Lupus-associated endothelial dysfunction, disease activity and arteriosclerosis

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Abstract
Objective
To analyze endothelial function in systemic lupus erythematosus (SLE), and its relationship with disease activity and subclinical arteriosclerosis.

Methods
We studied a group of 26 patients with SLE and 21 age- and sex-matched controls. None of the patients or controls had had any ischemic event. Data were recorded on medical history, anthropometrics, prior treatment and the lupus activity index (LAI). Endothelial function was quantified by flow-mediated dilatation in the brachial artery. The presence of subclinical arteriosclerosis was assessed by the average intima-media thickness (IMT) on carotid ultrasound.

Results
The patients and the controls had a similar degree of carotid IMT (0.58±0.08 mm vs. 0.57±0.07 mm, NS) and a similar prevalence of carotid plaque (27% vs. 24%, NS). However, the SLE patients had worse endothelial function than the controls (FMD 12.4±4.4% vs. 16.9±5.5%, p<0.05). This difference remained after adjusting for age, smoking, body mass index, waist circumference, total cholesterol, triglycerides, HDL cholesterol, apolipoproteins A-I and B100 and postmenopausal status. A significant association was found in the SLE patients between FMD and LAI (Spearman Rho -0.462, p<0.05).

Conclusion
SLE-associated endothelial dysfunction is present in patients who have no prior ischemic events and with the same degree of subclinical arteriosclerosis as controls. The endothelial dysfunction is significantly associated with the degree of disease activity.

Key words
Systemic lupus erythematosus, endothelial disfunction, carotid intima-media thickness, lupus activity index.
Endothelial function and atherosclerosis in lupus / P. Valdivielso et al.

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Introduction

Systemic lupus erythematosus (SLE) is associated with an increased risk for cardiovascular disease as compared with the general population of the same age and sex (1, 2). In fact, cardiovascular disease is the leading cause of death in SLE patients (3). This excess of cardiovascular disease in SLE patients has been attributed to a greater prevalence of the traditional risk factors (hypertension, dyslipidemia, obesity, sedentary life-style) (4, 5), as well as to the characteristic inflammatory background of the disease (6, 7).

The endothelium is a key element in the regulation of vascular homeostasis and its alteration is a precursor of vascular disease (8). Endothelial dysfunction is an inherent feature in most vascular risk factors and established arteriosclerosis (9-13). Of the methods available for the clinical assessment of endothelial function, flow-mediated dilation (change in brachial artery diameter) is currently the standard for noninvasive assessment of conduit artery endothelial function because of the considerable experience with clinical trials and its validation, as well as a firm link to biology and an association with vascular events (14).

Endothelial function is altered in SLE patients (15, 16), especially when coronary disease already exists (17, 18). As in the general population (19), the presence of subclinical arteriosclerosis, quantified as intima-media thickness (IMT), is an independent predictor of endothelial dysfunction in SLE patients (20). Arteriosclerosis is a systemic disease that, except in cases of stable angina or intermittent claudication, does not manifest itself until the appearance of a thrombotic event. The exclusion of persons with ischemic clinical events does not therefore guarantee the absence of subclinical vascular disease. Hence the importance of non-invasive diagnostic techniques such as carotid ultrasound. Whether this endothelial function, assessed as flow mediated dilation (FMD) in SLE patients, is due to the presence of greater subclinical vascular disease remains to be established.

The aim of this study was to evaluate the endothelial function in SLE patients and controls free of known vascular disease, and its association with subclinical arteriosclerosis, determined by the presence of plaque in the carotid tree, and the level of disease activity.

Subjects

All the SLE patients were recruited from the Rheumatology Unit of Virgen de la Victoria Hospital, in Malaga, Spain. They fulfilled the revised criteria of the ACR for the diagnosis of the disease (21). The controls were selected from age- and sex-matched persons who were free of systemic disease symptoms and had no family history of SLE. Patients or controls were excluded if they had had prior vascular disease in any territory (coronary, cerebral or peripheral), had thrombocytopenia (present level <70000/mm³), any disorder predisposing to hemorrhage, Raynaud’s phenomenon, digital ischemia or being treated with statins or other lipid-lowering drugs. No patient or control had diabetes. Patients with secondary antiphospholipid syndrome were included just in case of fetal losses or venous thrombosis.

All the SLE patients and the controls signed the consent form. The study was approved by the Ethics and Research Committee of Virgen de la Victoria Hospital, Malaga.

The study included a structured, medical interview, a physical examination performed by a rheumatologist, laboratory analyses, and, for the patients, a review of their clinical history and the database. Historical data were also recorded on clinical manifestations and cumulative laboratory analyses, treatments received to date, and the number of flares experienced during follow-up. Data were recorded at the time of study on clinical manifestations and the last treatment. For corticoids, in addition to noting the last dose, we also recorded the cumulative and mean doses over the previous six months. Lupus activity index (LAI) and SLEDAI were recorded in the last 15 days of the study.

Cardiovascular risk factors were recorded for both patients and controls during the interview. Blood pressure was measured as the average of two measurements separated by at least 5 minutes. High blood pressure was defined

Competing interests: none declared.
according to the criteria of the Sixth Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (22). Hypercholesterolemia was considered to be present when LDL cholesterol levels were greater than 160 mg/dl, hypertriglyceridemia if total triglycerides surpassed 150 mg/dl, and HDL cholesterol values were defined as low when less than 50 mg/dl, according to international criteria (23). Anthropometric measurements included waist and hip circumferences, height and weight, and calculation of the body mass index (BMI) as the ratio of weight in Kg to the square of the height in meters. A BMI greater than 30 was considered to indicate obesity.

**Laboratory tests**

A blood sample was drawn between 8:30 and 9:30 AM after 12 hours fasting by venopuncture from the forearm of all subjects, both patients and controls, using vacuum tubes. An automated blood chemistry profile was performed (glucose, urea, creatinine, ions, transaminases, creatinine kinase and lactic dehydrogenase), as well as a full blood count, and measurements of the erythrocyte sedimentation rate (ESR), C reactive protein (CRP), basic coagulation, protein panel and antinuclear antibodies (ELISA ANA Screening Corméda SA, Palex Group).

In addition, the C3 and C4 fractions of the serum complement, fresh urine sediment, and routine urinalysis were performed in the patients. The anti-double-stranded DNA was assessed by Crichtid lucullae kinetoplast assay. IgG and IgM anti-cardiolipin (aCL) antibodies were measured by ELISA (Reads Anti-Cardioliplin Test Kit) and levels were deemed to be positive if they were greater than 40 GPL for IgG and 25 for IgM. Lupus anticoagulant (LA) was measured using an activated partial thromboplastin time-sensitive test (Dade Actin FSL Activated Thromboplastin Time Reagent) and the Russell Viper Venom Screen (LA 1 screening reagent and LA 2 screening reagent, Dade Behring).

**Plasma lipoproteins**

A full lipoprotein analysis was performed in both patients and controls. The plasma, obtained from blood anticoagulated with EDTA-K3 (1.2 mg/ml) and following centrifugation at 3,000 rpm for 15 minutes, was stored in plastic tubes with Sodium Azide (1 mg/ml) at 4°C until subsequent processing. Plasma lipoproteins were separated in accordance with the standard Lipid Research Clinic method: the VLDL were isolated by ultracentrifugation for 18 h at 10°C, 40,000 rpm in a Beckman L8-70 ultracentrifuge; the remainder, containing LDL and HDL, underwent precipitation with heparin-manganese (24), thereby obtaining a supernatant containing HDL. The plasma lipids and various lipoprotein fractions (VLDL, LDL, and HDL) were measured using enzymatic techniques (Unimate, Roche) and following reagent calibration and control (ApoA1 SPQ II Test System Antibody Reagent Set DiaSorin and ApoB SPQ II Test System Antibody Reagent Set DiaSorin). Values were established using a Cobas Mira autoanalyzer (Roche).

**Carotid ultrasound**

This was done using a Toshiba Power Vision 6000 SSA-370 A device equipped with a PLM 703 linear 6-11 MHz/32 mm transducer at 9 MHz frequency for the evaluation of the carotid arteries and focus centered on the intimal line. Each ultrasound recording was stored digitally and IMT measurements were analyzed using VISILOG 6.0 (Noesis, Velizy, France) in the University of Malaga Digital Imaging Laboratory. IMT was measured bilaterally and in triplicate at the level of a) the deep wall of the common carotid artery, b) the superficial wall of the common carotid artery, c) the carotid bulb and d) the internal carotid artery. Each measurement was of immunoturbidimetric analysis, following reagent calibration and control (ApoA1 SPQ II Test System Antibody Reagent Set DiaSorin). Values were established using a Cobas Mira autoanalyzer (Roche).

**Table I. Clinical and anthropometric data (shown as mean ± SD).**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women/Men</td>
<td>20/1</td>
<td>25/1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 ± 9</td>
<td>34 ± 10</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.2 ± 3.1</td>
<td>25.8 ± 5.1</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82 ± 7</td>
<td>81 ± 12</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.83 ± 0.04</td>
<td>0.83 ± 0.05</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113 ± 15</td>
<td>115 ± 19</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68 ± 9</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>Current smokers n (%)</td>
<td>7 (33%)</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>Obesity n (%)</td>
<td>2 (9%)</td>
<td>6 (23%)</td>
</tr>
<tr>
<td>Hypertension n (%)</td>
<td>2 (9%)</td>
<td>6 (23%)</td>
</tr>
<tr>
<td>Low HDL cholesterol n (%)</td>
<td>11 (52%)</td>
<td>13 (50%)</td>
</tr>
<tr>
<td>Mean steroid dose (mg)</td>
<td>ND</td>
<td>5.11 ± 1.23</td>
</tr>
<tr>
<td>LAI</td>
<td>ND</td>
<td>0.52 ± 0.40</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>ND</td>
<td>5.58 ± 8.60</td>
</tr>
<tr>
<td>Anti-phospholipid Ab</td>
<td>ND</td>
<td>5 (19%)</td>
</tr>
<tr>
<td>Anti-phospholipid syndrome</td>
<td>ND</td>
<td>5 (12%)</td>
</tr>
</tbody>
</table>

*p<0.05  
ND: not done or not applicable.

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**Thromboplastin Time Reagent**

Plasma lipoproteins and the residual lipoproteins after separation of HDL were measured using the plasma collected, by means of immunoturbidimetric analysis, following reagent calibration and control (ApoA1 SPQ II Test System Antibody Reagent Set DiaSorin). Values were established using a Cobas Mira autoanalyzer (Roche).
recorded in mm and the mean of all the measurements (a total of 24 measurements for each case and control) was recorded as the mean IMT, as pooling measurements across artery walls often yields the most efficient analyses (25). Plaque was considered present when the IMT>1 mm. When present, its volume was also measured.

**Endothelial function**

Brachial endothelial function was studied with an Acuson Aspen® (Acuson, Mountain View, CA) equipped with a 7.0 MHz linear transducer, as described (9, 26). The diameter of the brachial artery was measured in two-dimensional mode. Recordings were made at rest, during reactive hyperemia and again at rest. Longitudinal sections of the brachial artery were recorded 2-15 cm above the elbow. The first recording was made at rest and arterial flow velocity was measured using the Doppler signal. Increased flow was induced by means of a pneumatic tourniquet around the arm (pressure of 250 mmHg for 5 min), which was then deflated. A second recording was obtained for 90 s after deflation, measuring the flow velocity in the first 15 s. After a 10-15 min wait for vascular recovery, a further recording was made at rest. A sublingual dose of 400 µg nitroglycerin was given and 3-4 min later the final recording was made to measure the dilatation independent of the endothelium, measured by nitrates (DIE).

The vascular diameter was measured by an independent observer blinded to the clinical characteristics of the patient. The measurements were obtained from the anterior to the posterior end-diastolic “m” line, coinciding with the R wave of the simultaneous electrocardiographic recording. For reactive hyperemia, the diameters were measured 45-60 s after deflating the cuff. The vascular diameter after the reactive hyperemia and the administration of nitroglycerin were expressed as the percentage of cases or as mean ± standard deviation. Comparisons between groups were studied with the Student t-test. Comparisons between qualitative variables were studied with the χ² test. The association between two continuous variables was measured by Spearman’s correlation coefficient. The General Linear Model was used to adjust the analysis between the SLE and the control groups for co-variables. Statistical significance was set at p<0.05. The statistical program used was SPSS 14.0 (Chicago, IL).

**Results**

Table I shows the clinical and anthropometric data for the 26 SLE patients and 21 controls who fulfilled the study criteria. Overall, the SLE patients had the disease for less than a mean of 10 years and had a low disease activity. Most were receiving low dose steroids and hydroxychloroquine. Compared with the controls, the patients more often had dyslipidemia and hypertension and were more frequently overweight.

Table II shows the biochemical characteristics. The SLE patients had notably higher CRP and ESR than the controls, but lower hemoglobin and leukocyte levels. Compared with the controls, the SLE patients had markedly higher levels of total triglycerides, cholesterol and VLDL triglycerides, HDL triglycerides, apolipoprotein B100 and a triglycerides/HDL cholesterol ratio.

Table III summarizes the main results of the study. The endothelial function was significantly reduced in the SLE patients as compared with the controls (Fig. 1), although no differences were detected in endothelial-independent arterial dilatation, in the carotid IMT, or in the presence or severity of carotid atheroma. The differences in FMD between the patients and the controls remained significant after adjusting for

| Biochemical data and lipoprotein profile (shown as mean ± SD). |
|------------------|------------------|
| **Hemoglobin (g/L)* | 13.9 ± 1.1 |
| **Leucocytes (cells/mm³)* | 6609 ± 1862 |
| **C₁ (mg/dL) ND | 114 ± 36 |
| **C₂ (mg/dL) ND | 20 ± 8.6 |
| **Positive anti-DNA ND | 4 (15%) |
| **Glycemia (mg/dL) 92 ± 10 | 89 ± 12 |
| **Urea (mg/dL) 31 ± 6 | 37 ± 23 |
| **Creatinine (mg/dL) 0.78 ± 0.13 | 0.84 ± 0.22 |
| **CRP (mg/dL)* 0.26 ± 0.29 | 0.80 ± 0.91 |
| **ESR (mm)* 10.7 ± 4.3 | 22.3 ± 22.3 |
| **Cholesterol (mg/dL) 203 ± 32 | 250 ± 82 |
| **Triglycerides (mg/dL) 88 ± 23 | 151 ± 64 |
| **Apo A1 (mg/dL) 124 ± 22 | 157 ± 41 |
| **Apo B100 (mg/dL)* 71 ± 16 | 84 ± 22 |
| **Lp(a) (mg/dL) 22.6 ± 15.6 | 28.4 ± 19.3 |

* p<0.05
ND: not done or not applicable.
age, smoking, BMI, waist circumference, total cholesterol, triglycerides, HDL cholesterol, apolipoproteins A-I and B100, and postmenopausal status. The FMD was significantly correlated in both groups and inversely correlated with BMI (Spearman’s Rho - 0.462), waist circumference (Spearman’s Rho - 0.462), and VLDL cholesterol (Spearman’s Rho - 0.327) and triglycerides (Spearman’s Rho - 0.407). No correlation was seen between FMD and other study variables, such as age, IMT, CRP, ESR or lipid variables. No association was found in the SLE patients between FMD and IMT, although there was an association between LAI and FMD (Spearman’s Rho-0.479, p<0.05) (Fig. 2) and a marked tendency for association between SLEDAI and FMD (Spearman’s Rho - 0.380, p=0.073). Among patients, FMD was lower in those taking steroids (11.3±4% vs. 15.1±4%, p=0.066) or immunosuppressants (8.5±3% vs. 14.0±4%, p<0.05). No changes in FMD was found among those treated with hydroxychloroquine.

Discussion
The results of this study clearly indicate that patients with SLE have an anomaly in their endothelial function, in agreement with previous results from other groups (15-18, 20). The cause, or causes, of this poor endothelial function in SLE patients remain unclear. One explanation may be the arteriosclerosis itself; given the systemic nature of arteriosclerosis, a sick vascular bed is a dysfunctional bed (29). In SLE, Johnson et al. showed a marked reduction in the FMD in 5 women with SLE and symptomatic ischemic heart disease as compared with SLE patients with silent ischemic involvement or no vascular involvement (17). In the study by El Magadmi et al., endothelial function was reduced by 50% in 62 premenopausal women with SLE in comparison with 38 age- and sex-matched controls, although 16% of the sample had already had an ischemic event, mostly cerebrovascular (20). Likewise, one out of every four patients in the study by Kiss et al. had already had an arteriosclerotic ischemic event (18). Only two studies (15, 16) analyzed endothelial function in SLE patients excluding patients with a history of angina or myocardial infarction; however, neither of these studies looked for the presence of subclinical arteriosclerosis in either patients or controls. The subject of this study is of great significance because subclinical involvement of the carotid and the coronary arteries in SLE is very common. Indeed, the presence of atheroma plaque in the carotid occurs in 9%-37% of women with SLE when they are examined with ultrasound (30-32). Myocardial perfusion defects studied by scintigraphy

<table>
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<tr>
<th>Carotid ultrasonography and endothelial function in controls and SLE patients (shown as mean ± SD).</th>
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<tbody>
<tr>
<td>Controls</td>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Average carotid IMT (mm)</td>
</tr>
<tr>
<td>Carotid plaque n (%)</td>
</tr>
<tr>
<td>Plaque volume (μ)</td>
</tr>
<tr>
<td>FMD (%)</td>
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<td>FIND (%)</td>
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*p<0.05
have been shown in 28% of women with SLE (33) and calcification of the coronary arteries using electron-beam computed tomography in 30% of patients with SLE (34). Our study was designed to eliminate this bias. The SLE patients and the controls, unlike what was initially expected, had the same mean IMT and the same prevalence of plaque. This may be partly due to exclusion of patients who had had a clinical event, as the presence of plaque in the carotid arteries correlates well with the presence and severity of lesions in other territories (35). The marked difference in FMD between the SLE patients and the controls cannot therefore be attributed to a greater atherosclerotic burden in the former, and other causes must thus be sought.

Of the factors analyzed in the two groups, FMD was significantly associated with the BMI, waist circumference, increased cholesterol lipids and VLDL triglycerides. These findings are not surprising because fat distribution is one of the determinant factors of FMD in the healthy population, probably due to its association with inflammatory markers (IL-6, TNF-α, CRP) and insulin resistance (36). Nonetheless, the fat distribution does not appear to explain the differences in FMD between the two groups. Although the SLE patients were exposed to a situation of greater inflammation, as demonstrated by the higher levels of CRP and ESR than the controls, no evidence was found for a significant association in the SLE patients between these two variables and FMD. The greater presence in the SLE patients of dyslipidemia could have contributed, at least partly, to the different endothelial function observed. However, the most interesting result was the inverse relation between the degree of lupus activity, measured with the LAI and SLEDAI nearest to the analysis of endothelial function, and FMD. The finding of lower FMD in patients taking steroids or immunosuppressants probably reflects higher disease severity and activity. Previous studies on endothelial function and lupus have either failed to examine this association (17), found it absent (15) or found it positive but not significantly so (20).

It seems reasonable to assume that disease activity is related negatively with FMD, even though the SLE patients were selected from among patients with no known previous vascular disease and with a low atherosclerotic burden, as evidenced in the carotid study. As mentioned earlier, the selection criteria make our study of SLE patients different to other studies.

Despite the consistency of our results, the study does have certain limitations that may weaken its conclusions. The first concerns the number of cases and controls, but this is justified by the strict exclusion criteria as the study involved just one center. The second limitation relates to the fact that our evaluation of asymptomatic arterial disease was restricted to the carotid tree. No non-invasive study was undertaken of the coronary or peripheral vascular territories. It is possible, though very unlikely, that the number of patients or controls with atheroma plaque would have been greater if other vascular territories had been included. In summary, our study reinforces the idea that anomalies of endothelial function in patients with SLE are independent of clinical and subclinical atherosclerosis and at least partly associated with the degree of disease activity.

Reference


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