Risk Factors for Cardiovascular Disease in Systemic Lupus Erythematosus

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Background—Cardiovascular disease (CVD) is overrepresented in patients with systemic lupus erythematosus (SLE). We determined the prevalence of traditional and nontraditional risk factors for CVD in SLE patients with and without CVD compared with controls.

Methods and Results—Twenty-six women (aged 52±8.2 years) with SLE and a history of CVD (SLE cases) were compared with 26 age-matched women with SLE but without manifest CVD (SLE controls) and 26 age-matched population-based control women (population controls). Common carotid intima-media thickness (IMT) was measured by B-mode ultrasound as a surrogate measure of atherosclerosis. SLE cases had increased IMT compared with SLE controls (P=0.03) and population controls (P<0.001), whereas IMT of SLE controls did not differ from population controls. SLE cases had raised plasma concentrations of circulating oxidized LDL (OxLDL; P=0.03), as measured by the monoclonal antibody EO6, and autoantibodies to epitopes of OxLDL (P=0.001); dyslipidemia with raised triglycerides (P<0.001) and lipoprotein(a) (P=0.002) and decreased HDL-cholesterol concentrations (P=0.03); more frequent osteoporosis (P=0.03); and a higher cumulative prednisolone dose (P=0.05) compared with SLE controls. Disease duration, smoking, blood pressure, body mass index, and diabetes mellitus did not differ significantly between the groups.

Conclusions—A set of distinct CVD risk factors separate SLE cases from SLE controls and population controls. If confirmed in a prospective study, they could be used to identify SLE patients at high risk for CVD in order to optimize treatment. (Circulation. 2001;104:1887-1893.)

Key Words: cardiovascular diseases ■ risk factors ■ atherosclerosis ■ ultrasonics

Systemic lupus erythematosus (SLE) is a systemic inflammatory disease that mainly affects women. Although treatment has improved during recent decades, patients with SLE appear to have increased morbidity and mortality from cardiovascular disease (CVD).1

See p 1876

Among established risk factors for atherosclerosis, only dyslipoproteinemia has been demonstrated in SLE, in which enhanced plasma triglycerides and decreased HDL levels have been described while the LDL concentration is similar to that of controls in most patients.2 Lipoprotein(a) (Lp(a)) has also been reported to be elevated in SLE.3 Hypertension is not generally present in SLE, although it may be a feature of SLE nephritis. Comparatively little is known about diabetes mellitus in SLE, although both chronic inflammation4 and steroid treatment may be associated with diabetes.

Inflammation is a prominent feature of atherosclerotic lesions,5 and systemic inflammation, as reflected by a raised serum concentration of C-reactive protein (CRP), is associated with enhanced risk of CVD.6 Oxidized LDL (OxLDL) plays an important role in atherogenesis and may contribute to the immune activation and inflammation present in the atherosclerotic lesions, because it has chemotactic, immune-stimulatory, and toxic properties and is taken up by macrophages and other cells in the atherosclerotic plaque, which develop into foam cells.7,8 To elucidate the relationship between SLE and arterial disease and possible causes of the increased risk of CVD present in SLE, we studied the prevalence of traditional and nontraditional risk factors for CVD in SLE patients with and without CVD compared with controls.
The investigation included a written questionnaire, an interview, and a physical examination by a rheumatologist; laboratory determinations; and blinded ultrasound examination of the carotid arteries. SLE disease activity was determined with the Systemic Lupus Activity Measure (SLAM).\(^9\) Organ damage was determined with the Systemic Lupus International Collaborating Clinics (SLICC) damage index.\(^1,1\) Osteoporosis was considered present if osteoporotic fractures had occurred or bone mineral density measurement was clinically indicated and showed \(-2.5\) SD (T-score) as determined by dual-energy x-ray absorptiometry.

### Methods

#### Study Group

The study group consisted of 26 women with SLE surviving 1 or more manifestations of CVD, defined as a history of myocardial infarction (MI), angina (n=15), cerebral infarction (n=9), or claudication (peripheral atherosclerosis confirmed by angiogram). The SLE cohort at the Karolinska Hospital comprises 206 SLE patients. Of these, 24 women had a history of arterial disease, 1 of whom declined to participate in the study. Three SLE cases were also selected from Huddinge University Hospital. Twenty-six age-matched women with SLE but without manifestations of CVD, defined as a history of myocardial infarction (MI; n=11), angina (n=9), cerebral infarction (n=4). The SLE cohort at the Karolinska Hospital comprises 206 SLE patients. Of these, 24 women had a history of arterial disease, 1 of whom declined to participate in the study. Three SLE cases were also selected from Huddinge University Hospital. Twenty-six age-matched women with SLE but without manifest CVD were included from the cohort at Karolinska Hospital, and 26 control women were recruited randomly from the population registry; none of the control women had arterial disease or SLE. All patients fulfilled the 1982 revised criteria of the American Rheumatism Association for classification of SLE.\(^9\) CVD was defined as thrombembolic and not hemorrhagic or vasculitic stroke (confirmed by computed tomography or MRI), MI (confirmed by electrocardiography and a rise in creatine kinase), angina pectoris (coronary insufficiency confirmed by exercise stress test), or intermittent claudication (peripheral atherosclerosis confirmed by angiogram). Of the 35 CVD events, 27 occurred before menopause.

The study was approved by the local Ethics Committee of the Karolinska Hospital. All subjects gave informed consent before entering the study.

#### Study Protocol

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### Routine Laboratory Tests

Anti–double-stranded DNA (dsDNA) antibodies were determined by immunofluorescence with *Crithidia lucillae* kinetoplast assay. Anticardiolipin antibodies (aCLs) were measured by ELISA with ethanol-fixed cardiolipin (Sigma) and horseradish peroxidase–conjugated fractionated rabbit immunoglobulins against human IgG and IgM, respectively (Dako). Lupus anticoagulant was determined by a modified Dilute Russel Viper Venom method (Biopool) with Bioclot lupus anticoagulant. \(\beta_2\)-Glycoprotein I (\(\beta_2\)GPI) antibodies were determined with ELISA (R&D Systems).

#### Plasma Lipoproteins

Plasma lipoprotein concentrations were determined by a combination of preparative ultracentrifugation followed by lipid analyses of the lipoprotein fractions as described previously.\(^1,5\) Lp(a) was determined by use of ELISA [TintELIZE Lp(a), Biopool Int]. LDL was isolated from pooled plasma of healthy donors by sequential preparative ultracentrifugation under conditions to minimize oxidation and proteolysis and subsequently oxidized by copper or modified by malondialdehyde (MDA) as described previously.\(^1,3\)

**Chemiluminescent Immunoassay for Autoantibody Binding to OxLDL**

The chemiluminescent assay was performed with modifications as described previously\(^1,4\) on plasma dilutions of 1:250. Data are...
expressed as relative light units per millisecond (RLU/ms). Each determination was done in triplicate, and all samples were measured in a single assay. The coefficients of variation for low and high standards were 6% to 8%.

### Determination of OxLDL Epitopes

The EO6 epitope concentration on apolipoprotein (apo) B-100–containing particles was measured by a chemiluminescent modification of a previously described assay.\(^\text{14}\)\(^,\)\(^\text{15}\) This sandwich assay uses an anti-human apoB-100 monoclonal antibody, MB47, to capture apoB-containing lipoproteins and a biotin-labeled anti-OxLDL antibody, EO6, to measure the amount of the EO6 epitope present on the apoB-containing lipoproteins captured. The number of apoB-containing particles should saturate the binding capacity of the plated apoB-containing lipoproteins (mainly LDL) from SLE cases except for those who did not. Plaque occurrence was 17/26 in SLE cases versus 3/26 in population controls. The common carotid IMT of SLE cases had significantly more plaques than population controls (Table 2).

### Carotid Ultrasound

The right and left carotid arteries were examined with a duplex scanner (Acuson Sequoia), and the intima-media thickness (IMT) was determined as described previously.\(^\text{16}\) A plaque was defined as a local intimal-medial thickening, with a thickness >1 mm.\(^\text{17}\)

### Statistical Methods

For skewed variables, nonparametric tests were used for comparisons between groups (Friedman test), whereas ANOVA was used for normally distributed variables, with paired \(t\) test used as post hoc analysis. McNemar’s test was used for comparison of nominal variables between study groups. Nonparametric continuous variables were logarithmically or reciprocally transformed. The significance level was put at \(P<0.05\).

### Results

### Basic Characteristics of Study Groups

Disease duration and present disease activity (SLAM) did not differ significantly between the SLE groups (Table 1). Organ damage (SLICC) was higher in SLE cases than in controls (median value of 4 and 1, respectively), but SLICC includes CVD and is therefore biased when SLE cases and SLE controls are compared.

SLE manifestations including nephritis, vasculitis, serositis, skin involvement, and central nervous system affections did not differ between SLE groups. Osteoporosis was more frequent in SLE cases than in SLE controls (\(P=0.027\)). Both SLE groups were subject to long-term prednisolone treatment. The cumulative dose (evaluated through interview and retrospective review of patient charts) was significantly higher in SLE cases and was also significantly associated with the plasma triglyceride concentration (\(P=0.01\)) in the whole SLE group. Exposure time (months of prednisolone use) and current dosage did not differ significantly between the 2 SLE groups (data not shown).

Medication with cyclophosphamide, chloroquine, azathioprine, or antihypertensive drugs did not differ between SLE groups (data not shown). However, lipid-lowering compounds were taken by 9 SLE cases, 1 SLE control, and 1 healthy control.

### IMT and Traditional Risk Factors

There were no differences in blood pressure, smoking habits, body mass index, or prevalence of diabetes mellitus between groups. SLE cases had a greater common carotid IMT than SLE controls and population controls, whereas SLE controls did not differ from population controls in this respect. SLE cases had significantly more plaques than population controls, and a similar trend was present in relation to SLE controls. Furthermore, SLE controls had significantly more plaques than population controls (Table 2).

Plasma concentrations of major lipoproteins and lipids are presented in Table 3. A dyslipoproteinemia was present in SLE cases but not in SLE controls that comprised significantly decreased HDL cholesterol and increased triglyceride concentrations in both VLDL and LDL fractions. LDL cholesterol did not differ between the SLE groups. The plasma Lp(a) concentration was significantly higher in SLE patients than in SLE controls or population controls (Table 1).

### Nontraditional Risk Factors

The erythrocyte sedimentation rate and plasma concentrations of orosomucoid, \(\alpha_1\)-antitrypsin, and CRP were significantly higher in SLE cases than in SLE controls (Table 4). aCLs, anti-\(\beta_2\)GPI antibodies, and lupus anticoagulant were more common in SLE cases than in population controls (Table 4). In addition, lupus anticoagulant was significantly higher in SLE cases than in SLE controls (Table 4). Homocysteine levels discriminated strongly between groups, with SLE cases having significantly higher levels than either SLE controls or population controls.

### Oxidation-Associated Factors

IgG and IgM autoantibodies to OxLDL and MDA-LDL were increased in both SLE groups. Both anti-OxLDL and anti-MDA-LDL antibodies of the IgG subclass discriminated between SLE cases and SLE controls (Table 5). ApoB-containing lipoproteins (mainly LDL) from SLE cases expressed significantly higher levels of EO6-specific epitopes (oxidized phospholipid) than SLE controls. This also tended to be true for population controls (Table 5).

### Discussion

In this study, we sought to determine the prevalence of risk factors in SLE patients who developed CVD compared with those who did not. The common carotid IMT of SLE cases...
TABLE 3. Plasma Lipid and Lipoprotein Concentrations

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>SLE Cases: 1</th>
<th>SLE Controls: 2</th>
<th>Population Controls: 3</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>4.99±0.95 (4.85)</td>
<td>5.09±1.14 (4.96)</td>
<td>5.06±0.93 (4.85)</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.57±0.51 (0.37)</td>
<td>0.26±0.16 (0.22)</td>
<td>0.26±0.16 (0.26)</td>
<td>1:2 P=0.007 1:3 P=0.006 2:3 NS</td>
</tr>
<tr>
<td>LDL</td>
<td>2.74±0.81 (2.71)</td>
<td>2.82±0.91 (2.75)</td>
<td>2.82±0.91 (2.85)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL</td>
<td>1.51±0.53 (1.51)</td>
<td>1.78±0.45 (1.67)</td>
<td>1.78±0.77 (1.49)</td>
<td>1:2 P=0.03 1:3 NS 2:3 NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>1.64±1.00 (1.26)</td>
<td>0.96±0.37 (0.94)</td>
<td>1.01±0.37 (0.96)</td>
<td>1:2 P=0.001 1:3 P=0.005 2:3 NS</td>
</tr>
<tr>
<td>VLDL</td>
<td>1.15±0.93 (0.75)</td>
<td>0.60±0.32 (0.55)</td>
<td>0.64±0.34 (0.53)</td>
<td>1:2 P=0.004 1:3 P=0.02 2:3 NS</td>
</tr>
<tr>
<td>LDL</td>
<td>0.31±0.11 (0.30)</td>
<td>0.21±0.08 (0.20)</td>
<td>0.22±0.11 (0.20)</td>
<td>1:2 P=0.001 1:3 P=0.001 2:3 NS</td>
</tr>
<tr>
<td>HDL</td>
<td>0.23±0.05 (0.21)</td>
<td>0.20±0.06 (0.18)</td>
<td>0.20±0.05 (0.20)</td>
<td>NS</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>352±351 (239)</td>
<td>177±212 (113)</td>
<td>159±208 (74)</td>
<td>1:2 P=0.05 1:3 P=0.02 2:3 NS</td>
</tr>
</tbody>
</table>

Values are given as mean±SD (median).

The antiphospholipid antibody syndrome is characterized by both arterial and venous thrombosis and is common in SLE.22 In the present study, lupus anticoagulant showed a significant association with CVD in SLE. In addition, both aCLs and anti-β2GP1 antibodies tended to be associated with arterial disease in SLE. It is possible, therefore, that the increased risk of CVD in SLE is to some extent caused by thrombosis.

Homocysteine is increasingly recognized as a risk factor in the general population23 and in SLE.24 How homocysteine is related to arterial disease is not completely clear, but one interesting possibility is an association with increased LDL oxidation.25

Steroid treatment is often believed to be atherogenic, due to effects on plasma lipoproteins. Because inflammation is implicated in atherosclerosis, cortisone could actually prevent atherosclerosis as well. Indeed, one animal study supports this notion.26 In spite of high cumulative prednisolone doses, the SLE control group did not have increased common carotid IMT. SLE cases, on the other hand, had higher total prednisolone consumption than SLE controls, which most likely reflects a raised cumulative disease activity among SLE cases. Clearly, the role of prednisolone treatment in development of arterial disease in SLE deserves further study.

was greater than that of SLE controls and population controls, a finding that validates our selection of patients and also indicates that atherosclerosis plays an important role in arterial disease in SLE. The IMT of the SLE controls was not different from that of population controls. SLE cases tended to have more plaques than SLE controls, and both SLE cases and SLE controls had significantly more plaques than population controls.

Despite a more common use of hypolipidemic drugs, dyslipidemia (elevated triglycerides and decreased HDL cholesterol) was more common in SLE cases than in either SLE controls or healthy controls. In contrast, the LDL-cholesterol concentration did not differ between groups. Dyslipidemia was present only in SLE cases with manifest CVD, whereas the lipoprotein pattern in SLE controls was identical to that of healthy controls.

In line with recent findings3,18 our data indicate that the plasma concentration of Lp(a) is significantly enhanced in SLE cases compared with SLE controls and healthy controls, whereas the latter 2 groups do not differ. aPLs predict an increased risk for MI, and their levels are increased in young survivors of MI.19,20 β2GP1 is a cofactor for antibody binding to cardiolipin,17 and recent studies indicate that many aCLs recognize oxidized CL (OxCL) and/or adducts of OxCL with β2GP1.21
Of note, no association between disease duration and arterial disease was found.

Recently, an association between increased bone loss and progression of atherosclerotic calcification in women was reported.27 We found that osteoporosis was more frequent in SLE cases than in SLE controls. Whether this is related to prednisolone treatment only or to other underlying mechanisms remains to be shown.

We confirm previous observations that autoantibodies related to OxLDL are elevated in SLE.21,28,29 We also report that anti-MDA-LDL and anti-OxLDL antibodies of IgG type appear to discriminate between SLE cases and SLE controls. Such autoantibodies could be mere markers of disease or could play an important role in SLE-associated arterial disease. In one previous report,29 anti-MDA-LDL antibodies were found not to be associated with arterial disease in an SLE cohort of 118 patients, in contrast to our findings. Apart from methodological differences, this discrepancy may be related to differences in populations tested, because our selection procedure allowed us to match both SLE controls and population controls with SLE cases on the basis of age.

CRP and other markers of inflammatory activity were elevated in SLE cases. Another novel observation was that oxidized phospholipid epitopes were significantly more frequent on apoB particles from SLE cases than on those from population controls.8 It is thus possible that OxLDL may contribute to the progression of atherosclerotic calcification in women.

Because of this selection process, it is not possible to reach any conclusions about the prevalence of atherosclerosis in our cohort of SLE patients. The design is aimed at studying survivors of CVD and does not allow any conclusions about risk factors for mortality in CVD among SLE patients.
Nevertheless, it is interesting to observe that women with SLE of close to 20 years’ duration without CVD had risk factors that distinguished them from SLE patients with CVD and furthermore had an IMT that did not differ from that of healthy women. Thus, it could be hypothesized that having SLE does not inherently predispose an individual to an enhanced risk of CVD.

Taken together, this study identifies a variety of risk factors for CVD in SLE patients, not only traditional factors such as dyslipidemia and Lp(a) but also a range of factors reflecting acute and chronic inflammation, including indices of enhanced LDL oxidation. It will be important to determine in a prospective study whether these factors can predict future CVD. If so, they can be used to identify a high-risk group that would be eligible for intensive intervention, for example, with potent antioxidants and anti-inflammatory agents.

Acknowledgments

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References


### Table 5. Antibodies Against OxLDL and Oxidation Epitopes in LDL

<table>
<thead>
<tr>
<th>SLE Cases: 1</th>
<th>SLE Controls: 2</th>
<th>Population Controls: 3</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>aMDA-LDL IgG</td>
<td>31 583±20 551</td>
<td>20 747±11 916</td>
<td>12 435±6 113</td>
</tr>
<tr>
<td>(26 026)</td>
<td>(16 441)</td>
<td>(11 076)</td>
<td></td>
</tr>
<tr>
<td>aMDA-LDL IgM</td>
<td>43 014±24 101</td>
<td>46 248±25 778</td>
<td>31 278±14 217</td>
</tr>
<tr>
<td>(42 803)</td>
<td>(41 527)</td>
<td>(27 294)</td>
<td></td>
</tr>
<tr>
<td>a0xLDL IgG</td>
<td>16 411±14 949</td>
<td>80 444±42 444</td>
<td>67 365±28 956</td>
</tr>
<tr>
<td>(13 026)</td>
<td>(74 711)</td>
<td>(61 486)</td>
<td></td>
</tr>
<tr>
<td>a0xLDL IgM</td>
<td>24 214±18 230</td>
<td>25 149±22 201</td>
<td>13 731±8 620</td>
</tr>
<tr>
<td>(21 306)</td>
<td>(18 055)</td>
<td>(10 263)</td>
<td></td>
</tr>
<tr>
<td>E06/apoB</td>
<td>0.135±0.097</td>
<td>0.113±0.095</td>
<td>0.114±0.085</td>
</tr>
<tr>
<td>(0.087)</td>
<td>(0.066)</td>
<td>(0.078)</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean±SD (median). aMDA-LDL indicates anti-MDA LDL antibodies; a0xLDL, anti-Ox LDL antibodies. E06/apoB is an index of the content of E06 epitopes per apoB100 particle (see Methods).


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