoimmune vascular injury [14].

In patients with systemic *lupus erythematosus*, atherosclerosis has a long period of subclinical evolution. The first reversible step in the atherogenesis process is represented by the endothelial dysfunction [16]. Endothelial dysfunction

appears when the normal functions of the endothelial cells (control of vascular tone and blood pressure, regulation of leucocytes traffic from the blood to the tissues, and platelet adhesion and aggregation, maintenance of the balance between blood coagulation and fibrinolysis, control of growth, development and differentiation of the vessel wall cells) are lost or dysregulated [15]. A non-invasive method for the assessment of endothelial dysfunction is represented by flow mediated vasodilation (endothelium dependent dilation) [4].

Several authors studied endothelial dysfunction in SLE patients. The first study, performed by Lima *et al*. [10], showed that SLE patients presented lower FMD than sex and age-matched controls, even in subjects without traditional cardiovascular risk factors [10].

In our study, FMD in SLE patients was significantly lower than in control subjects ($p < 0.001$). Tani *et al.* [16] identified the same pattern of FMD in SLE patients. The reduced values of FMD in patients with SLE were found by Piper and Turner in their studies, too [13, 17].

We found a strong inverse correlation between FMD and SLEDAI ($r = -0.7321$, $p < 0.001$). This index comprises 24 items (clinical, biological, immunological): seizure, psychosis, organic brain syndrome, visual disturbance, cranial nerve disorder, lupus headache, cerebrovascular accident, vasculitis, arthritis, myositis, urinary casts, hematuria, proteinuria, pyuria, rash, alopecia, mucosal ulcers, pleurisy, pericarditis, low complement, increased DNA binding, fever, thrombocytopenia, leucopenia [8]. This correlation between FMD and the disease activity was identified in other studies [5, 10, 13]. Endothelial dysfunction is caused by several factors such as: anti dsDNA antibodies ($r = 0.7201$, $p < 0.001$), circulating immune complexes ($r = -0.4891$, $p < 0.01$), activated complement products ($r = 0.7117$, $p < 0.001$), systemic inflammation, other antibodies (antiribonucleoproteins, anti endothelial cells, antiphospholipids), total cholesterol ($r = -0.4450$, $p < 0.01$), arterial hypertension. The small number of studied patients represents a drawback in statistical analysis, but this study will continue in the future.

FMD using vascular ultrasonography on brachial artery represents a useful, non-invasive method for the assessment of the endothelial dysfunction. Reactive hyperemia produces a shear stress stimulus that induces the normal endothelium to release nitric oxide (NO), which acts as a vasodilator. Impaired endothelial function is associated with a reduced release of NO and a lower vasodilation [4].

CONCLUSION

Endothelial dysfunction is present in SLE patients even in the absence of traditional cardiovascular risk factors. It is due to disease activity. FMD using vascular ultrasonography on brachial artery represents a non-invasive, repeatable and useful method for the assessment of endothelial dysfunction.

REFERENCES

1. AHMAD, Y., J. SHELMERDINE, H. BODILL, *et al*., Subclinical atherosclerosis in systemic *lupus erythematosus* (SLE): the relative contribution of classic risk factors and the lupus phenotype, *Rheumatology*, 2007, **46**(6), 983–988.
2. BHATT, S.P., R. HANDA, G.S. GULTI, *et al*., Atherosclerosis in Asian Indians with systemic *lupus erythematosus*, *Scand. J. Rheumatol*., 2006, **35**, 128–132.
3. BRUCE, I.N., D.D. GLADMAN, M.B. UROWITZ, Detection and modification of risk factors for coronary artery disease in patients with SLE: a quality improvement study, *Clin. Exp. Rheumatol.*, 1998, **16,** 435–440.
4. CORRETTI, M.C., T.J. ANDERSON, E.J. BENJAMIN, *et al*., Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation at the brachial artery, *J. Am. Coll. Cardiol.*, 2002, **39,** 257–265
5. EL-MAGADMI, M., H. BODILL, Y. AHMAD, *et al*., Systemic lupus erythematosus – an independent risk factor for endothelial dysfunction in women, *Circulation,* 2004, **110,** 399–404.
6. ESDAILE, J.M., M. ABRAHAMOWICZ, T. GRODZICKY, *et al*., Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus, *Arthritis Rheum*., 2001, **44**(10), 2331–2337.
7. HAHN, B.H., Systemic lupus erythematosus and accelerated atherosclerosis, *N. Engl. J. Med*., 2003, **349,** 2379–80.
8. IONESCU, R., Lupusul eritematos sistemic, in: Ionescu Ruxandra, *Esențialul în reumatologie*, ed. a 2-a. Editura Medicală Amaltea, București, 2007, pp. 348–371.
9. JOHNSON, S.R., P.J. HARVEY, J.S. FLORAS, *et al*., Impaired brachial artery endothelium dependent flow mediated dilation in systemic lupus erythematosus: preliminary observations, *Lupus*, 2004, **13,** 590–593.
10. LIMA, D.S., E.I. SATO, V.C. LIMA, *et al*., Brachial endothelial function is impaired in patients with systemic lupus erythematosus, *J. Rheumatol*., 2002, **29**(2), 292–297.
11. MANZI, S., E.N. MEILAHN, J.E. RAIRIE, Age-specific incidence rates of myocardial infarction and angina in women with systemic *lupus erythematosus*: comparison with the Framingham study, *Am. J. Epidemiol*., 1997, **145,** 408–415.
12. PEARSON, J.D., Normal endothelial cell function, *Lupus*, 2000, **9,** 183–188.
13. PIPER, M.K., K. RAZA, S.L. NUTTALL, *et al*., Impaired endothelial function in systemic lupus erythematosus, *Lupus*, 2007, **16**(2), 84–88.
14. ROMAN, M.J., B.A. SHANKER, A. DAVIS, Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus, *N. Engl. J. Med*., 2003, **349,** 2399–2406.
15. ROSS, R., Atherosclerosis-an inflammatory disease, *N. Engl. J. Med*., 1999, **340,** 115–126.
16. TANI, C., M. MOSCA, A. d'ASCANIO, *et al.,* Chronic inflammation and endothelial dysfunction: analysis of a cohort of patients with SLE and UCTD, *Reumatismo*, 2006, **58**(3), 212–218.
17. TURNER, E., V. DISHY, C.P. CHUNG, *et al*., Endothelial function in systemic lupus erythematosus: relationship to disease activity, cardiovascular risk factors, corticosteroid therapy, and coronary calcification, *Vasc. Health. Risk. Manag*., 2005, **1**(4), 357–360.
18. UROWITZ, M.B., A.A. BOOKMAN, B.E. KOEHLER, *et al*., The bimodal mortality pattern of systemic lupus erythematosus, *Am. J. Med*., 1976, **60,** 221–225.
19. YAZICI, Z.A., E. RASCHI, A. PATEL, *et al*., Human monoclonal anti-endothelial cell IgG-derived from a systemic lupus erythematosus patient binds and activates human endothelium *in vitro*, *Int. Immunol.*, 2001, **13,** 349–357.

2. Magadmi2004 (truncated)

Systemic Lupus Erythematosus
An Independent Risk Factor for Endothelial Dysfunction in Women

Masoud El-Magadmi, MB; Helena Bodill, MSc; Yasmeen Ahmad, MB, MRCP; Paul N. Durrington, MD, F Med Sci; Michael Mackness, PhD; Michael Walker, ChM, FRCS; Robert M. Bernstein, MA, FRCP; Ian N. Bruce, MD, FRCP

Background—Systemic lupus erythematosus (SLE) patients have a significantly increased risk of coronary heart disease (CHD) that is not fully explained by classic risk factors. Endothelial dysfunction is an early stage in the process of atherogenesis. Our aim was to determine whether endothelial dysfunction occurs in SLE and whether it is associated with the occurrence of classic Framingham risk factors.

Methods and Results—We studied 62 women with SLE (1997 revised criteria) and 38 healthy women. Demographic and risk factor data were collected. In patients, disease activity and treatment-related parameters were also assessed. Endothelial function was assessed by flow-mediated dilation (FMD) in the brachial artery in response to reactive hyperemia. Carotid intima-media thickness (IMT) and the presence of carotid plaques were also assessed in SLE patients. FMD was impaired in SLE patients (median, 3.6%; range, −6.3% to 13.7%; versus median, 6.9%; range, −6.6% to 17.8%, $P<0.01$). Using multiple regression analysis that included all subjects in which we retained all the classic CHD risk factors, we found that systolic blood pressure ($P=0.019$) and SLE ($P=0.017$) were significantly associated with impaired FMD. Within SLE patients, IMT showed a negative correlation with percent FMD ($r=-0.37$, $P<0.01$). In stepwise multiple regression of SLE patients only that also included SLE factors and IMT, IMT alone was independently associated with FMD ($P=0.037$).

Conclusions—Patients with SLE have endothelial dysfunction that remained significant even after adjustment for other classic CHD risk factors. Within SLE patients, endothelial dysfunction correlates negatively with IMT, another marker of early atherosclerosis. Understanding the mechanism(s) of endothelial dysfunction in SLE may suggest novel strategies for CHD prevention in this context. **(*Circulation*. 2004;110:399-404.)**

Key Words: coronary disease ■ endothelium ■ lupus erythematosus, systemic

Systemic lupus erythematosus (SLE) is associated with an increased risk of coronary heart disease (CHD). The rate ratio for myocardial infarction in women with SLE 35 to 44 years of age was found to be 52 times that of a comparative population.[1] In several large clinical series, the cumulative prevalence of CHD-related events was 6% to 10%, and the annual incidence rate of CHD in SLE populations was ≈1.3% to 1.5%.[2] In addition, several studies of subclinical atherosclerosis have identified that 30% to 40% of women with SLE have carotid plaques or myocardial perfusion abnormalities.[3,4] Framingham risk factors do contribute to CHD risk, but even after adjustment for these factors, the risk remains increased by 8- to 10-fold.[5]

Endothelial dysfunction represents a widespread phenomenon, and in the context of CHD, abnormalities of the brachial and femoral arteries have been noted. Endothelial dysfunction is also present in patients with CHD risk factors such as smoking, diabetes mellitus, and hypercholesterolemia.[6]

SLE is a complex disease, and in addition to atherosclerosis, it is characterized by several vascular processes, namely inflammation, Raynaud's phenomenon, and a propensity to vascular thrombosis associated with anti-phospholipid antibodies. Lima et al[7] have found that patients with SLE have evidence of endothelial dysfunction, but patients with significant CHD risk factors were excluded from this study and the presence of atheroma was not assessed. Recently, endothelial dysfunction has also been found in patients with systemic vasculitis and has been reversed by administration of immunosuppressive therapy.[8] Because endothelial dysfunction may represent an early stage in atherogenesis, it is important to understand the mechanisms of its development in a condition such as SLE. It is also important to determine whether it is associated with other CHD risk factors or early atheroma. Therefore, our aims were to determine whether endothelial dysfunction occurred in women with SLE, whether it was

Received February 9, 2004; de novo received April 13, 2004; accepted May 15, 2004.

From the University of Manchester arc Epidemiology Unit (M.E.-M., Y.A.), Department of Vascular Surgery (H.B., M.W.), Rheumatism Research Centre (Y.A., R.M.B., I.N.B.), and Department of Medicine (P.N.D., M.M.), Manchester Royal Infirmary, Central Manchester and Manchester Children's University Hospitals NHS Trust, Manchester, UK.

Correspondence to Dr I.N. Bruce, MD, FRCP, Senior Lecturer in Rheumatology, University of Manchester, Rheumatism Research Centre, Central Manchester and Manchester Children's University Hospitals NHS Trust, Oxford Rd, Manchester, M13 9WL UK. E-mail ian.bruce@man.ac.uk

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000136807.78534.50**

explained by the presence of classic CHD risk factors, and whether any of the vascular processes associated with SLE influence endothelial dysfunction.

Methods

Subjects

Patients were recruited from the Lupus Clinic at the University of Manchester Rheumatism Research Centre. Patients fulfilled at least 4 classification criteria (1997 revised criteria[9]) for SLE or had 3 criteria with no other alternative diagnosis. Healthy control subjects were recruited from the secretarial and support staff at the University of Manchester Rheumatism Research Centre and School of Medicine, as well as from friends of patients. Subjects were excluded if they had had infection within the past 4 weeks or had been pregnant or lactating in the previous 6 months. All subjects gave written informed consent to take part in this study, which was approved by the Central Manchester Local Research Ethics Committee.

Clinical and Laboratory Assessment

All subjects had a complete history and physical examination. Classic CHD risk factors were recorded. In patients, disease activity and cumulative damage were assessed by the SLE Disease Activity Index (SLEDAI)[10] and the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI),[11] respectively. In addition, any history of Raynaud's phenomenon or previous thrombosis was sought and recorded. Blood was drawn for measurement of plasma glucose and lipid profile. Antibodies to ds-DNA and cardiolipin were measured with commercially available ELISA kits. The lupus anticoagulant was measured with the dilute Russell Viper Venom Test; C3 and C4 complement levels were also measured.

Measurement of Lipids and Lipoproteins

An ultracentrifugation method was used to remove VLDL cholesterol from the plasma.[12] HDL cholesterol was determined after precipitation of LDL from the resulting supernatant by heparin/Mn^{2+} sulfate.[12] Total serum cholesterol, HDL cholesterol, and infranatant cholesterol were determined by the CHOD-PAP method (ABX Diagnostics). LDL cholesterol was calculated as the difference between infranatant cholesterol and HDL cholesterol. Serum triglycerides were determined by the GPO-PAP method (ABX Diagnostics).

Assessment of Endothelial Function

Endothelial function was assessed with high-resolution B-mode Doppler (ATL HDI 5000 with a 7.4-MHz linear-array transducer) examination of the brachial artery using the protocol described by Celermajer et al.[13] We measured flow-mediated dilation (FMD) in response to reactive hyperemia (endothelium dependent), and endothelium-independent dilation was measured in response to glyceryl trinitrate (GTN). All subjects were studied between 8 and 11 AM after a12-hour overnight fast. They were asked not to smoke on the morning of study and to avoid alcohol for 48 hours. Antihypertensive medications were also omitted for 24 hours before the study. The brachial artery was scanned longitudinally 5 to 15 cm above the antecubital fossa. The scans were recorded on a super VHS videotape for later measurement of resting diameter and blood velocity. A blood pressure cuff was then inflated around the forearm to 300 mm Hg for 4.5 minutes. A further scan recorded from 30 seconds before to 1 minute after cuff release. Measurement of the maximal diameter was taken 45 to 60 seconds after cuff release. After 15 minutes, further measurement was taken at rest and at 3 minutes after sublingual spray of 400 μg GTN. All measurements were taken at the end of diastole coincident with the R-wave on an ECG monitor. Distance measured was from anterior to posterior M lines (media-adventitia interface), and every measurement was taken as an average of 5 consecutive cardiac cycles. The vascular technologist performing the scans was given only the subject's name; no diagnostic information was available. The scans were read in batches every 3 to 4 weeks, and again the technologist was unaware of the diagnostic classification of each subject at the time that the scans were read.

FMD is calculated as follows: 100%×[(postdeflation diameter−resting diameter)/resting diameter]. To assess reproducibility of our technique, we looked at the reliability of reading scans on 2 separate occasions by a single blinded observer. For this, 15 scans from patients or control subjects were chosen at random. The intraclass correlation coefficients for resting diameter and FMD were 0.93 (95% CI, 0.56 to 0.95) and 0.82 (95% CI, 0.30 to 0.97), respectively.

Carotid Artery Intima-Media Thickness and Plaque

Patients but not control subjects also had the intima-media thickness (IMT) of their carotid artery measured. The common carotid artery was scanned longitudinally, and the IMT measurement was taken in the proximal part of the common carotid artery, 1 cm proximal to the carotid bulb as the maximum distance between the intima-lumen and adventitia-media interfaces in areas without carotid plaque.[14] IMT was determined as the average of 6 measurements, 3 each from the left and right common carotid arteries. We also noted the presence or absence of carotid plaques, with plaque being defined using the criteria described by Li et al.[15] The intraclass correlation coefficient for IMT measurements, assessed in 15 subjects on 2 separate occasions 2 weeks apart, was 0.92 (95% CI, 0.84 to 1.00).

Statistical Analysis

We used version 10.1 of the SPSS statistical package. Values are quoted as median (range). Differences between numeric variables were tested with the Mann-Whitney *U* test. Correlation was tested with Spearman's rank-order or Pearson's correlation coefficient. A significance level was set at $P<0.05$. For comparison of categorical variables or percentages, we used Fisher's exact and χ^2 tests when appropriate. Multiple linear regression analysis was used to test for independent associations between FMD and various factors.

Results

Endothelial Function in SLE and Healthy Control Subjects

We studied 62 SLE patients and 38 healthy control subjects. In the patient group, age and disease duration were 48 years (range, 21 to 73 years) and 11 years (range, 1 to 23 years), respectively. Fifty-five patients (88%) were white. The SLEDAI and SDI scores were 2 (range, 0 to 12) and 0 (range, 0 to 4), respectively. Nine patients (14.5%) had an SLEDAI ≥6 at the time of study. Twenty-six patients (42%) had ≥1 item of damage on the SDI. With regard to clinical features, 10 patients (16%) had a history of renal involvement, and 34 (56%) had a history of Raynaud's phenomenon. On autoantibody profiles, 29 patients (46.8%) currently had antibodies to native ds-DNA, and 23 (37.7%) had anti-cardiolipin antibodies (IgG or IgM anticardiolipin and/or lupus anticoagulant) on the day of study. Eight patients (13%) had a previous stroke or transient ischemic attack, and 2 patients (3%) had a previous myocardial infarction. Current therapy included steroids in 31 (50%), antimalarial drugs in 31 (50%), and immunosuppressants in 17 (27%), of whom 13 (21%) were on azathioprine and 4 (6%) were taking methotrexate. In addition, 11 patients were on aspirin, 9 were on nonsteroidal anti-inflammatory drugs (8 on conventional, 1 on COX-2–specific inhibitor), and 3 were on both aspirin and a nonsteroidal anti-inflammatory drug. One patient was on a statin, 10 patients were on hormone replacement therapy, and 3 were on contraceptive pills at the time of study.

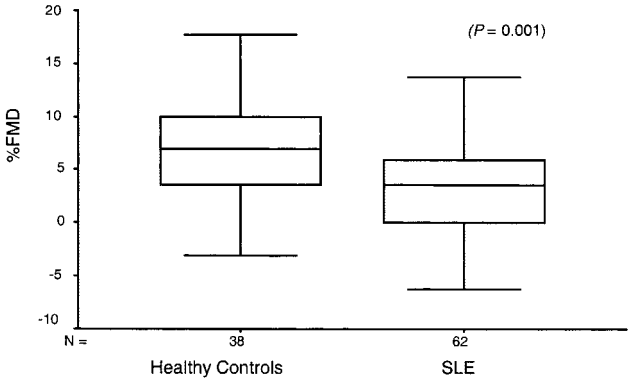

Figure 1. Comparison of FMD in SLE patients and control subjects. Boxes represent IQR; horizontal line inside box is median value. Whiskers extend to the lowest value within 1.5 times IQR below the first quartile and the highest value within 1.5 times IQR above the third quartile.

we retained classic risk factors, menopause, ethnicity, resting diameter, and SLE, independent factors associated with FMD were SBP (B=−0.108; P=0.019) and SLE (B=−2.75; P=0.017) (R^2=0.30, P=0.01) (Table 3). After adjustment for other factors, SLE was associated with a 2.7% lower FMD compared with control subjects, and each increase of 10 mm Hg in SBP was associated with a 1.0% reduction in FMD. A stepwise multiple regression model confirmed these associations.

Endothelial Function in SLE Patients

For regression analysis of only SLE patients, we included demographic details and classic CHD risk factors. We also looked at specific factors associated with the different vascular manifestations of SLE. These included Raynaud's phenomenon, anti-phospholipid antibodies, disease activity (SLEDAI), and early atherosclerosis (carotid IMT). We also included corticosteroid treatment and serum creatinine as additional variables. Neither Raynaud's or anticardiolipin/lupus anticoagulant status was associated with endothelial dysfunction (Figures 2 and 3). Similarly, there was no difference in FMD or percent GTN dilation between patients taking and not taking steroid therapy. The SLEDAI correlated positively with FMD (r=0.37, P<0.01) and negatively with resting diameter (r=−0.33, P=0.01). FMD also correlated negatively with IMT (r=−0.37, P<0.01) (Figure 4). In stepwise multiple regression analysis, we examined those variables that showed a degree of association in the univariate analysis (P<0.2). IMT only was independently associated with FMD (P=0.026) (Table 4). The association of SLEDAI with FMD was no longer significant in this model. After adjustment for other factors, each 0.01-cm increase in IMT is associated with a 0.92% decrease in FMD. Twelve SLE patients (19%) also had carotid plaque. These patients had higher resting diameter (0.36 cm; range, 0.28 to 0.43 cm; versus 0.31 cm; range, 0.24 to 0.39 cm; P=0.014) and a trend

TABLE 3. Multivariate Analysis in 62 SLE Patients and 38 Control Subject Combined Into a Single Group

	B*	95% CI	P
SBP	−0.108	−0.20–−0.02	0.019
DBP	−0.057	−0.053–0.17	0.300
Hypertension	1.12	−1.36–3.51	0.360
Resting diameter	−8.35	−32.6–15.0	0.488
Age	−0.084	−0.21–0.04	0.193
Menopausal state	2.14	−0.64–4.73	0.124
Ethnicity	2.18	−1.2–5.40	0.185
Fasting glucose	0.173	−0.86–1.24	0.744
Total cholesterol	0.271	−1.1–1.68	0.700
HDL cholesterol	−1.41	−3.9–1.10	0.267
LDL cholesterol	−0.282	−1.80–1.34	0.818
Smoking	−0.282	−2.1–1.25	0.709
SLE	−2.75	−4.97–−0.50	0.017

DBP indicates diastolic blood pressure. Multiple coefficient of determination of this model (R^2)=0.30, P=0.01.

*Unstandardized regression coefficient.

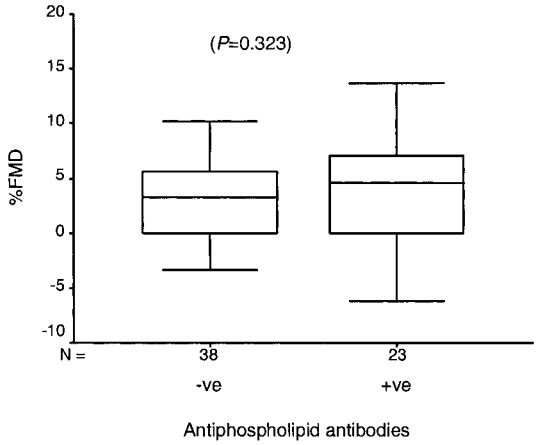

Figure 2. Comparison of FMD in SLE patients with and without anti-phospholipid antibodies. Boxes represent IQR; horizontal line inside box is median value. Whiskers extend to the lowest value within 1.5 times IQR below the first quartile and the highest value within 1.5 times IQR above the third quartile.

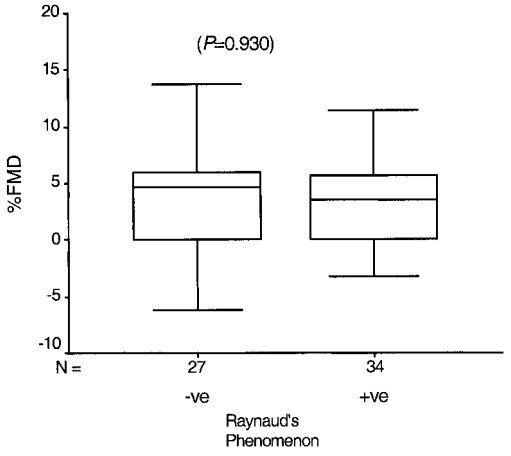

Figure 3. Comparison of FMD in SLE patients with and without a history of Raynaud's phenomenon. Boxes represent IQR; horizontal line inside box is median value. Whiskers extend to the lowest value within 1.5 times IQR below the first quartile and the highest value within 1.5 times IQR above the third quartile.

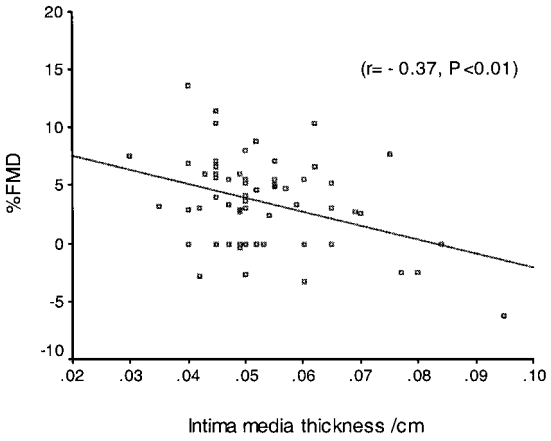

Figure 4. Relationship between FMD and IMT in patients with SLE.

toward lower FMD (2.7%; range, −6.3% to 7.1%; versus 4.7%; range, −3.3% to 13.7%; $P=0.1$).

Discussion

Endothelial dysfunction is believed to represent a widespread phenomenon that occurs at an early stage in the atherogenic process. There is a correlation between endothelial function measured in the brachial and coronary circulations.[16] We have found evidence of endothelial dysfunction in SLE patients that is not fully explained by classic CHD risk factors. Also, within SLE patients, endothelial dysfunction is associated with carotid IMT, an early marker of atherosclerosis. Two other studies have reported similar findings. In a study from Sao Paulo, Lima et al[7] noted that the mean±SD FMD in SLE was 5.0±5.0% compared with 12.0±6.0% in healthy control subjects. In this study, postmenopausal women and subjects with known CHD risk factors were excluded. Piper et al[17] found in a UK cohort that SLE women had a median FMD of 5.6 (interquartile range [IQR], 3.1% to 7.2%) compared with 8.0% (IQR, 6.3% to 9.3%) in control subjects. The differences in subject selection and interlaboratory variations in technique will clearly influence the absolute values between studies. The median percent FMD in our control subjects appears low. Potential reasons for this include the wide age range of control subjects studied and the lack of exclusions based on prevalent CHD risk factors. In a subgroup of premenopausal control subjects, the median percent FMD was 9.1% (IQR, −3.1% to 17.8%), which accords with other studies.[7,17] Nevertheless, endothelial function is significantly impaired in SLE. The role of classic CHD factors in this process is less clear from these studies. Piper et al[17] found a significant negative correlation between total cholesterol and FMD ($r=-0.442$). In contrast, Lima et al[7] found no association with cholesterol or blood pressure, although it should be noted that this study excluded patients with established hypertension or hyperlipidemia. We chose not to exclude patients with known risk factors to examine the full range of association with FMD in SLE. A recent large cohort control study found that SLE patients have differences in several key cardiovascular risk factors. Specifically, SLE patients are more likely to have hypertension and to be postmenopausal at a particular age; they also have more risk factors per patient.[18] In our population, patients also had lower HDL cholesterol. To adjust for these differences, we performed a multivariate analysis on all patients and control subjects. Of the classic risk factors, SBP was most strongly associated with reduced FMD. Each 10–mm Hg increase in SBP was associated with a 0.89% reduction in FMD. Importantly, however, even after adjustment for classic risk factors, SLE remains independently associated with endothelial dysfunction. This finding is important because SLE is associated with an increased risk of CHD compared with the general population. It also accords with the clinical observation by Esdaile et al[5] that even after adjustment for Framingham risk factors, SLE patients still have increased CHD risk. It may also suggest that interventions that improve endothelial function in the context of SLE will have the potential to improve long-term CHD outcomes.

TABLE 4. Stepwise Multiple Regression Analysis for Variables Associated With FMD in SLE Patients

	B*	β†	95% CI of B	P
Independent variable				
IMT	−92.3	−0.305	−173–−11.3	0.026
Excluded variables				
Resting diameter		−0.187		0.177
SBP		−0.190		0.248
Carotid plaque		−0.126		0.375
Fasting glucose		0.038		0.781
LDL cholesterol		0.239		0.081
SLEDAI		0.209		0.173
Hypocomplementemia		0.216		0.114
Serum creatinine		0.025		0.855

Multiple coefficient of determination (R^2)=0.093, $P=0.026$.
*Unstandardized regression coefficient.
†Standardized coefficient.

Multivariate analysis of SLE patients showed that FMD was associated with carotid IMT. Twelve patients had carotid plaque, and in this group, there was also a trend toward lower FMD. We did not measure IMT in our control subjects, but this finding in SLE confirms that, as in the general population, endothelial dysfunction in SLE correlates with other markers of atherosclerosis development. Piper et al[17] found an association of FMD with total cholesterol. We could not confirm this finding, but IMT as an early marker of atherosclerosis will represent the final common pathway of several other risk factors and will make it difficult for any single risk factor to remain in a multivariate analysis. Endothelial function may act in a similar fashion as an integrated index of all atherogenic and atheroprotective factors present in an individual.[19] Interventions that modify atherosclerosis risk factors may therefore be of benefit in SLE.

In univariate analysis, we noted a positive association between disease activity and FMD. It should be noted that SLEDAI was associated with a reduced resting diameter, which itself predicts increased FMD; after adjustment for resting diameter, the effect of SLEDAI was lost. Inflammation itself may be associated with endothelial dysfunction.

This has been suggested by studies of endotoxin-induced experimental inflammation and in patients with primary systemic vasculitis.[20,21] Therefore, it would be reasonable to expect that inflammatory disease activity in SLE should impair endothelial function, but this was not found in this study or in the study by Lima et al.[7] Both of these studies, however, were cross-sectional and included patients mainly with low disease activity. A prospective study of patients in a clinical flare before and after therapy is therefore warranted to study the influence of SLE inflammation on endothelial function. It is still an attractive hypothesis that chronic inflammation is the key factor contributing to atherosclerosis risk in SLE, yet other mechanisms mediating endothelial dysfunction—eg, insulin resistance, hyperhomocysteinemia, or ADMA, a recently described inhibitor of nitric oxide synthase—could be more important in SLE.[22] These require further investigation in the context of SLE.

This study has several limitations. First, the cross-sectional nature may fail to estimate the true magnitude of the contribution of variables such as disease activity and therapy; a prospective study might demonstrate a greater effect over time. Second, this study was underpowered to determine the association of endothelial function with clinical outcomes of relevance. Even so, we did find a significant association with carotid IMT and a trend toward lower FMD in patients with carotid plaque. Carotid IMT is itself a valid surrogate for early atherosclerosis and has value in predicting future CHD events in the general population. This association, as well as the trend to lower FMD in patients with plaque, supports the validity of endothelial dysfunction as a marker of interest, and we plan to follow up our cohort to determine whether endothelial dysfunction does indeed predict future CHD events.

In conclusion, we have found impaired endothelial function in SLE that is not fully explained by classic CHD risk factors. Within SLE patients, endothelial dysfunction is associated with carotid IMT and plaque. Interventions to improve endothelial function in SLE may therefore have an impact on the risk of future CHD. A better understanding of the factors underlying endothelial dysfunction that may be peculiar to SLE is also required to develop novel approaches to reducing the CHD burden in these patients.

Acknowledgment

This study was funded by the arc Epidemiology Unit, University of Manchester, and Research Endowment funds from Central Manchester and Manchester Children University Hospitals NHS Trust, Manchester, UK.

References

1. Manzi S, Meilahn EN, Rairie JE, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham study. *Am J Epidemiol*. 1997; 145:408–415.
2. Bruce IN, Gladman DD, Urowitz MB. Premature atherosclerosis in systemic lupus erythematosus. *Rheum Dis Clin North Am*. 2000;26: 257–278.
3. Manzi S, Selzer F, Sutton-Tyrrell K, et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum*. 1999;42:51–60.
4. Bruce IN, Gladman DD, Ibanez D, et al. Single photon emission computed tomography dual isotope myocardial perfusion imaging in women with systemic lupus erythematosus, II: predictive factors for perfusion abnormalities. *J Rheumatol*. 2002;30:288–291.
5. Esdaile JM, Abrahamowicz M, Grodzicky T, et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum*. 2001;44:2331–2337.
6. Celermajer DS, Sorensen KE, Bull C, et al. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol*. 1994;24: 1468–1474.
7. Lima DS, Sato EI, Lima VC, et al. Brachial endothelial function is impaired in patients with systemic lupus erythematosus. *J Rheumatol*. 2001;29:292–297.
8. Raza K, Thambyrajah J, Townend JN, et al. Suppression of inflammation in primary systemic vasculitis restores vascular endothelial function: lessons for atherosclerotic disease? *Circulation*. 2000;102:1470–1472.
9. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1997;40:1725.
10. Bombardier C, Gladman DD, Urowitz MB, et al. Derivation of the SLEDAI: a disease activity index for lupus patients: the Committee on Prognosis Studies in SLE. *Arthritis Rheum*. 1992;35:630–640.
11. Gladman DD, Urowitz MB, Goldsmith CH, et al. The reliability of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index in patients with systemic lupus erythematosus. *Arthritis Rheum*. 1997;40:809–813.
12. Mackness MI, Durrington PN. Lipoprotein analysis for clinical studies. In: Converse CA, Skinner ER, eds. *Lipoprotein Analysis: A Practical Approach*. Oxford, UK: IRL Press; 1992:1–42.
13. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111–1115.
14. Sidhu PS, Desai SR. A simple and reproducible method for assessing intimal-medial thickness of the common carotid artery. *Br J Radiol*. 1997;70:85–89.
15. Li R, Duncan BB, Metcalf PA, et al. B-mode-detected carotid artery plaque in a general population: Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Stroke*. 1994;25:2377–2383.
16. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235–1241.
17. Piper MJ, Heaton SJ, Gardner-Medwin JM, et al. A study of endothelial function in systemic lupus erythematosus (SLE). *Rheumatology*. 2001; 40(suppl 1):113.
18. Bruce IN, Urowitz M, Gladman DD, et al. A cohort-control study of risk factors for coronary artery disease (CAD) in women with systemic lupus erythematosus (SLE): the Toronto Risk Factor Study. *Arthritis Rheum*. 2003;48:3159–3167.
19. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol*. 2003;23:168–175.
20. Hingorani AD, Cross J, Kharbanda RK, et al. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation*. 2000;102:994–999.
21. Filer AD, Gardner-Medwin JM, Thambyrajah J, et al. Diffuse endothelial dysfunction is common to ANCA associated systemic vasculitis and polyarteritis nodosa. *Ann Rheum Dis*. 2003;62:162–167.
22. Cooke JP. Does ADMA cause endothelial dysfunction? *Arterioscler Thromb Vasc Biol*. 2000;20:2032–2037.

Rheumatology 2007;46:983–988
Advance Access publication 23 March 2007
doi:10.1093/rheumatology/kem002

Subclinical atherosclerosis in systemic lupus erythematosus (SLE): the relative contribution of classic risk factors and the lupus phenotype

Y. Ahmad[1,2], J. Shelmerdine[1], H. Bodill[3], M. Lunt[2], M. G. Pattrick[4], L. S. Teh[5], R. M. Bernstein[1], M. G. Walker[3] and I. N. Bruce[1,2]

Objectives. We aimed to examine the strength of association between traditional cardiovascular risk factors and carotid plaque development in systemic lupus erythematosus (SLE) patients and controls. We also aimed to determine which lupus-related factors are associated with carotid plaque and whether SLE sensitizes patients to the effects of traditional factors.
Methods. We studied 200 women with SLE and 100 controls. Demographic and risk factor data were collected and SLE features, including autoantibody profiles and therapy were noted. All subjects had B- mode ultrasound of their carotid arteries examined for the presence and distribution of plaque.
Results. SLE patients <55 years old had more plaque (21% *vs* 3% $P < 0.01$) and more SLE patients had plaque in the internal carotid artery (11% *vs* 4%; $P < 0.05$). Traditional risk factor models performed less well in SLE compared with controls [area under Receiver Operator Characteristic curves (AUC ROC) = 0.76 *vs* 0.90; $P < 0.01$]. A multivariable model using SLE factors only, performed significantly better (AUC ROC = 0.87; $P < 0.01$). The final model in SLE included age and cigarette pack-years smoking as well as azathioprine exposure ever, antiphospholipid antibodies (APLA) and previous arterial events (AUC ROC = 0.88).
Conclusions. SLE patients have a higher prevalence and different distribution of carotid plaque than controls. SLE factors perform significantly better than traditional risk factors in their association with atherosclerosis in SLE and these factors add to the influence of traditional risk factors rather than sensitizing lupus patients to traditional factors. The SLE phenotype helps identify patients at increased risk of atherosclerosis.

KEYWORDS: Systemic lupus erythematosus, atherosclerosis, risk factors, smoking.

Introduction

Premature coronary heart disease (CHD) is a major cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE) and in younger women, the excess risk may be >50-fold [1]. SLE patients also have an increased prevalence of subclinical atherosclerotic disease detected using several modalities [2–4]. Hypertension and diabetes mellitus are also more prevalent in SLE [5] and traditional risk factors, e.g. hypercholesterolaemia, contribute to the development of atherosclerosis in SLE [1, 2, 6]. However, these risk factors alone do not explain the excess CHD risk and after adjusting for traditional risk factors, SLE itself remains independently associated with both clinical and subclinical outcomes [2, 4, 7]. Several lupus-related factors may contribute to the development of accelerated atherosclerosis, for example, there is growing evidence that atherosclerosis itself has a chronic inflammatory component [8]. In addition to chronic inflammation, patients with SLE frequently have lupus anticoagulant (LAC) or associated antiphospholipid antibodies (APLA) and there is *in vitro* evidence that certain APLA may be pro-atherogenic as well as pro-thrombotic [9]. Corticosteroid therapy has also been associated with clinical and subclinical disease in several studies [1, 6]. However, more recently Roman *et al.* [2] suggested that less steroid exposure was associated with carotid plaque.

The aim of this current study was to assess the prevalence of carotid plaque in an ethnically homogenous cohort of women with SLE compared with healthy controls. We specifically aimed to compare the strength of association between traditional CHD risk factors and carotid plaque development in patients and controls. We also aimed to determine which lupus-related factors are associated with carotid plaque and assess whether SLE sensitizes patients to the effects of traditional factors or whether any SLE effect is additive to the risk of atherosclerosis development.

Patients and methods

The study was approved by the North West Multi-Centre Research Ethics Committee and written informed consent was obtained from each participant.

SLE patients

SLE patients were recruited from out-patient clinics in Manchester Royal Infirmary, North Manchester General Hospital, Blackburn Royal Infirmary, South Manchester University Hospital and Stepping Hill Hospital, Stockport. We included SLE patients >18 yrs old who fulfilled ≥4 1997 updated ACR criteria for SLE [10] and also patients with three criteria for SLE in the absence of any alternative diagnosis. All cases were white Caucasian females of British Isles descent; we limited recruitment to this ethnic group as we were also collecting DNA for future genetic studies. Patients were on stable therapy for at least 2 months and we excluded women who were pregnant or lactating mothers within 6 months. Patients underwent a clinical interview and examination according to a standard protocol that included demographic information, family history and lifestyle factors. We also assessed the presence of traditional coronary risk factors, anthropomorphic measures and history of previous

[1]The University of Manchester, Rheumatism Research Centre, [2]ARC Epidemiology Unit, and [3]Department of Vascular Surgery, Manchester Royal Infirmary, Central Manchester and Manchester Children's University Hospitals NHS Trust, Oxford Road, Manchester, [4]North Manchester General Hospital, Crumpsall Road, Manchester and [5]Blackburn Royal Infirmary, Blackburn, Lancashire, UK.

Submitted 21 June 2006; revised version accepted 3 January 2007.

Correspondence to: I. N. Bruce, MD, FRCP, Reader in Rheumatology, University of Manchester, Rheumatism Research Centre, Central Manchester and Manchester Children's University Hospitals NHS Trust, Oxford Road, Manchester M13 9WL, UK. E-mail: ian.bruce@manchester.ac.uk

Therefore our key finding of a differential influence of traditional and lupus factors is likely to be generalizable to other SLE populations. The other key limitation is the cross-sectional nature of the study that limits our ability to identify the direction of association of some of these factors with carotid plaque. This is particularly true for azathioprine exposure and antiphospholipid antibodies; prospective follow-up of this population is now underway.

In this study of subclinical atherosclerosis in SLE, we have therefore found an increased prevalence of plaque occurring at a younger age and starting at a lower carotid IMT in patients compared with ethnically matched controls. There was also a higher prevalence of plaque in the internal carotid artery in SLE patients. The strength of association between traditional risk factors and plaque was significantly stronger in controls than patients. A statistical model employing only lupus-related factors showed a much stronger association with plaque in SLE and knowledge of these factors may help identify patients at increased risk of atherosclerosis in SLE. Our study also demonstrates that lupus adds to the influence of traditional risk factors rather than simply sensitizing lupus patients to classic risk factors. Addressing classic risk factors and SLE-related factors will therefore both be required to reduce the burden of atherosclerosis in SLE. Prospective studies are now needed to examine the mechanism by which these factors mediate plaque development in lupus and these may suggest novel preventative strategies for this population.

Acknowledgements

The authors would like to acknowledge the help and support of Drs Neil Snowden, Beverley Harrison, Patricia Smith, Jeff Marks, Paul Sanders, Faieza Qasim, Colin Short and Mike Venning for helping provide patients for this study. We also acknowledge the support of the Wellcome Trust Clinical Research Facility, Central Manchester Health Care Trust for hosting the study and we acknowledge the funding support of the Arthritis Research Campaign, The Wellcome Trust and Lupus, UK.

The authors have declared no conflicts of interest.

References

1 Manzi S, Meilahn EN, Rairie JE *et al.* Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. Am J Epidemiol 1997;145:408–15.
2 Roman MJ, Shanker BA, Davis A *et al.* Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. N Eng J Med 2003;349:2399–406.
3 Bruce IN, Burns RJ, Gladman DD, Urowitz MB. Single photon emission computed tomography dual isotope myocardial perfusion imaging in women with systemic lupus erythematosus. I. Prevalence and distribution of abnormalities. J Rheumatol 2000;27:2372–7.
4 El Magadmi M, Bodill H, Ahmad Y *et al.* Systemic lupus erythematosus: an independent risk factor for endothelial dysfunction in women. Circulation 2004;110:399–404.
5 Bruce IN, Urowitz MB, Gladman DD, Ibanez D, Steiner G. Risk factors for coronary heart disease in women with systemic lupus erythematosus: the Toronto Risk Factor Study. Arthritis Rheum 2003;48:3159–67.
6 Petri M, Perez-Gutthann S, Spence D, Hochberg MC. Risk factors for coronary artery disease in patients with systemic lupus erythematosus. Am J Med 1992; 93:513–9.
7 Esdaile JM, Abrahamowicz M, Grodzicky T *et al.* Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. Arthritis Rheum 2001;44:2331–7.
8 Hansson GK. Inflammation, atherosclerosis and coronary artery disease. N Eng J Med 2005;352:1685–95.
9 Hasunuma Y, Matsuura E, Makita Z, Katahira T, Nishi S, Koike T. Involvement of beta 2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. Clin Exp Immunol 1997;107:569–73.
10 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
11 Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol 2002;29:288–91.
12 Gladman DD, Urowitz MB, Goldsmith CH *et al.* The reliability of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index in patients with systemic lupus erythematosus. Arthritis Rheum 1997;40:809–13.
13 Wilson WA, Gharavi AE, Koike T *et al.* International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arthritis Rheum 1999;42:1309–11.
14 Sidhu PS, Desai SR. A simple and reproducible method for assessing intimal-medial thickness of the common carotid artery. Br J Radiol 1997;70:85–9.
15 Li R, Duncan BB, Metcalf PA *et al.* B-mode-detected carotid artery plaque in a general population. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Stroke 1994;25:2377–83.
16 Hanley JA, McNeill BJ. The meaning and use of the area under a receiver operating characteristic 'ROC' curve. Radiology 1982;143:29–36.
17 DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.
18 Durrington PN. Risk in cardiovascular disease. Joint British Societies recommend their computer program for risk calculations. Br Med J 2000;321:174–5.
19 Asanuma Y, Oeser A, Shintani AK *et al.* Premature coronary-artery atherosclerosis in systemic lupus erythematosus. N Eng J Med 2003;349:2407–15.
20 Svenungsson E, Jensen-Urstad K, Heimburger M *et al.* Risk factors for cardiovascular disease in systemic lupus erythematosus. Circulation 2001;104: 1887–93.
21 Doria A, Shoenfeld Y, Wu R *et al.* Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. Ann Rheum Dis 2003;62:1071–7.
22 Toloza SM, Uribe AG, McGwin G, Jr *et al.* Systemic lupus erythematosus in a multiethnic US cohort (LUMINA). XXIII. Baseline predictors of vascular events. Arthritis Rheum 2004;50:947–57.
23 Kobashigawa J, Miller L, Renlund D *et al.* A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipents. Transplantation 1998; 66:507–15.
24 Lahita RG, Rivkin E, Cavanagh I, Romano P. Low levels of total cholesterol, high-density lipoprotein, and apolipoprotein A1 in association with anticardiolipin antibodies in patients with systemic lupus erythematosus. Arthritis Rheum 1993;36:1566–74.
25 Delgado Alves J, Kumar S, Isenberg DA. Cross-reactivity between anti-cardiolipin, anti-high-density lipoprotein and anti-apolipoprotein A-I IgG antibodies in patients with systemic lupus erythematosus and primary antiphospholipid syndrome. Rheumatology 2003;42:893–9.
26 Ames PR, Margarita A, Delgado Alves J, Tommasino C, Iannaccone L, Brancaccio V. Anticardiolipin antibody titre and plasma homocysteine level independently predict intima media thickness of carotid arteries in subjects with idiopathic antiphospholipid antibodies. Lupus 2002;11:208–14.
27 Nicolo D, Goldman BI, Monestier M. Reduction of atherosclerosis in low-density lipoprotein receptor-deficient mice by passive administration of antiphospholipid antibody. Arthritis Rheum 2003;48:2974–8.
28 Van Oostrum AJHHM, van Wijk JPH, Cabezas MC. Lipaemia, inflammation and atherosclerosis: novel opportunities in the understanding and treatment of atherosclerosis. Drugs 2004;64:19–41.
29 Huang ZS, Jeng JS, Wang CH, Yip PK, Wu TH, Lee TK. Correlations between peripheral differential leukocyte counts and carotid atherosclerosis in non-smokers. Atherosclerosis 2001;158:431–6.
30 Selzer F, Sutton-Tyrrell K, Fitzgerald SG, Pratt JE, Tracy RP, Kuller LH, Manzi S. Comparison of risk factors for vascular disease in the carotid artery and aorta in women with systemic lupus erythematosus. Arthritis Rheum 2004;50:151–9.
31 Tell GS, Howard G, McKinney WM. Risk factors for site specific extracranial carotid artery plaque distribution as measured by B-mode ultrasound. J Clin Epidemiol 1989;42:551–9.
32 Crouse JR, Goldbourt U, Evans G *et al.* Risk factors and segment-specific carotid arterial enlargement in the Atherosclerosis Risk in Communities (ARIC) cohort. Stroke 1996;27:69–75.