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SLE patients show an increased arterial stiffness that is predicted by IgM-anti-ß$_2$-glycoprotein I and small dense HDL particles

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ABSTRACT

Background: Patients with systemic lupus erythematous (SLE) show increased cardiovascular mortality and morbidity. The accelerated atherosclerosis observed in SLE patients has been measured by the carotid intima media thickness (c-IMT) and functional vascular tests, such as the arterial stiffness that occurs before plaque formation.

Aims: To investigate the metabolic and immunological factors associated with the presence of central arterial stiffness measured by the augmentation index (AIx).

Methods: Cross-sectional study of 69 SLE patients compared with a control group of 34 healthy women. On the same day of the study, clinical, vascular and biochemical data were obtained. The AIx was assessed by peripheral arterial tonometry. The analysis of lipoprotein populations was performed using ^{1}H nuclear magnetic resonance (NMR).

Results: Patients with SLE showed increased arterial stiffness with respect to the control group [20.30(21.54)% *versus* 10.84(11.51)%;p=0.0021]. The values of the AIx were correlated with the Framingham risk score (*r*=0.486,p <0.001), the c-IMT (*r*=0.503;p <0.001), systolic blood pressure (*r*=0.456;p <0.001) and age (*r*=0.456;p<0.001). Patients taking antimalarial drugs showed a lower AIx [11.74(11.28)% vs 24.97(20.63)%;p=0.024]. The AIx was correlated with the atherogenic lipoproteins analyzed by NMR. The immunological variables associated with the AIx were C4 (*r*=0.259;p=0.046) and IgM-ß$_2$-glycoprotein I (*r*=0.284,p=0.284). In the multivariate analysis, age [ß=0.347(0.020-0.669);p=0.035], IgM-ß$_2$-GLP-I [ß=0.321(0.024-0.618);p=0.035] and small dense HDL particles [ß=1.288(0.246-2.329);p=0.017] predicted the AIx.

Conclusions: SLE patients show increased arterial stiffness compared to a healthy population. Patients treated with antimalarial drugs show lower arterial stiffness. Age, IgM-ß2-GLP-I and the number of small dense HDL particles predicted the AIx.

SIGNIFICANCE & INNOVATIONS (2-4 bullet points)

- The AIx is a non-invasive method to evaluate arterial stiffness in SLE patients that is well correlated with other surrogate markers of atherosclerosis such as the c-IMT.
- SLE patients show increased arterial stiffness compared to healthy control patients, although there are no differences with respect to the classic cardiovascular risk factors.
- Patients under antimalarial treatment show significantly decreased levels of the AIx, similar to the control group.
- Age, the antiphospholipid antibody, IgM-anti-ß2-anti-glycoprotein I, and pro-inflammatory small HDL particles were the variables that predicted the levels of the AIx.

INTRODUCTION

Patients affected by systemic lupus erythematous (SLE) show increased cardiovascular morbidity and mortality despite improvements in the control of disease activity and its complications (1-4).

The accelerated atherosclerosis observed in patients with SLE cannot be entirely explained by the traditional cardiovascular risk factors. Other non-classical cardiovascular risk factors related to inflammation have been associated with this accelerated atherosclerotic process (5-8).

The cardiovascular risk scales used in the general population underestimate the risk of cardiovascular events in SLE patients because they do not consider the other non-classic cardiovascular risk factors associated with SLE disease (9). These data are supported by the results of several studies showing a higher prevalence of subclinical atherosclerosis in this young population measured by carotid intima media thickness (c-IMT), arterial stiffness and endothelial dysfunction (10-14).

Thus, in SLE patients, the study of the arterial wall structure and its function is of particular interest in order to improve the evaluation and optimization of the individual cardiovascular risk of each patient (15-16).

Endothelial dysfunction is considered to be the earliest alteration of the arteries that leads to atherosclerosis. Another vascular alteration that precedes atherosclerosis is increased rigidity of the arterial wall (14). Several indices have been developed to assess arterial stiffness, including aortic pulse wave velocity (PWV) and the augmentation index (AIx) (17). The AIx is defined as an increase in pressure after the first systolic shoulder to the peak of aortic pressure, and it is expressed as a percentage of the aortic pulse pressure. The AIx is associated with several cardiovascular risk factors, including age, hypertension, diabetes, hyperhomocysteinaemia and cigarette smoking. In the Framingham cohort, arterial stiffness was associated with an increased risk for developing a first cardiovascular event, thereby improving the risk prediction of classical risk factors (18,19).

It has been reported in several studies that SLE patients show impaired arterial stiffness measured by the AIx, and this has been associated with inflammatory factors and SLE disease activity and duration (20-26).

Our group has focused on the research of surrogate markers of subclinical atherosclerosis in SLE patients and a more detailed method to analyze the lipid profile by ^{1}H-nuclear magnetic resonance (NMR) because standard laboratory methods could not reflect the real atherogenic lipid profile in these patients (27-29). We have also observed in our cohort that some specific SLE factors, such as complement system factors and antiphospholipid antibodies, are also associated with carotid intima media thickness and some lipoproteins measured by NMR, such as IDL particles and the small dense HDL particles (30,31).

The aim of the present study was to investigate if an earlier functional vascular test of atherosclerosis in SLE patients, such as arterial stiffness measured by the AIx, is associated with other subclinical markers of atherosclerosis, such as SLE-associated factors and the lipoprotein populations analyzed by NMR.

PATIENTS AND METHODS

Subjects

This was a cross-sectional study. Sixty-nine women with SLE attending the autoimmune diseases outpatient program at Sant Joan University Hospital (Reus, Spain) were recruited. Patients fulfilled at least four classification criteria for SLE as defined by the American College of Rheumatology, as revised in 1997 (32).

None of the subjects presented with active disease as defined by an SLE disease activity index (SLEDAI) >4. Thirty-four healthy women adjusted for age were recruited as controls from the same region.

Neither diabetes mellitus nor impaired renal function had been evident in these patients, and none of them presented ischemic or adverse cardiovascular events.

All patients provided fully informed consent to participate, and the Ethics Committee of "Sant Joan" University Hospital approved the study.

Biochemical analyses

Fasting venous blood samples were collected in EDTA or sera tubes and centrifuged at 1500 x g immediately for 15 min at 4ºC. The samples were then divided into aliquots and stored at -80ºC until analysis.

Standard laboratory methods were used to quantify glucose, HbA1c, total cholesterol, triglycerides and HDL cholesterol. LDL cholesterol was calculated by the Friedewald formula (33).

Apolipoprotein measurements were performed with immunoturbidimetric assays using antisera specific for apoA-1 and apoB (Hoffman-La Roche, Basel, Sweden) (Incstar Corporation, Stillwater, MN, USA). High-sensitivity C-reactive protein (hs-CRP) was measured with the highly sensitive near-infrared particle immunoassay (NIPIA) rate methodology (Beckman Coulter, Fullerton, CA) on a Synchron LXi PRO automated autoanalyzer (Beckman Coulter, Fullerton, CA). Insulin was measured in fasting sera by commercial ELISA kits (Mercodia AB, Uppsala, Sweden and BioVendor Laboratory Medicine Inc., Brno, Czech Republic, respectively). Insulin resistance (IR) was estimated using the homeostasis model assessment index (HOMA-IR), calculated as fasting glucose (in mmol/l) times fasting insulin (in mIU/l) divided by 22.5.

2D Nuclear Magnetic Resonance (NMR) lipoprofile and separation and quantification of Remnants Lipoproteins cholesterol (RLPc)

Total plasma lipids and the distribution of subclasses of lipoproteins were analyzed by nuclear magnetic resonance spectroscopy (NMR Lipoprofile, Raleigh, USA). Subclasses were of a given

average size. This technique allows for the determination of three discrete subclasses of very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), four low-density lipoprotein (LDL) and three high-density lipoprotein (HDL) subclasses. NMR was carried out on EDTA plasma stored at -80ºC and thawed just prior to the analysis (27).

Additionally, RLPc was measured in plasma using the method described by Nakajima et al., using RLP Cholesterol Assay Kits (Jimro-II, Otsuka, Japan, Immunoresearch Laboratories, Japan (34).

Cardiovascular (CVD) risk assessment

The 10-year CVD risk was assessed in all SLE patients by applying SCORE, Framingham risk score (FRS) and REGICOR scales.

Carotid Intima-Media Thickness assessment

We used a My Lab 50 X-Vision sonograph (Esaote SpA, Indianapolis, U.S.A) with a linear array ultrasound probe of 8-12 MHz transducer to identify the intimae media complex of the far wall of the common carotid, the bulb and the internal branch of the left and right carotid arteries. The images were digitalized and stored. Assessment of the c-IMT was performed by radiofrequency in *in vivo* images.

The images were obtained and measured by a single operator to reduce observer variability. We averaged the measurements of three images of the left and the right carotid arteries to obtain the mean IMT (c-IMT) (35).

Arterial Function Measurements

Arterial stiffness was measured by the augmentation index (AIx) using peripheral artery tonometry (PAT) technology (Endo-PAT 2000; Itamar Medical Ltd., Israel) (36). Patients were in a fasting state and had refrained from smoking or strenuous exercise in the previous 12 h. The test was performed

in a quiet room at 22-24°C. To perform the measurements, two probes that detect pulse wave amplitude were placed on a finger on both hands. After a stabilization period, a five-minute period of ischemia was induced by inflating a blood pressure cuff in one arm, and then the differences in pulse wave amplitude were analyzed before and after ischemia in comparison to the control arm. The software calculated the reactive hyperemia index (RHI) as an indicator of microvascular reactivity and endothelial function and the AIx, an indicator of systemic arterial stiffness. The AIx was obtained from the comparison of the systolic and diastolic waveform. Increased stiffness is expressed in higher values of the AIx in %. We used the AIx adjusted to 75 beats per minute for the analyses.

Statistical analyses

Analyses were performed using SPSS (version 24.0, SPSS Inc., Chicago, IL, USA). All data are presented as the means (SD) except when otherwise stated. Normality distribution was assessed with the Kolmogorov-Smirnov test. Differences between means were assessed by ANOVA. We performed Pearson's correlation tests to compare the AIx and other continuous variables. Multiple linear stepwise regression analyses were performed to find the variables that predicted levels of the AIx. We included those variables associated with the AIx in the univariate analyses and the bivariate correlations. We excluded those lipoproteins subclasses with a bivariate correlation >0.7 between them to avoid confounding factors. Two-tailed *P* values <0.05 were considered statistically significant.

RESULTS

Differences in central arterial stiffness (AIx) between SLE patients and the control group.

The general characteristics and the differences between SLE patients and the control group are shown in Table 1.

Patients with SLE showed significantly increased arterial stiffness (AIx) with respect to the control group [(20.30 SD 21.54)% *versus* (10.84 SD 11.51)%; p = 0.0021] (Figure 1A).

SLE patients showed a higher c-IMT than the control group [0.702(0.147) mm vs. 0.633(0.891) mm; p=0.012], although we did not find differences in the cardiovascular risk assessed by SCORE, FRS or REGICOR scales.

With respect to the classic cardiovascular risk factors, SLE patients presented higher triglycerides levels than the controls 0.97 (0.5) mmol/dL vs. 0.77 (0.3) mmol/dL; p = 0.048.

Variables associated with the AIx.

Bivariate correlations between continuous variables and the AIx are shown in Table 1.

In both groups, the values of the AIx were correlated with age, SBP, FRS, REGICOR and SCORE.

We could confirm that the AIx was also well correlated with the c-IMT, another surrogate marker of subclinical atherosclerosis in SLE patients (*r* = 0.503; p< 0.001) and the control group (*r* = 0.376; p = 0.034). We observed that greater tertiles of arterial stiffness were associated with greater values of the c-IMT (Figure 2).

With respect to lipids measured by standard laboratory methods, we found that in SLE patients the AIx was correlated with triglycerides (*r* = 0.480; p<0.001) and apoB100 (r = 0.290; p<0.001). However, in the control group, the AIx was correlated with the LDL-c levels (*r* = 0.428; p = 0.013), apoB100 (*r* = 0.379; p<0.001) and ApoA1 levels (*r* = 0.482; p<0.001).

The only immunological variables that correlated with the AIx were the levels of complement component C4 (*r*=0.259; p=0.046) and IgM- $ß_2$-glycoprotein I antibodies (*r*= 0.284; p = 0.284) (Table 1).

Differences in the AIx and the different treatments in SLE patients

We analyzed the differences between the AIx and the different treatments, such as antimalarial drugs, corticoids, immunosuppressive drugs, antihypertensive drugs, and statins.

With respect to the different SLE therapies, we only found differences regarding the antimalarial treatment. No other differences regarding the presence of corticoids or immunosuppressive therapies were found.

Patients treated with antimalarial drugs showed significantly lower central arterial stiffness (11.44(11.28)% vs. 24.97(20.63)%; *P*=0.024) (Figure 1B).

In Table 2, the general characteristics and the differences regarding the antimalarial treatment are described.

The results show that patients under antimalarial treatment did not show any difference regarding age, classic cardiovascular risk factors or c-IMT levels.

It is also interesting that the HDL cholesterol levels measured by standard laboratory methods were higher in the group of antimalarial-treated patients [1.85(0.49) mmol/dL vs. 1.57(0.3) mmol/dL; p = 0.006].

With respect to the lipid profile analyzed with NMR, we only found differences regarding the number of large HDL particles, which were higher in the antimalarial-treated patients [11.32(3.8) nmol/dL vs. 9.08(3.09) nmol/dL; p < 0.001]. No other differences regarding the other lipoprotein subpopulations were found.

Antimalarial-treated patients showed differences mainly regarding the immunological variables associated with SLE activity. They showed higher titers of anti-DNA antibodies by IFI (46.3(90.1) vs. 12.5(21.7); p = 0.025). They showed positive anti-DNA antibodies by chytridia in a higher proportion of patients with a near statistical significance (42.1% vs. 18,6%, *P* = 0.053) and lower levels of C3

[0.91(0.29) g/L respect 1.1(0.3) g/L; p = 0.011].

With respect to the pharmacological treatments for the cardiovascular risk factors, we found that patients treated with statins showed significantly increased levels of the AIx [32.24(27.69)% vs. 12.05(18.9)%; p = 0.033] and c-IMT [0.79(0.18) mm vs. 0.67(0.11) mm; p = 0.012].

Antihypertensive treatment was associated with a higher c-IMT but did not influence the AIx in SLE patients.

Correlation between the AIx and the lipoprotein subclasses analyzed with ^{1}H-NMR

When we analyzed the lipid profile in SLE patients by ^{1}H-NMR, we observed that the AIx was positively associated in the bivariate correlations with the main apoB-containing lipoproteins such as remnants (r = 0.441; p<0.001), total chylomicron and VLDL particles (r = 0.407; p<0.001), large VLDL particles (r = 0.269; p= 0.035), medium VLDL particles (r = 0.446; p<0.001), small VLDL particles (r = 0.307; p<0.015), IDL particles (r = 0.374; p= 0.003) and medium-small LDL particles (r = 0.261; p<0.041). On the other hand, we also found that the small HDL particles were associated with the AIx (r = 0.449; p<0.001). Data are shown in Table 3.

Variables that predict arterial stiffness (AIx) in SLE patients

Multivariate stepwise linear regression analysis was performed to assess the main predictors of arterial stiffness using the AIx as a dependent variable. We included in the model the following variables: age, systolic blood pressure (SBP), IgM-anti-ß$_2$- glycoprotein I, complement component C4, treatment with hydroxychloroquine and statins, apo-B100 levels, remnants, IDL particles, total number of VLDL, chylomicrons and small HDL particles. Using this multivariate model (*Durbin-Watson* = 1.915; R^2 = 0.541), we found that age [ß = 0.347 (95% IC 0.020-0.669); p = 0.035), IgM- ß$_2$-GLP I levels (ß = 0.321 (95% IC 0.024-0.618); p = 0.035] and small dense HDL particles (ß =1.288 (95% IC 0.246-2.329); p = 0.017) predicted the levels of the AIx (Figure 3).

DISCUSSION

The present study shows that SLE patients present increased arterial stiffness compared to the control group. No differences were found according to the presence of classic cardiovascular risk factors between both groups that could explain this increased arterial stiffness in SLE patients and an increase in the c-IMT.

AIx is a good surrogate marker for atherosclerosis in SLE patients

We found a good correlation between the AIx and the c-IMT, age, the systolic blood pressure and some lipid parameters in both SLE patients and in the control group. These results show that arterial stiffness measured by the AIx could be a good surrogate marker of atherosclerosis in SLE patients considering that this is a premature atherosclerotic step and that the cardiovascular risk estimation in these SLE patients does not seem to reflect the real vascular damage using cardiovascular risk scores from the general population.

Recent efforts have standardized methods to evaluate arterial elasticity. From the non-invasive methods, the use of the pulse-wave velocity (PWV) has been generalized (37,38). One of the limitations of this study is the use of the AIx to evaluate arterial stiffness. The AIx is obtained from a comparison of the systolic and diastolic waveform, and this is the result of several factors. The AIx reflects the systemic vascular stiffness not only in a regional territory such as PWV. To avoid some confounding factors, we corrected the AIx by the cardiac frequency and examined whether it was correlated with other surrogate markers of atherosclerosis such as the c-IMT.

Arterial stiffness is associated with immunological variables

We found that the AIx was associated with some specific immunological variables associated with SLE, such as the antiphospholipid antibody IgM-β_2-anti-glycoprotein I and the complement system. Activation of the immune system in SLE patients could affect vascular elasticity as a trigger of the atherosclerotic process (39,40). The AIx could reflect these changes that are not shown in studies

with other methods such as PWV, since the AIx reflects alterations in the wave reflection analysis from vascular territories from the small to the bigger vessels (41).

We did not find an association between the systemic inflammatory markers such as ESR and CRP, as has been published previously (42,43,20,22). This result could be related to the selection of the SLE patients for this study. Patients were under clinical remission six months before the study, and we excluded patients with impaired renal function and diabetes because these are already conditions with high cardiovascular risk.

Activation of the immune system and inflammation throughout the evolution of SLE could affect vascular elasticity as a trigger of the atherosclerotic process.

We found a positive correlation between the C4 complement compound and the AIx in the bivariate correlation, although we could not confirm this result in the multivariate analysis. Interestingly, the activation of the complement system is related to pathogenic processes such as SLE disease and atherosclerosis (5-8,31,40,43). SLE patients with a more active disease showing lower plasma levels of complement but high levels of C3 and C4 have also been linked to atherosclerosis and metabolic syndrome. On the basis of these data, we could consider that in patients with normal levels of complement, inflammation and activation of the complement system in the subendothelial space that leads to an increase in cardiovascular complications in SLE patients still persists.

Antimalarial treatment and statins affect the AIx

SLE patients under hydroxychloroquine treatment presented significantly lower AIx levels. The cardiovascular and metabolic protective effects of hydroxychloroquine in SLE have been associated with diverse mechanisms, such as an improvement in endothelial function, anti-inflammatory properties and reducing the presence of atherogenic dyslipidemia and insulin resistance (44,45). We did not find a clear relationship between hydroxychloroquine and a better cardiovascular risk profile in our patients. Although patients showed higher levels of HDL cholesterol, we could not confirm

that this association was related to the decrease in the AIx because we only found an association between AIx and the small dense HDL particles.

Antimalarial-treated patients showed differences mainly regarding the immunological variables associated with SLE activity. They showed higher levels of anti-DNA antibodies and lower levels of the complement compound C3.

These data indicate that antimalarial treatment was indicated in patients who were more immunologically active, although they were under clinical remission. Antimalarial treatment has been demonstrated to improve the risk of flares and long-term survival in SLE patients (46). Previous studies have associated disease activity with arterial stiffness and hydroxychloroquine (47), so perhaps the effect of hydroxychloroquine against disease activity could be monitored through the arterial stiffness measurement.

Although we did not find that hydroxychloroquine predicted the AIx in multivariate analyses, this result may be because we could not include other variables that affect arterial stiffness, such as the time under treatment and total dose administered, in the analyses. The cross-sectional observational study design, small sample size and selection criterion for patients to have been inactive six months before the study are limitations to confirm this hypothesis

We found that patients being treated with statins showed increased levels of the AIx but also increased levels of the c-IMT. This finding seems to reflect that patients with statins have a higher cardiovascular risk or worse lipid profiles.

AIx and lipoprotein subpopulations measured by NMR

The fourth result of the study is that we could confirm an association between the atherogenic lipoprotein subclasses and the arterial stiffness in SLE patients that has not been confirmed before using a more detailed analysis of the lipoprotein subclasses by NMR. This result is important because

the cardiovascular risk profile determined by the lipids in this population again seems to underestimate the real atherogenic profile in SLE patients.

We observed that the atherogenic apoB-containing lipoproteins and the small dense HDL particles that have been reported before to be pro-inflammatory and proatherogenic in SLE patients were associated with a worse AIx (48,49).

Variables that predicted the AIx

Finally, the multivariate model showed that only age and variables associated with SLE such as IgM-β_2-anti-glycoprotein I and the atherogenic small HDL particles predicted the levels of the AIx. We did not find that the AIx was associated with the classic cardiovascular risk factors, such as levels of blood pressure, glucose or apoB-containing lipoproteins.

The association that we have described between the small dense HDL particles and arterial stiffness is according to previous results that indicate a pro-atherosclerotic and pro-inflammatory function of HDL in SLE patients (50). HDL lipoproteins contain proteins involved in the innate immune system as part of the complement system (32). The analysis of HDL lipoprotein subpopulations by NMR in SLE patients can detect those lipoprotein subclasses with other functions than to reverse cholesterol transport. These HDL particles from SLE patients have a pro-atherogenic action from the initial states with an increase in arterial stiffness maybe reflecting the activation of the immune system and inflammation in the vascular territory.

We found the association in the multivariate analysis between the antiphospholipid antibodies IgM-β2-anti-glycoprotein I and the AIx to be of particular interest. Although the association between antiphospholipid antibodies and the risk of thrombosis is evident, the association between the increased c-IMT and arterial stiffness has not been well established (39, 50). It is necessary to validate these findings in a greater cohort of patients to confirm these results. Investigating the impact of non-classic cardiovascular factors such as SLE-related antibodies in the progression of the

atherosclerosis process from earlier stages could demonstrate the immunologic activation as a trigger of the atherosclerotic process.

In conclusion, SLE patients show increased arterial stiffness with respect to a healthy population as measured by the AIx. Patients treated with antimalarial drugs show lower arterial stiffness that is determined by age, the level of IgM-$ß_2$-anti-glycoprotein I and the number of small dense HDL particles.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for its intellectual content, and all authors approved the final version to be submitted for publication. Dr. Parra had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design: Castro, Parra.

Data acquisition: Parra, Castro, Ibarretxe, de las Heras, Català, Benavent, Garcés, Navarro, Ibarretxe.

Data analysis and interpretation: Parra, Lopez-Dupla, Castro, Amigó.

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Table 1. Differences between the general characteristics of SLE women and age-matched controls and its correlations with the AIx

Variable	SLE women group N = 69		Control women group N = 34		
	Mean(SD)	*r*	Mean(SD)	*r*	*P*
Anthropometric					
Age	49(16.8)	0.365**	48.7(13.2)	0.479**	NS
BMI, kg/m^2	26.3(5.8)	NS	24.5(3.2)	NS	NS
SBP, mmHg	118.5(19.3)	0.270*	111.26(14.8)	0.406*	NS
DBP, mmHg	75.7(11)	NS	72.2(7.7)	NS	NS
Mean BP, mmHg	89.26(12.62)	0.248*	85.59(9.2)	0.255*	NS
Waist circumference, cm	86.7(11.7)	NS	81.2(8.9)	NS	NS
Surrogate markers of subclinical atherosclerosis and cardiovascular risk estimation					
Mean c-IMT, mm	0.702(0.147)	0.503**	0.633(0.891)	0.376*	<0.001**
RHI	1.75(0.47)	NS	1.92(0.63)	NS	NS
REGICOR	2.19(1.86)	0.480**	1.66(1.16)	0.357**	NS
FRS	4.03(3.95)	0.418**	3.56(1.95)	0.454**	NS
SCORE	0.67(1.15)	0.349**	1.66(1.16)	0.426**	NS
Metabolism					
Gluc, mmol/L	5.1(0.6)	NS	5.0(0.6)	0.499	NS
Insulin, mIU/L	8.0(5.3)	NS	6.6(3.1)	0.203	NS
HOMA-IR	1.7(1.2)	NS	1.5(0.9)	0.346	NS
HbA_{1c}, %	4.7(0.6)	NS	-	-	-
Apo-A1, g/L	1.45(0.1)	NS	1.51(0.4)	0.482**	NS
Apo-B100, g/L	0.86(0.2)	0.290*	0.94(0.2)	0.379*	NS
Tot Chol, mmol/L	4.9(1.1)	NS	5.01(0.8)	0.460**	NS
TG, mmol/L	0.97(0.5)	0.480**	0.77(0.3)	NS	0.048*
LDL-c, mmol/L	2.82(0.8)	NS	3.1(0.7)	0.428*	NS
HDL-c, mmol/L	1.7(0.4)	NS	1.54(0.4)	NS	NS
Creatinine, µmol/L	67.5(15.4)	NS	64.7(10.6)	NS	NS
Factors of disease activity and inflammation					
Anti-DNA antibodies, IFI	23.9(5.7)	NS	-	-	-
C3, g/L	1.049(0.3)	NS	-	-	-
C4, g/L	0.174(0.1)	0.259*	-	-	-
CH50, U arb CH50	49.55(16.1)	NS	-	-	-
IgM-anticardiolipin, MPL-U/mL	8.87(12.84)	NS	-	-	-
IgG-anticardiolipin, GPL-U/mL	17.69(32.0)	NS	-	-	-
IgG-anti-β2-glicoprotein, I U/mL	7.21(12.7)	NS	-	-	-
IgM-anti-β2-glicoprotein I, U/mL	6.42(14.8)	0.289*	-	-	-
ESR, mm/h	17.98(2.5)	NS			
Hs-CRP, mg/L	2.47(2.6)	NS	1.93(1.71)	NS	NS

r: Bivariate relationship between the AIx and the variable with a statistical significance of *P <0.05 or **P < 0.001; *P:* significant differences between the mean of the control group and the SLE patients;NS: no significant difference; SBP: systolic blood pressure; DBP: Diastolic blood pressure; ESR erythrocyte sedimentation rate; FRS: Framingham risk score; RHI: Reactive hyperemia index.

Table 2: Differences between SLE patients regarding the presence of antimalarial treatment.

	WITH HYDROXYCHLOROQUINE N = 22	NO HYDROXYCHLOROQUINE N = 47	*P*
Age	47.8(16.1)	49.2(17.1)	NS
BMI, kg/m^2	25.4(4.1)	26.5(6.5)	NS
SBP, mmHg	114.6(19.3)	111.26(14.8)	NS
DBP, mmHg	75.7(11)	74.3(7.7)	NS
Waist circumference, cm	86.7.7(18.9)	89.6(11.3)	NS
Mean c-IMT, mm	707(150)	692(142)	NS
RHI	1.85(0.68	1.97(0.62)	NS
REGICOR	1.79(1.49)	2.4(2.0)	NS
FRS	3.41(2.26)	4.38(4.4)	NS
SCORE	0.41(0.61)	0.78(1.28)	NS
Gluc, mmol/L	4.8(0.6)	5.2(0.6)	NS
Insulin, mIU/L	9.2(6.4)	7.5(4.7)	NS
HOMA-IR	1.9(1.2)	1.6(1.1)	NS
HbA_{1c}, %	4.8(0.8)	4.7(0.5)	NS
Apo-A1, g/L	1.48(0.1)	1.41(0.2)	0.066
Apo-B100, g/L	0.86(0.2)	0.82(0.3)	NS
Tot Chol, mmol/L	5.1(1.3)	4.8(0.9)	NS
TG, mmol/L	0.89(0.51)	0.98(0.45)	NS
LDL-c, mmol/L	2.37(1.4)	2.5(1.07)	NS
HDL-c, mmol/L	1.85(0.49)	1.57(0.3)	0.006*
Creatinine, µmol/L	67.5(15.4)	64.7(10.6)	NS
Anti-DNA antibodies, IFI	46.3(90.1)	12.5(21.73)	0.025*
Anti-DNA antibodies +; %	42.1	18.6	0.053
C3, g/L	0.91(0.29)	1.1(0.3)	0.011*
C4, g/L	0.15(0.07)	1.1(0.24)	NS
CH50, U arb CH50	46.7(17.9)	50.1(14.9)	NS
IgM-anticardiolipin, MPL-U/mL	9.68(8.5)	8.96(10.8)	NS
IgG-anticardiolipin, GPL-U/mL	17.3(30.8)	17.3(32.1)	NS
IgG-anti-β2-glicoprotein I, U/mL	9.01(15.7)	6.1(11.1)	NS
IgM-anti-β2-glicoprotein I, U/mL	5.1(7.4)	7.25(16.9)	NS
Anti-lupus anticoagulant antibodies +,%	10.5	17.2	NS
ESR, mm/h	16.21(10.03)	19.03(14.01)	NS
Hs-CRP, mg/L	3.1(4.5)	3.7(3.2)	NS

P: significant differences between groups; NS: no significant difference;
SBP: systolic blood pressure; DBP: Diastolic blood pressure; ESR erythrocyte sedimentation rate; FRS: Framingham risk score; RHI: Reactive hyperemia index.

Table 3: Correlations between the AIx and lipoprotein particles analyzed by NMR in SLE patients (N = 69).

Variable		*r*
Remnant lipoprotein cholesterol, mg/dL		0.441**
VLDL and chylomicron concentrations, nmol/L	Total VLDL and chylomicron	0.407**
	Large VLDL and chylomicrons	0.269*
	Medium VLDL	0.440**
	Small VLDL	0.307**
LDL concentrations, nmol/L	Total LDL	0.290*
	IDL	0.374*
	Large LDL	NS
	Small LDL	NS
	Medium-small LDL	0.261*
	Very small LDL	NS
HDL concentrations, nmol/L	Total HDL	0.307**
	Large HDL	NS
	Medium HDL	NS
	Small HDL	0.449**
Mean sizes, nm	VLDL	NS
	LDL	NS
	HDL	NS

***p<0.05, **p<0.001**

FIGURE LEGENDS

Fig. 1. Differences between SLE patients and the control group (N = 34) according the levels of the AIx (N= 69) (A) and differences between the AIx in SLE patients according to hydroxychloroquine (HCQ) treatment (B).

Fig. 2. Differences in the AIx according to the c-IMT mean tertiles in SLE patients.

Fig. 3. Multivariate analyses and predictors of the augmentation index in SLE patients.