

## PAPER

# Oxidized low-density lipoprotein and $\beta$ 2-glycoprotein I in patients with systemic lupus erythematosus and increased carotid intima-media thickness: implications in autoimmune-mediated atherosclerosis

LR Lopez<sup>1\*</sup>, M Salazar-Paramo<sup>2</sup>, C Palafox-Sanchez<sup>3</sup>, BL Hurley<sup>1</sup>, E Matsuura<sup>4</sup> and I Garcia-De La Torre<sup>3</sup>  
<sup>1</sup>Corgenix, Inc., Westminster, Colorado, USA; <sup>2</sup>Research Division, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Mexico; <sup>3</sup>Department of Immunology and Rheumatology, Hospital General de Occidente and Department of Physiology, CUCS, University of Guadalajara, Guadalajara, Mexico; and <sup>4</sup>Department of Cell Chemistry, University of Okayama Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Oxidative stress and LDL modification (oxLDL) are early pro-atherogenic events. OxLDL binds  $\beta$ 2GPI producing immunogenic oxLDL/ $\beta$ 2GPI complexes. Antibodies to these complexes have been associated with arterial thrombosis in patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS). Circulating oxLDL/ $\beta$ 2GPI complexes, IgG and IgM antibodies to these complexes were measured by ELISA in 30 SLE patients asymptomatic for cardiovascular disease (mean age 31 years) and 27 age/sex matched healthy controls. Carotid intima-media thickness (IMT) was measured by ultrasound in all patients and controls. Forty-seven percent of SLE presented plaques (median IMT of  $0.65 \pm 0.12$  mm) while only 7% of the controls had plaques (median IMT of  $0.50 \pm 0.04$  mm,  $P < 0.001$ ). Median optical density (OD<sub>450nm</sub>) for oxLDL/ $\beta$ 2GPI complexes in SLE was  $0.244 \pm 0.07$ , higher than controls ( $0.174 \pm 0.09$ ,  $P < 0.001$ ). Median OD for IgG anti-oxLDL/ $\beta$ 2GPI antibodies was also higher in SLE ( $0.297 \pm 0.26$ ) compared to controls ( $0.194 \pm 0.07$ ,  $P < 0.001$ ) while the median OD for IgM antibodies in SLE ( $0.444 \pm 0.46$ ) was not different than controls ( $0.326 \pm 0.22$ ,  $P = 0.267$ ). There was no correlation between IMT and oxLDL/ $\beta$ 2GPI complexes, IgG or IgM antibodies, possibly reflecting the complex interrelationship between these serologic elements and tissue factors in the arterial wall. These results support the hypothesis that oxLDL/ $\beta$ 2GPI complexes and IgG (not IgM) anti-oxLDL/ $\beta$ 2GPI antibodies contribute to the development of autoimmune-mediated atherosclerosis *Lupus* (2005) 15, 80–86.

**Key words:** autoimmune-mediated atherosclerosis;  $\beta$ 2-glycoprotein I; intima-media thickness; oxidized low-density lipoprotein; systemic lupus erythematosus

## Introduction

Today's prolonged survival rates may have exposed the presence of significant atherosclerotic cardiovascular (CVD) morbidity and mortality in patients with systemic lupus erythematosus (SLE).<sup>1–3</sup> Traditional Framingham risk factors failed to fully account for the premature (or accelerated) development of atherosclerosis in these patients.<sup>4</sup> Several inflammatory and immunologic factors have been established as important contributors to atherogenesis.<sup>5</sup> Among these, lipid

peroxidation and low-density lipoprotein modification (oxLDL) play a central role in the initiation and progression of atherosclerotic lesions.<sup>6–8</sup> Several autoimmune mechanisms have also been implicated as significant contributors in the accelerated or premature development of atherosclerosis observed in patients with systemic autoimmune diseases. Shoenfeld *et al.*<sup>9</sup> reviewed the experimental evidence and clinical studies recently published that confirm the early onset and severity of atherosclerosis in various rheumatic disorders.

Recent prospective studies have shown that antiphospholipid antibodies,  $\beta$ 2GPI-dependent anti-cardiolipin (aCL) or anti- $\beta$ 2GPI, were associated with arterial thrombosis in patients with antiphospholipid syndrome (APS).<sup>10,11</sup> The finding that some aCL antibodies from APS patients cross-react with

\*Correspondence: Luis R Lopez, Corgenix Inc., 12061 Tejon Street, Westminster, Colorado 80234, USA. E-mail: llopez@corgenix.com  
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oxLDL,<sup>12</sup> along with the colocalization by immunohistochemical staining of oxLDL,  $\beta$ 2GPI, CD4/CD8 lymphocytes and immunoglobulins in human and rabbit atherosclerotic lesions,<sup>13,14</sup> have stimulated renewed support to the hypothesis that autoimmune mechanisms play an active role in atherogenesis. The development of larger fatty streaks in mice that received syngeneic lymphocytes from  $\beta$ 2GPI-immunized LDL-receptor deficient mice compared to control mice that received lymphocytes from mice immunized with BSA, provided direct evidence that antigen  $\beta$ 2GPI-reactive T cells promote atherogenesis in murine models.<sup>15</sup>

Generalized immunological vascular injury with a decreased in the antioxidant activity of the plasma enzyme paraoxonase (PON1) may cause chronic oxidative stress and LDL modification (oxLDL). Ongoing oxidative stress have been demonstrated in patients with systemic autoimmune disorders with vascular complications.<sup>16–19</sup> OxLDL binds  $\beta$ 2GPI *in vitro* to form oxLDL/ $\beta$ 2GPI complexes.<sup>20,21</sup> The coexistence of oxLDL/ $\beta$ 2GPI complexes in SLE and APS patients with antibodies to these complexes suggests that oxLDL/ $\beta$ 2GPI complexes are atherogenic autoantigens.<sup>22,23</sup> The physiologic relevance of these findings has been illustrated by the enhanced *in vitro* macrophage uptake of oxLDL/ $\beta$ 2GPI complexes in the presence of anti- $\beta$ 2GPI antibodies,<sup>24,25</sup> likely mediated by macrophage Fc $\gamma$  receptors. This observation provides an explanation for the accelerated foam cell formation in autoimmune-mediated atherosclerosis.

Recent experimental evidence suggests a pro-atherogenic role for IgG anti-oxLDL/ $\beta$ 2GPI antibodies. Elevated serum levels of these antibodies were associated with the history of arterial thrombosis in SLE and APS patients.<sup>23,26</sup> OxLDL/ $\beta$ 2GPI complexes and antibodies to these complexes seem to be formed mainly in the atherosclerotic lesion (arterial intima) and released into the circulation. Further, these complexes may be found in circulation combined with immunoglobulins as immune complexes. We hypothesized that these elements may serve as serologic markers for atherosclerosis reflecting tissue inflammation (oxidation) at the level of the arterial intima. In this study, we measured oxLDL/ $\beta$ 2GPI complexes and antibodies to these complexes in a group of SLE patients to study their association with a surrogate marker of atherosclerosis (intima-media thickness, IMT).

## Material and methods

### Study population

The study subjects consisted of a cohort of 30 SLE patients attending the Department of Immunology and

Rheumatology outpatient clinic at the Hospital General de Occidente, Guadalajara, Mexico. All patients fulfilled the revised American College of Rheumatology (ACR) classification criteria for SLE.<sup>27</sup> Patients with evident clinical cardiovascular disease (myocardial infarction, angina, congestive heart failure due to coronary heart disease, transient ischemic attacks, stroke), with history of antiphospholipid syndrome, renal, liver, thyroid or infectious disease were excluded from the study. The study was approved by IRB (Ethical Committee) of the Hospital General de Occidente, and all patients signed written consent to participate in the study. Demographic and baseline clinical information and blood samples to be used for routine or experimental serologic testing were obtained from each patient and controls upon enrollment. Serum samples were stored frozen at  $-70^{\circ}\text{C}$  until tested. Twenty-seven age and sex matched healthy subjects (blood bank donors from Hospital General de Occidente) with similar (Mexican) ethnicity were enrolled in the study and used as controls.

### Intima-media thickness

Carotid intima media thickness (IMT) was evaluated in all SLE and controls subjects at the University of Guadalajara within 15–30 days of study entry. IMT was measured by B-mode ultrasonography using a Toshiba SSA-270A ultrasound (Tustin, California, USA) equipped with a linear transducer (5-MHz). Three IMT measurements of diastolic images on each side at 10 mm before or after the carotid bifurcation were obtained. Mean IMT was calculated for each point and the highest value (maximum IMT) recorded for each subject. IMT results were expressed in millimeters (mm). IMT was defined as the distance from lumen-intima interface to the intima-adventitia interfaces. A plaque (atheroma) was defined as a focal (localized) protrusion into the vessel lumen more than 50% of the vessel diameter. The ultrasound operator was unaware of subject's diagnosis or status at the time of IMT measurements. Ultrasonography measurements were found to be reproducible.

### Clinical and laboratory assessment

Information about the traditional risk factors for atherosclerotic CVD was obtained by interrogation and physical exam from each subject at the time of enrollment. The following variables were recorded: age, sex, body weight, height, body mass index (BMI), blood pressure, cigarette smoking, total cholesterol, HDL and LDL-cholesterol, triglycerides, diabetes mellitus and menopause. Demographics and clinical

characteristics of SLE patients and controls studied are summarized in Table 1.

Lipid profile including total cholesterol, HDL, LDL-cholesterol and triglycerides were measured in fasting blood samples by standardized colorimetric laboratory tests. SLE autoantibody (ANA) profile was measured by standard indirect immunofluorescence on HEp-2 cell substrate and ELISA for antinuclear antibodies immunospecificities (Binding Site, Ltd., San Diego, California, USA) in the rheumatology laboratory of the Hospital General de Occidente following the manufacturer's instructions. Antiphospholipid antibodies, anticardiolipin (aCL) and anti- $\beta$ 2GPI antibodies, were measured by commercially available ELISA kits (Corgenix, Inc., Westminster, Colorado, USA). Other serologic markers of disease activity ie, CRP, ESR (Westergren), C3, were also measured by standard laboratory techniques. SLE disease activity was assessed using the Mexican modification of the Systemic Lupus Erythematosus Disease Activity Index, (Mex-SLE-DAI).<sup>28</sup> According to the index, patients with scores <2 were considered inactive and with scores  $\geq$ 6 had active disease. The accumulative dose of prednisone ranged from 10–20 mg/day. None of the patients were taking lipid-lowering drugs (statins) at the time of the study. Immunologic and clinical characteristics of SLE patients are summarized in Table 2.

**Table 1** General characteristics of study cohort<sup>a</sup>

	SLE (n = 30)	Controls (n = 27)	P-value
Sex (F/M)	27/3	27/0	
Age, mean $\pm$ SD, years (range)	31 $\pm$ 9 (18–56)	31 $\pm$ 7 (18–56)	
Body weight, mean $\pm$ SD (kg)	67 $\pm$ 15	61 $\pm$ 9	0.16
Height, mean $\pm$ SD (cm)	160 $\pm$ 8	161 $\pm$ 5	0.58
BMI, mean $\pm$ SD (kg/m <sup>2</sup> )	26 $\pm$ 5.4	23 $\pm$ 2.8	0.02
Hypertension (%)	27	4	0.001
Systolic BP, mean $\pm$ SD (mmHg)	117 $\pm$ 19	105 $\pm$ 9	0.012
Diastolic BP, mean $\pm$ SD (mmHg)	75 $\pm$ 11	64 $\pm$ 4	0.001
Total cholesterol, mean $\pm$ SD (mg/dL)	180 $\pm$ 42	194 $\pm$ 28	0.19
HDL cholesterol, mean $\pm$ SD (mg/dL)	36.2	42.4	0.02
LDL cholesterol, mean $\pm$ SD (mg/dL)	115.4	138.1	0.01
Triglycerides, mean $\pm$ SD (mg/dL)	144 $\pm$ 83	97 $\pm$ 39	0.01
Smoking (%)	17	20	0.21
DM (%)	7	4	0.3
Menopause (%)	18	9	0.87

<sup>a</sup>SLE = systemic lupus erythematosus; BMI = body-mass index is body weight (in kg) divided by square of height (in m); BP = blood pressure; DM = type 2 diabetes mellitus.

**Table 2** Immunologic characteristics of SLE patients<sup>a</sup>

Mean $\pm$ SD disease duration, years (range)	10 $\pm$ 5 (5–25)
Mean $\pm$ SD SLE-DAI(mex) score	1.9 $\pm$ 2.5
Prednisone, mg/day (daily dose range)	10–20
Antinuclear antibodies (%)	98
Anti-double stranded DNA (%)	44
Anti-Sm (%)	29
Anti-RNP (%)	43
IgG aCL (%)	7
IgM aCL (%)	3
IgG anti-B2GPI (%)	13
IgM anti-B2GPI (%)	10

<sup>a</sup>SLE = systemic lupus erythematosus; SLE-DAI(mex) = SLE disease activity index, Mexican modification, scores of <2 indicated inactive and scores  $\geq$ 6 indicated active disease; aCL = anticardiolipin antibodies; anti-B2GPI = anti-B2GPI antibodies.

### ELISA for oxLDL/ $\beta$ 2GPI complexes

Murine IgG monoclonal antibodies against human  $\beta$ 2GPI (WB-CAL-1) were coated onto Nunc MaxiSorp<sup>TM</sup> 96-micro-well plates (Nunc, Denmark). WB-CAL-1 monoclonal antibody used in this assay only reacts with  $\beta$ 2GPI bound to oxLDL, not with free  $\beta$ 2GPI. Serum samples diluted in buffer containing 2 mM MgCl<sub>2</sub> were added to the coated microwells and incubated for one hour at room temperature. MgCl<sub>2</sub> dissociates intermediate or electrostatically bound oxLDL/ $\beta$ 2GPI complexes, allowing the specific detection of non-dissociable or stable covalently bound complexes. Horseradish peroxidase (HRP)-conjugated anti-human Apo B100 murine monoclonal antibody (2E10) was added to the microwells and incubated for an additional hour. 2E10 monoclonal antibody reacted equally well to native and oxidized Apo B100. Colour was developed with tetramethyl-bezidine (TMB)/H<sub>2</sub>O<sub>2</sub>, and the reaction stopped with 0.36 N sulfuric acid. Optical density was read at a wavelength of 450 nm (650 nm reference). Reactivity of the assay was assessed with a curve built with oxLDL/ $\beta$ 2GPI complex solution. The complexes were prepared in advance by incubating equal amounts of Cu<sup>2+</sup>-oxLDL and purified human  $\beta$ 2GPI. A normal cut-off value for the assay was established by testing 260 serum samples from healthy blood donors (95th percentile). Coefficient of variation (intra and inter-assay precision) for weak and strong reactive samples ranged between 2.5–5.9% and 3.4–7.8%, respectively.

### ELISA for IgG and IgM anti-oxLDL/ $\beta$ 2GPI antibodies

OxLDL/ $\beta$ 2GPI complexes prepared by incubating equal amounts of Cu<sup>2+</sup>-oxLDL and purified human  $\beta$ 2GPI were coated onto Immunlon 1B<sup>TM</sup> 96-micro-well

plates (Dynex Technologies Inc., Virginia). These oxLDL/ $\beta$ 2GPI complexes served as antigenic substrates to capture patient's antibodies. Diluted serum samples were added to the microwells and incubated for one hour at room temperature. HRP-conjugated anti-human IgG (or IgM) antibody was added to the microwells and incubated for one hour. Colour was developed with TMB/ $H_2O_2$  and the reaction stopped with 0.36 N sulfuric acid. Optical density was read at a wavelength of 450 nm (650 nm reference). Reactivity of the assay was assessed with a curve built with serial dilutions of selected positive sera for IgG or IgM anti-oxLDL/ $\beta$ 2GPI antibodies. A normal cut-off value for the assay was established by testing 260 serum samples from healthy blood donors (95th percentile). Coefficient of variation (intra and inter-assay precision) for weak and strong reactive samples ranged between 2.8–6.3% and 3.6–8.1%, respectively.

### Statistics analysis

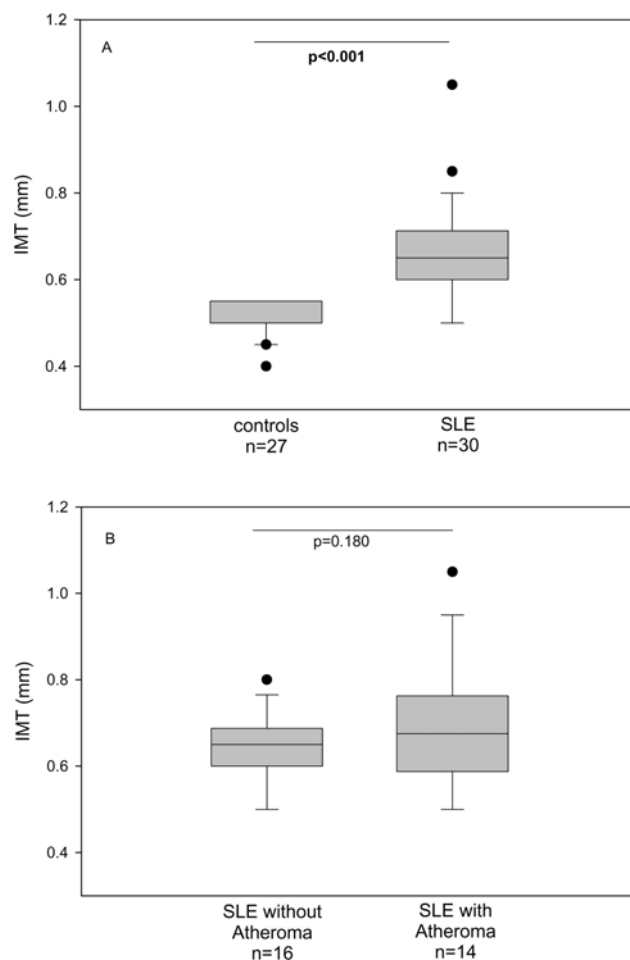
Comparisons between patient and control subjects were made by non-parametric *t*-test (Mann–Whitney rank sum test). Correlation (*r*) between two variables was assessed by Pearson's product moment correlation. *P*-values <0.05 were considered statistically significant.

## Results

Compared to the age and sex matched control group, this cohort of SLE patients presented the following classic risk factors for atherosclerosis: increased BMI, hypertension, both systolic and diastolic, low HDL and high triglycerides serum levels. Blood levels of total cholesterol and LDL were higher in the control group. The mean age of the SLE patients was 31 years, a young population for CVD. The mean disease duration was also relatively short at 10 years. In addition, this was a population with relative inactive disease at the time of the study as judged by a mean SLE-DAI(mex) score of 1.9, and presented with a low prevalence of antiphospholipid antibodies (aCL and anti- $\beta$ 2GPI).

### Intima-media thickness (IMT)

Forty-seven percent of SLE patients had plaques with a median IMT of  $0.65 \pm 0.12$  mm while two of the controls (7%) had plaques with a median IMT of  $0.50 \pm 0.04$  mm ( $P < 0.001$ ). SLE patients with plaques had a median IMT of  $0.67 \pm 0.15$  mm compared to  $0.65 \pm 0.08$  mm of those without plaques, but this difference was not statistically different ( $P = 0.180$ ) (Figure 1). Further, blood levels of total



**Figure 1** Carotid intima-media thickness (IMT) in SLE patients and healthy controls. Box plot of IMT measurements made by ultrasonography in a) SLE patients and age/sex-matched healthy controls; and b) SLE patients with and without atherosclerotic plaques (atheroma). IMT measurements expressed in mm. Boxes represent 75/25 percentiles and horizontal lines the median. Dots represent samples outside the 10/90 percentile bars. *P*-values (Mann–Whitney rank sum test) statistically significant if less than 0.05.

cholesterol, HDL, LDL or triglycerides were similar in SLE patients with and without plaques, possibly suggesting different mechanisms for the increased IMT and plaque formation. IMT measurements of SLE patients did correlate with total cholesterol ( $r = 0.500$ ,  $P = 0.006$ ), LDL ( $r = 0.375$ ,  $P = 0.045$ ), and triglycerides ( $r = 0.514$ ,  $P = 0.004$ ).

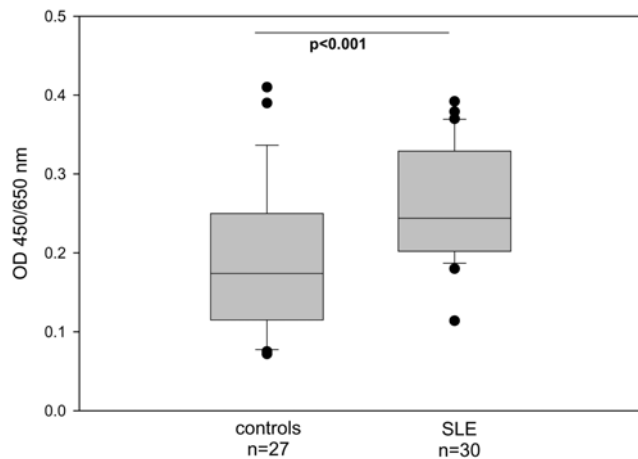
### oxLDL/ $\beta$ 2GPI complexes

Fifteen SLE patients (50%) had positive serum levels of oxLDL/ $\beta$ 2GPI complexes with a median OD of  $0.244 \pm 0.06$ , compared to five positives in the control group (18%) and a median OD of  $0.174 \pm 0.09$

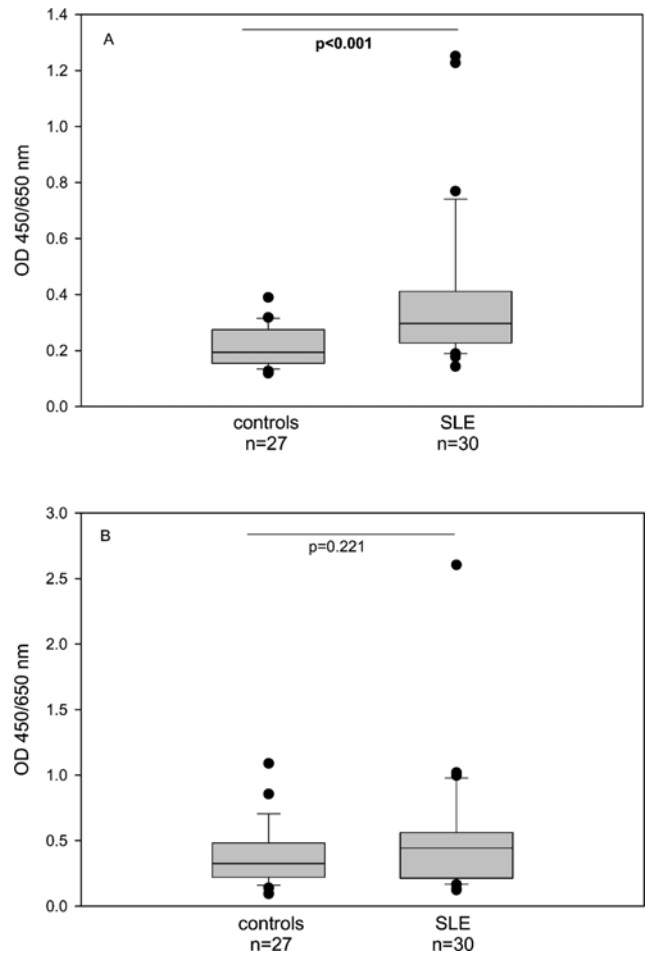
( $P < 0.001$ ) (Figure 2). SLE patients with plaques had lower complex levels (median  $0.221 \pm 0.06$ ) than those patients without plaques ( $0.281 \pm 0.08$ ), but this difference was not significant ( $P = 0.135$ ). There was no correlation between oxLDL/ $\beta$ 2GPI complex levels and IMT measurements in SLE patients ( $r = -0.002$ ,  $P = 0.456$ ).

### IgG and IgM anti-oxLDL/ $\beta$ 2GPI antibodies

Eighteen SLE patients (60%) had positive serum levels of IgG anti-oxLDL/ $\beta$ 2GPI antibodies with a median OD of  $0.297 \pm 0.27$ , compared to three positives (11%) in the control group and a median OD of  $0.194 \pm 0.07$  ( $P < 0.001$ ) (Figure 3). There was no difference in IgG anti-oxLDL/ $\beta$ 2GPI antibody levels between SLE patients with plaques (median  $0.284 \pm 0.09$ ) and patients without plaques (median  $0.306 \pm 0.34$ ,  $P = 0.271$ ). Eleven SLE patients (36%) had positive serum levels of IgM anti-oxLDL/ $\beta$ 2GPI antibodies with a median OD of  $444 \pm 0.46$ , compared to five positives (18%) in the control group and a median OD of  $0.326 \pm 0.22$ , but this difference was not significant ( $P = 0.267$ ). There was no difference in IgM anti-oxLDL/ $\beta$ 2GPI antibody levels between SLE patients with ( $0.514 \pm 0.29$ ) and without plaques ( $0.368 \pm 0.58$ ,  $P = 0.442$ ). There was no correlation between IgG or IgM anti-oxLDL/ $\beta$ 2GPI antibody levels and IMT measurements in SLE patients ( $r = -0.205$ ,  $P = 0.271$ ;  $r = -0.279$ ,  $P = 0.128$ ).



**Figure 2** OxLDL/ $\beta$ 2GPI complexes in SLE patients with increased carotid IMT. Box plot of serum levels of oxLDL/ $\beta$ 2GPI complexes measured by ELISA in SLE patients and age/sex-matched healthy controls. OxLDL/ $\beta$ 2GPI complex levels expressed in optical density (OD) units measured at 450/650 nm. Boxes represent 75/25 percentiles and horizontal lines the median. Dots represent samples outside the 10/90 percentile bars.  $P$ -values (Mann–Whitney rank sum test) statistically significant if less than 0.05.



**Figure 3** Antibodies to oxLDL/ $\beta$ 2GPI complexes in SLE patients with increased carotid IMT. Box plot of serum levels of a) IgG anti-oxLDL/ $\beta$ 2GPI antibodies and b) IgM anti-oxLDL/ $\beta$ 2GPI antibodies measured by ELISA in SLE patients and age/sex-matched healthy controls. Antibody levels expressed in optical density (OD) units measured at 450/650 nm. Boxes represent 75/25 percentiles and horizontal lines the median. Dots represent samples outside the 10/90 percentile bars.  $P$ -values (Mann–Whitney rank sum test) statistically significant if less than 0.05.

## Discussion

The results of this cross sectional study of young patients with significantly increased carotid IMT measurements confirm previous reports of CVD morbidity in SLE,<sup>29,30</sup> increased prevalence of carotid and femoral plaques in premenopausal APS and SLE women,<sup>31</sup> and increased carotid IMT in primary APS patients.<sup>32</sup> About half (47%) of our SLE patients presented atherosclerotic plaques (atheromas). IMT measurements were higher in SLE patients with plaques compared to patients without plaques, however, this difference was not statistically significant. Because the SLE patients were asymptomatic for CVD at the time of the study, our results clearly indicate the

presence of subclinical atherosclerosis. Several traditional risk factors for atherosclerosis (BMI, hypertension, low HDL, high triglycerides) were present suggesting an increased risk for CVD.<sup>4,5</sup> High serum levels of oxLDL/ $\beta$ 2GPI complexes and IgG antibodies to these complexes found in SLE patients in this and previous studies<sup>21–23,26</sup> strongly suggest that these immunologic factors be added to the list of atherosclerotic risk factors, and taken into consideration for therapeutic and preventive programmes aimed at reducing the risk of atherosclerotic CVD morbidity in systemic autoimmune diseases.

The relatively short disease duration (mean 10 years) may explain the absence of obvious clinical CVD in our SLE patients. We can also speculate that if our patients have had more active disease or other immunologic risk factors such as antiphospholipid antibodies, we would have seen other clinical events ie, venous thrombosis. Two of the healthy controls subjects had plaques with normal IMT and elevated oxLDL/ $\beta$ 2GPI complex or antibody levels, suggesting that these immunologic risk factors may be observed and perhaps clinically relevant in some non-autoimmune conditions.

The occurrence of oxidative stress in patients with systemic autoimmune diseases has been reported by several groups of investigators. The presence of oxidative stress was assessed by different methods: urinary excretion of arachidonic acid metabolites (isoprostanes F<sub>2</sub> $\alpha$ ) which is regarded as a marker of *in vivo* lipid peroxidation,<sup>16</sup> chemiluminescence using a monoclonal antibody E06 specific for oxidized phospholipids,<sup>17</sup> and multiple serum markers of protein oxidation by ELISA, acid hydrolysis and high-performance liquid chromatography.<sup>18</sup> The LDL modification into oxLDL is a central event in atherogenesis that leads to a cycle of inflammation, endothelial dysfunction and foam cell (atherosclerotic plaque) formation. Because oxLDL/ $\beta$ 2GPI complexes are formed in the arterial wall and released into the circulation, the elevated serum levels of oxLDL/ $\beta$ 2GPI complexes found in our SLE patients indicate an underlying oxidative injury and strongly suggest that these complexes contribute to increased IMT and plaque formation. It is possible that under physiological conditions,  $\beta$ 2GPI binds oxLDL to counteract its inflammatory (oxidative) effect and to promote macrophage uptake via scavenger receptors. Under chronic inflammatory conditions, the excess of oxLDL (and oxLDL/ $\beta$ 2GPI complexes) can promote foam cell formation, as scavenger receptors are not downregulated. In this study, we found no correlation between carotid IMT measurements and serum oxLDL/ $\beta$ 2GPI complex levels. In addition to the small number of patients studied, another explanation for the lack of correlation is that

we measured circulating (not tissue) oxLDL/ $\beta$ 2GPI complexes while IMT measurements represent a complex tissue (arterial wall) event.

OxLDL/ $\beta$ 2GPI complexes have been implicated as atherogenic autoantigens<sup>21</sup> and IgG anti-oxLDL/ $\beta$ 2GPI antibodies in SLE and APS patients have shown stronger correlation with arterial than with venous thrombotic events.<sup>22,26</sup> Serum levels of IgG anti-oxLDL/ $\beta$ 2GPI antibodies were significantly higher in our SLE patients when compared to the healthy controls. However, serum levels of IgM anti-oxLDL/ $\beta$ 2GPI antibodies were not different when compared to the controls. Whether IgG and IgM anti-oxLDL/ $\beta$ 2GPI antibodies play different roles remain to be investigated. Anti-oxLDL antibodies have been demonstrated in healthy individuals as well as in patients with atherosclerosis and CVD. Human atherosclerotic lesions contain immunoglobulins that recognize oxLDL, and reports that circulating anti-oxLDL antibodies predict atherosclerosis and CVD, suggest a pathogenic role. It has been shown that IgG antibodies induced by active immunization with oxLDL accelerated the development of atherosclerosis in a murine experimental model of APS.<sup>33</sup> In contrast, reduced atherosclerosis correlated with IgG anti-oxLDL antibodies in rabbits and mice suggesting a protective role. Similar protective effect has been reported with a monoclonal IgM anti-oxLDL antibody (E06) indicating an anti-atherogenic role of the innate immune response by blocking macrophage oxLDL uptake.<sup>34</sup> Thus, we hypothesized that IgM anti-oxLDL/ $\beta$ 2GPI antibodies in humans play a protective role (anti-atherogenic) while IgG anti-oxLDL/ $\beta$ 2GPI antibodies are pro-atherogenic.

*In vitro* macrophage uptake of oxLDL/ $\beta$ 2GPI complexes was significantly enhanced when IgG anti- $\beta$ 2GPI antibodies were added, suggesting the participation of a more efficient macrophage uptake via Fc $\gamma$  receptors.<sup>24,25</sup> These results support the hypothesis that IgG anti-oxLDL/ $\beta$ 2GPI antibodies or oxLDL/ $\beta$ 2GPI/IgG immune complexes are pro-atherogenic. Thus, it appears that autoimmunity accelerates the development of atherosclerosis. Our results support the hypothesis that oxLDL/ $\beta$ 2GPI complexes and IgG (not IgM) anti-oxLDL/ $\beta$ 2GPI antibodies are pro-atherogenic and may contribute to the development of autoimmune-mediated atherosclerosis.

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