Complement system and small HDL particles are associated with subclinical atherosclerosis in SLE patients

Sandra Parra[1,4] MD, PhD, Gloria Vives[1,4]MD, Raimon Ferré[1,2]MD, PhD, Marta González[3], Montse Guardiola[3]PhD, Josep Ribalta[3]PhD, Antoni Castro[1,4]MD, PhD.

[1] Internal medicine department. Hospital Universitari Sant Joan de Reus. Institut Investigació Sanitaria Pere Virgili (IISPV). Universitat Rovira i Virgili. Reus, Spain.
[2]Unitat de Medicina Vascular i Metabolisme (UVASMET). Unitat de Recerca de Lipids i Arteriosclerosis (URLA). Hospital Universitari Sant Joan de Reus, Internal Medicine. IISPV. Universitat Rovira i Virgili, Reus, Spain.
[3] URLA. CIBERDEM. Hospital Universitari Sant Joan de Reus. IISPV. Universitat Rovira i Virgili, Reus, Spain.
[4] Grupo de Enfermedades Autoinmunes Sistémicas (GEAS). Sociedad Española de Medicina Interna (SEMI).

Corresponding author
Antoni Castro, MD, PhD.
Internal medicine department
Hospital Universitari Sant Joan de Reus
Av/ Josep Laporte, 1, 43206, Reus, Spain
Telf: +34 977 310 300 ext 50417
Fax: +34 977 319 984
e-mail: antoni.castro@urv.cat

ABSTRACT

Background: The complement system is involved in the pathogenic course of SLE. These patients exhibit metabolic disturbances in lipoprotein metabolism characterized by a pro-inflammatory status and an accelerated atherosclerosis.
The aim of this study is to investigate whether levels of the complement are associated to the presence of subclinical atherosclerosis, lipid and glucose metabolism and inflammatory markers in SLE patients.
Methods: Sixty-nine consecutive patients with SLE were recruited for the study. Fasting venous blood samples were collected on the same day as the measurements of cIMT were performed. Total plasma lipids and the distribution of subclasses of lipoproteins were analyzed by nuclear magnetic resonance spectroscopy.
Results: We found direct correlations between cIMT values and the levels of C3, C4 and CH50. In multivariate analyses, the mean cIMT from the three territories were predicted by age (β= 0.005, 95% CI: 0.002-0.007, *P* <0.001) and the functional hemolytic assay of the complement activity CH50 (β= 0.003, 95% CI: 0.001-0.006, *P* <0.0013). The complement components were associated with BMI, SBP and levels of glucose. Small, dense HDL particles also correlated with the three complement components C3, C4 and CH50 in bivariate analyses. In multivariate analyses small HDL particles predicted levels of C3: β= 0.024, 95% CI: 0.013-0.035, *P* <0.001); and C4: β= 0.005, 95% CI: 0.002-0.008, *P* = 0.006.
Conclusions: Activation of the complement system measured by functional assay CH50 is related to subclinical atherosclerosis in quiescent lupus patients and is activated by the small dense HDL particles

Key words: complement, intimal medial thickness, small HDL particles, lupus

Abbreviations: SLE: systemic lupus erythematosus; CVR: cardiovascular risk; cIMT: carotid intima media thickness; ICA: internal carotid artery; BCA: bulb carotid artery; CCA: common carotid artery; NMR: nuclear magnetic resonance. Hs-CRP: highly-sensitive C reactive protein.

1. Introduction

Systemic Lupus Erythematosus (SLE) represents a chronic autoimmune inflammatory disease with an increased cardiovascular mortality and morbidity [1]. The estimated prevalence of cardiovascular disease in this population lies between 6-10%, with an annual incidence of 1.3-1.5 % [2-3].

This accelerated atherosclerosis observed in SLE patients cannot be entirely explained by classic cardiovascular risk (CVR) factors as hypertension, diabetes, smoking, obesity or dyslipidemia but also by some disease-related factors such as the duration of the disease, corticoid therapy and some inflammatory mechanisms. It has been proposed as ethiopatogenic factors of atheroscelrosis in SLE patients elevated levels of CRP, anti-beta2GPI antibodies, heat shock proteins, anti-oxidized low-density lipoprotein antibodies or complement activation [4,5,6].

It is well recognized that the complement system plays an important role in the clinical course of SLE disease as hipocomplementemia is associated with flares and worse evolution and is used in the clinical monitoring of patients [7]. SLE syndrome is often found associated with complement deficiency. It is thought that an absence of key complement proteins results in defective immune complex clearance and consequent deposition of the complexes into various organs that are related to severe clinical manifestations of this disease [8].

It is recognized that the pathogenic process of atherosclerosis resembles a chronic inflammatory reaction involving components of both innate and adaptive immunity [9]. The existence of T-cells within plaque lesions or the presence of auto-antibodies against the endothelium have been observed in the general population [5].

Several proteins of the complement system, activation products, receptors and regulatory proteins [10,11] have been detected in atherosclerotic lesions, and the deposition of C5b-9 has been shown to correlate with the disease state [12]. Modified lipoproteins and apoptotic/necrotic cells have been shown to activate both the alternative and the classical complement pathway by binding immunoglobulins and CRP *in vitro* [13], and signs of complement activation via these pathways have been detected in atherosclerotic lesions *in vivo* [14]. Furthermore, plasma levels of complement component 3 (C3) have been related to coronary artery disease measured

by coronary angiography and to clinical ischemic events [15,16]. These data suggest the relevance of the complement system in atherogenesis [17,18].

Lipid and glucose metabolism is also related to the complement system. Several studies had levels of C3 and C4 associated with higher levels of triglycerides, low levels of HDL and insulin resistance, which are the metabolic syndrome compounds [19-22]. This characteristic pro-atherogenic dyslipidaemia found in the metabolic syndrome is also characteristic of SLE patients [23,24].

Furthermore, a fragment of C3 (C3a-des-Arg) is common to the fraction of the acylation-stimulating protein (ASP), which is the most potent agent to stimulate triglyceride synthesis and glucose membrane transport in human adipocytes. This might help to explain the association between complement, lipid metabolism, insulin resistance and postpandrial lipemia [25-27].

Recently, new proteomic technologies applied to HDL composition have revealed that HDL plays a previously unsuspected role in regulating the complement system. The complement components C3, C4 and C9, and complement-regulatory proteins like vitronectin and clusterin have been found bound to HDL. These studies also showed that HDL from patients with CAD was enriched in C3 and C4 [28,29]. These studies also add evidence that distinct HDL subpopulations contain distinct apolipoproteins, conferring different anti-inflammatory and antiatherogenic properties.

In summary, the complement system is involved in the pathogenic course of SLE and patients exhibit metabolic disturbances in lipoprotein metabolism characterized by a pro-inflammatory status and accelerated atherosclerosis.

The aim of this study was to investigate whether levels of complement that plays a singularity in SLE was associated, as well, to the presence of subclinical atherosclerosis, lipid and glucose metabolism and inflammatory markers in SLE patients.

2. MATERIAL AND METHODS

2.1 Study population.

Sixty-nine consecutive patients with SLE were recruited from the outpatient clinic of autoimmune diseases from the *Hospital Universitari de Sant Joan de Reus*. Patients fulfilled at least four of the classification criteria of the American College of Rheumatology, as revised in 1997 [30]. None of them had an active disease, defined by

an SLE disease activity index (SLEDAI) >4 nor chronic organ damage using the SLICC/ACR Damage Index (SDI).
Neither diabetes mellitus nor impaired renal function was evident in these patients, and none of them presented any ischemic or adverse cardiovascular event.
All patients provided fully informed consent for participation, and the Ethics Committee of the *Hospital Universitari de Sant Joan de Reus* approved the study.

2.2 Biochemical analyses

Fasting venous blood samples were collected in EDTA tubes and centrifuged immediately for 15 min at 4ºC for 1500 x g. The samples were then divided into aliquots and stored at -80ºC until the determination of analytical variables.
Standard laboratory methods were used to quantify glucose, HbA_{1c}, total cholesterol, triglycerides and HDL cholesterol. LDL cholesterol was calculated by the Friedewald formula [31]. Measurement of apolipoproteins was carried out by immunoturbidometry using antisera specific for apoA-1 and apoB (Hoffman-La Roche) and lipoprotein (a) (Incstar Corporation, Stillwater, MN, USA). High-sensitive CRP (hsCRP) was measured with a high sensitive near-infrared particle immunoassay (NIPIA,Beckman Coulter) on a SYNCHRON LXi PRO System automated autoanalyzer (Beckman Coulter). C3 and C4 levels were measured using turbidimetric methodology (Beckman Coulter) and total complement activity in serum was measured by automatized methods based on the liposomes Autokit CH50 (Wako Chemicals GmbH, Neuss, Germany). Insulin levels were measured in fasting sera by the commercial ELISA kit (Mercodia AB, Uppsala, Sweden). Insulin resistance (IR) was estimated by 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.

2.3 Carotid intima-media thickness

The carotid intima-media thickness (cIMT) was measured on the same day as the blood samples were obtained. We used a My Lab 50 X-Vision sonograph (Esaote S.p.A, U.S.A) with a linear array ultrasound probe of 8-12 MHz transducer to identify the intima media complex of the far wall of the common carotid (CCA) (1 cm proximal to the bifurcation), the bulb (BCA) (in the bifurcation) and the internal branch (ICA) (1 cm

distal to the bifurcation) of the left and right carotid arteries. The images were digitalized and stored. Assessment of cIMT was made 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 cIMT (mIMT). An increased cIMT value was defined as those measurements above the 75th percentile of the general population mean cIMT values adjusted by age and gender [32,33].

2.4 Nuclear Magnetic Resonance (NMR) lipo-profile

Total plasma lipids and the distribution of subclasses of lipoproteins were analyzed by nuclear magnetic resonance spectroscopy (NMR Lipo-Profile, Raleigh, USA), whose subclasses were of a given average size. This technique allows for the determination of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). Then, the VLDL fraction is quantified in three discrete subclasses, LDL in four subclasses and HDL in three subclasses, all according to increasing molecular weight. The NMR was carried out on EDTA plasma stored at -80ºC and thawed just prior to the analysis.

Statistical analyses

All data is presented as mean and standard deviation in parenthesis except when otherwise stated. Normality distribution was assessed with the Kolmogorov-Smirnov test. Differences between means were assessed by ANOVA and Bonferroni post-hoc test or the non-parametric tests Mann-Whitney-U test for those variables without normal distribution. Spearman correlation coefficients between continuous variables were determined using a bivariate correlation test.

Backward stepwise multiple linear regression analyses were carried out to find the changes in variables with an independent significant association with changes in complement levels (C3, C4 and CH50) and the cIMT values in the different territories (mean cIMT, bulb, internal and common carotid artery). Variables with a high association ($r>0.700$, $P<0.05$) were not included in the same model. The accepted model was that with a higher adjusted R2 and lower number of variables included. We report the B coefficient values and their 95% confidence interval (95%CI). Two-tailed *P* values <0.05 were considered statistically significant. Analyses were carried out using SPSS (version 19.0, SPSS Inc., Chicago, IL, USA).

3. Results

3.1 General characteristics of the study population.

General clinic characteristics of the patients are described in Table 1. Briefly, the mean age of the patients recruited in the study was 48.89(16.53) years. The median of duration of disease was 12.44(10.05) years. As determined by routine biochemical analyses, the levels of glucose, lipid profile or hsCRP were within a normal range. Although the mean cardiovascular risk estimation was in the low range when calculated by Framingham risk score or SCORE, the mean of the cIMT value was significantly increased in respect to the general population in the same area after adjustment by age and gender (data not shown) [32,33].

3.2 Complement and subclinical atherosclerosis.

First of all, we checked the association between subclinical atherosclerosis measured by cIMT and the complement components to test the hypothesis that complement is related to atherosclerosis in SLE patients.
We found direct correlations between cIMT values in the three territories (common, bulb and internal carotid artery) and the levels of C3, C4 and the hemolytic functional assay of complement activity (CH50) (Figure 1).

3.3 Complement components, cardiovascular risk factors and inflammatory markers in SLE patients.

Results are described in Table 2.
We did not find any association between levels of complement and the classic cardiovascular risk factors as age, smoking, LDL, HDL or TG (measured by standard laboratory techniques).
Levels of glucose were correlated with levels of C3 ($\rho = 0.494$, $P<0.001$), C4 ($\rho = 0.341$, $P = 0.009$, and CH50 ($\rho = 0.320$, $P = 0.014$) but we did not find any correlation between levels of insulin or the HOMA index.

The BMI was also correlated with C3 (ρ = 0.335, P = 0.028) and C4 (ρ = 0.402, P = 0.008) levels and the SBP only with C3 levels (ρ = 0.335, P = 0.015).
Regarding the inflammatory factors, levels of ESR were associated with levels of C3 (ρ = 0.504, P <0.001), C4 (ρ = 0.323, P = 0.016) and CH50 (ρ = 0.272, P =0.045). No correlation was found between levels of complement, hsCRP, total leucocytes or lymphocyte cell count.
An inverse correlation was found between the complement components and the markers of disease activity in SLE as Anti-DNA antibodies (C3: $\rho = -0.307$, P = 0.018; C4: $\rho = -0.307$, P =0.069; Ch50: $\rho = -0.414$, P = 0.001) and IgM –anticardiolipin (C3: $\rho = -0.271$, P = 0.040; C4: $\rho = -0.359$, P = 0.006; CH50: $\rho = -0.296$, P = 0.024).

3.4 Complement componentss and NMR-lipoprofile.

Since no significant correlations were found between the complement components and the standard lipid profile, we decided to investigate whether a more detailed NMR lipoprotein profile in SLE patients might provide more information on the relationship between lipid metabolism and the complement system.
NMR fractionation revealed significant correlations between the complement components and the HDL particles. Between the other apoB-containing lipoproteins we only found correlations with the medium small LDL particles and the C3 (ρ = 0.261, P = 0.048), C4 (ρ = 0.270, P = 0.040) and CH50 (ρ = 0.268, P = 0.042).
The total number of HDL particles positively correlated with the three complement compounds C3 (ρ = 0.331, P = 0.011), C4 (ρ = 0.257, P = 0.005) and CH50 (ρ = 0.266, P = 0.04) (Figure 2).
Among the three populations of HDL particles (large, medium and small), the small dense HDL particles were associated with the three complement components C3 (ρ = 0.431, P = 0.001), C4 (ρ = 0.298, P = 0.023) and CH50 (ρ = 0.281, P = 0.032) (Figure 2).
We found that higher levels of total and small HDL particles were also significantly associated to higher cIMT values (Figure 3)**.**

*3.5 Variables that predict levels of complement and cIMT in SLE patients***.**

We observed that levels of mean cIMT from the three territories were predicted by age (β= 0.005, 95% CI: 0.002-0.007, *P* <0.001) and the CH50 (β= 0.003, 95% CI: 0.001-0.006, *P* <0.0013).

Variables included in the model that were associated to changes in C3 (R^2= 0.803, *P* <0.001), C4 (R^2= 0.703, *P*<0.001) and CH50 (R^2=0.789, *P*<0.001) were BMI, SBP, glucose, ESR, Anti-DNA antibodies, IgM- anticardiolipin and the number of small HDL particles, hsCRP, TG and HDL. Results are shown in Table 3.

Multivariate analyses were also carried out to investigate which variables were associated to changes in the mean cIMT. Variables included in the model (R^2=0.558, P=0.004) were age, BMI, SBP, LDL, HDL, hsCRP, C3, C4, CH50 and the number of small HDL particles.

4. Discussion

In patients with an established SLE regular measurement of complement activity is a helpful guide for detecting a flare-up of the disease [6]. The complement system has also been related to the pathogenic process of atherosclerosis in the general population. Several complement protein depositions have been found in atherosclerotic lesions and have been associated with some cardiovascular risk factors like insulin resistance and lipid metabolism[15,16,19,20-23]. In SLE patients, it has also been suggested the complement levels are associated to cardiovascular disease [4-6,34,35].

For all this, we investigated the presence of subclinical atherosclerosis using the c-IMT as a surrogate marker of atherosclerosis in a cohort of SLE patients despite having an estimated low cardiovascular risk as calculated by Framingham risk score and SCORE. Those patients showed significantly higher cIMT values compared to the general population [36]. We confirm that there was a correlation between cIMT values and the levels of C3, C4 and CH50 in all the territories (bulb, common and internal carotid artery and the mean of the three territories). In the multivariate analyses, the level of CH50 predicted levels of the mean of cIMT. This test might provide a more accurate estimation of the subendothelial inflammation related to this accelerated atherosclerotic process than the determination of the C3 and C4 complement components that reflect proper activation of the complement system by the classic pathway of immune complexes. This hypothesis might be supported by the fact that in multivariate analyses

we found that CH50 levels were predictable by inflammatory serum markers like ESR and hsCRP [37].

Besides the relationship between atherosclerosis, the complement system and inflammation, we have shown that activation of the complement system is also associated to some classical cardiovascular risk factors like BMI, SBP and levels of glucose. These data suggest a relationship between the complement and the metabolic syndrome compounds a metabolic disorder with a pro-inflammatory pathological substrate.

Another interesting result is the association that we have described between HDL subpopulations and the levels of the complement components. In bivariate and multivariate analyses, we observed that levels of C3, C4 and CH50 were associated to the number of total HDL particles, and mainly to the small HDL particles. In multivariate analyses, the number of small HDL particles predicted the levels of C3 and C4. These results are supported by previous studies about HDL composition provided by new laboratory techniques in proteomics. HDL lipoproteins have been shown to contain proteins involved in the innate immune system as complement system. HDL particles from patients with coronary artery disease are enriched with C3 and C4 proteins. [28,29]

Under inflammatory conditions, HDL lipoproteins undergo structural remodeling, which leads to functional and structural changes involved in the regulation of the acute phase response after inflammation and the complement activation [38,39]. In these conditions, HDL particles become pro-inflammatory and pro-atherogenic, as has been described in patients with SLE. The pro-inflammatory HDL found in SLE patients has been shown to be directly correlated with the ESR and the presence of carotid artery plaques and cIMT [40]. In our cohort of patients, we also found a direct correlation between cIMT and the number of the total and small HDL particles.

Furthermore, a significant decrease in HDL concentration is found in clinical outbreaks of SLE in the same way as complement levels [23,41]. These results could point to a close relationship between HDL particles and the complement system. This role of HDL particles in the innate immune system, the regulation of inflammation and atherosclerosis should be taken into account when HDL is the target of therapies that modify size and composition and might promote changes in HDL functionality with unexpected results as an increase of risk of infections [42].

These results suggest that in the pathogenic process of atherosclerosis in SLE there are biological mechanisms that promote subendothelial inflammation with a low systemic manifestation leading to an increase of cardiovascular complications in an advanced phase of SLE disease.

The complement system might be considered as a laboratory test for predicting SLE flares, but also as a surrogate marker of atherosclerosis in this population with an inactive disease. Complement components are influenced by HDL concentration and composition. Modifications of HDL subpopulations and its functionality might be considered a therapeutic target for regulating complement system activation and atherosclerosis in SLE patients.

6. References

[1] Bernatsky S, Boivin JF, Joseph L, Manzi S, Ginzler E, Gladman DD, Urowitz M, Fortin PR, Petri M, Barr S, Gordon C, Bae SC, Isenberg D, Zoma A, Aranow C, Dooley MA, Nived O, Sturfelt G, Steinsson K, Alarcón G, Senécal JL, Zummer M, Hanly J, Ensworth S, Pope J, Edworthy S, Rahman A, Sibley J, El-Gabalawy H, McCarthy T, St Pierre Y, Clarke A, Ramsey-Goldman R. Mortality in systemic lupus erythematosus. Arthritis Rheum. 2006;54:2550-2557.

[2] Bruce IN, Gladman DD, Urowitz MB. Premature atherosclerosis in systemic lupus erythematosus. Rheum Dis Clin North A.m 2000;26:2cx57-278.

[3] Manci S**,** Selzer F, Sutton-Tyrrell K. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. Arthritis Rheum. 1999;42:51-60.

[4] Esdaile JM, Abrahamowicz M, Grodzicky T, Li Y, Panaritis C, du Berger R, Côte R, Grover SA, Fortin PR, Clarke AE, Senécal JL. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. Arthritis Rheum. 2001;44:2331-2337.

[5] Sherer Y, Zinger H, Shoenfeld Y. Atherosclerosis in systemic lupus erythematosus; Autoimmunity.2010;43:98-102

[6] López-Pedrera C, Aguirre MÁ, Barbarroja N, Cuadrado MJ.Accelerated atherosclerosis in systemic lupus erythematosus: role of proinflammatory cytokines and therapeutic approaches. J Biomed Biotechnol. 2010;26. Review.

[7] Mosca M, Tani C, Aringer M, Bombardieri S, Boumpas D, Brey R, Cervera R, Doria A, Jayne D, Khamashta MA, Kuhn A, Gordon C, Petri M, Rekvig OP, Schneider M, Sherer Y, Shoenfeld Y, Smolen JS, Talarico R, Tincani A, van Vollenhoven RF, Ward MM, Werth VP, Carmona L. EULAR Recommendations for monitoring systemic lupus erythematosus patients in clinical practice and in observational studies. Ann Rheum Dis. 2010;69:1269-74

[8] Sarma JV, Ward PA. Complement system. Cell Tissue Res.2011;343:227–235

[9] Ross R. Atherosclerosis and inflammatory disease. N Engl J Med.1999;340:115-126.

[10] Vlaicu R, Rus HG, Niculescu F, Cristea A. Immunoglobulins and complement components in human aortic atherosclerotic intima. Atherosclerosis.1985;55:35-50

[11] Seifert PS, Hansson GK. Complement receptors and regulatory proteins in human atherosclerotic lesions. Atheriosclerosis.1989;9:802-811

[12] Vlaicu R, Niculescu F, Rus HG, Cristea A. Immunohistochemical localization of the terminal C5b-9 complement complex in human aortic fibrous plaque. Atherosclerosis 1985;57:163-177

[13] Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. Arterioscler Thromb Vasc Biol. 1999;19:2348-2354

[14] Oksjoki R, Jarva H, Kovanen PT, Laine P, Meri S, Pentikäinen MO. Association between complement factor h and proteoglycans in early human coronary atherosclerotic lesions: implications for local regulation of complement activation. Arterioscler Thromb Vasc Biol. 2003;23:630-636

[15] Ajjan R, Grant PJ, Futers TS, Brown JM, Cymbalista CM, Boothby M. Complement C3 and C-reactive protein levels in patients with stable coronary artery disease. Thrombosis and haemosthasis. 2005;94:897-1115.

[16] Muscari A, Massarelli G, Bastagli L, Poggiopollini G, Tomassetti V, Drago G, Martignani C, Pacilli P, Boni P, Puddu P. Relationship of serum C3 to fasting insulin, risk factors and previous ischaemic events in middle-aged men. Eur Heart Journal. 2000;21:1081-1090.

[17] Niculescu F, Rus H. The role of complement activation in atherosclerotic. Immunol Res. 2004;30:73-80

[18] Haskard DO, Boyle JJ, Mason JC. The role of complement in atherosclerosis. Curr Opin Lipidol. 2008;19:478-482.

[19] van Oostrom AJ, Alipour A, Plokker TW, Sniderman AD, Cabezas MC. The metabolic syndrome in relation to complement component 3 and postprandial lipemia in patients from an outpatient lipid clinic and healthy volunteers. Atherosclerosis. 2007;190:167-173.

[20] Ylitalo K, Porkka KV, Meri S, Nuotio I, Suurinkeroinen L, Vakkilainen J, Pajukanta P, Viikari JS, Peltonen L, Ehnholm C, Taskinen MR. Serum complement and familial combined hyperlipidemia. Atherosclerosis. 1997;129:271–277.

[21] Pasqui AL, Pucetti L, Bova G, Di Renzo M, Bruni F, Pastorelli M, Palazzuoli A, Auteri A. Relationship between serum complement and different lipid disorders. Clin Exp Med. 2002;2:33-38;

[22] Mamane YL, Chan C, Lavallee G, Morin N, Xu LJ, Huang J, Gordon R, Thomas W, Lamb J, Schadt E, Kennedy BP, and Mancini1 JA. The C3a Anaphylatoxin Receptor Is a Key Mediator of Insulin Resistance and Functions by Modulating Adipose Tissue Macrophage Infiltration and Activation. Diabetes. 2009;58:2006–2017

[23] de Carvalho JF, Bonfá E, Borba EF. Systemic lupus erythematosus and "lupus dyslipoproteinemia". Autoimmun Rev. 2008;7:246-50.

[24] Sabio JM, Vargas-Hitos J, Zamora-Pasadas M, Mediavilla JD, Navarrete N, Ramirez A, Grupo Lupus Virgen de las Nieves. Metabolic syndrome is associated with increased arterial stiffness and biomarkers of subclinical atherosclerosis in patients with systemic lupus erythematosus. J Rheumatol. 2009;36:2204-11.

[25] Baldo A, Sniderman AD, St-Luce S, Avramoglu RK, Maslowska M, Hoang B, Monge JC, Bell A, Mulay S and Cianflone K. The Adipsin-Acylation Stimulating Protein System and Regulation of Intracellular Triglyceride Synthesis. J Clin Invest. 1993; 92:1543-1547.

[26] Germinario R, Sniderman AD, Manuel S, Lefebvre SP, Baldo A, Cianflone K. Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. Metabolism. 1993;42:574-80.

[27] S. Meijssen, H. van Dijk, C. Verseyden, D.W. Erkelens, M. Castro Cabezas. Delayed and Exaggerated Postprandial Complement Component 3 Response in Familial Combined Hyperlipidemia Arterioscler Thromb Vasc Biol. 2002;22:811-816.

[28] Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao XQ, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the anti-inflammatory properties of HDL. The Journal Clin Inv. 2007;117:746-756.

[29] Gordon SM, Deng J, Lu LJ, Davidson WS. Proteomic characterization of human plasma high density lipoprotein fractionated by gel filtration chromatography. J Proteome Res. 2010;9: 5239-5249.

[30] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.40.

[31] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502.

[32] Coll B, Parra S, Alonso-Villaverde C, Aragonés G, Montero M, Camps J, Joven J, Masana L. The role of immunity and inflammation in the progression of atherosclerosis in patients with HIV infection. Stroke. 2007;38:2477-2484.

[33] Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS. American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr. 2008;21:93e111.

[34] Plazak W, Pasowicz M, Kostkiewicz M, Podolec J, Tomkiewicz-Pajak L, Musial J, Podolec P. Influence of chronic inflammation and autoimmunity on coronary calcifications and myocardial perfusion defects in systemic lupus erythematosus patients Inflamm Res. 2011;60:973-980.

[35] Rua-Figueroa I, Arencibia-Mireles O, Elvira M, Erausquin C, Ojeda S, Francisco F, Naranjo A, Rodríguez-Gallego C, Garcia-Laorden I, Rodríguez-Perez J, Rodríguez-

Lozano C. Factors involved in the progress of preclinical atherosclerosis associated with systemic lupus erythematosus: a 2-year longitudinal study. Ann Rheum Dis. 2010;69:1136-1139.

[36] Shiflett AM, Bishop JR, Pahwa A, Hadjuk SL. Human high density lipoproteins are platforms for the assembly of multi-component innate immune complexes. J Biol Chem 2005;280:32578-32585.

[37] B J Van Lenten, S Y Hama, F C de Beer, D M Stafforini, T M McIntyre, S M Prescott, B N La Du, A M Fogelman, and M Navab. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest 1995;96:2758-2767.

[38] McMahon M, Grossman J, FitzGerald J, Dahlin-Lee E, Wallace DJ, Thong BY, Badsha H, Kalunian K, Charles C, Navab M, Fogelman AM, Hahn BH. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 2006;54: 2541-2549.

[39] Borba EF, Borba E. Dyslipoproteinemias in systemic lupus erythematosus: influence of disease, activity, and anticardiolipin antibodies. Lupus.1997;6:533–539.

[40] Gonzàlez M, Ribalta J, Vives G, Iftimie S, Ferré R, Plana N, Guardiola M, Dallinga-Thie G, Masana L, Castro A. Nuclear Magnetic Resonance Lipoprotein Subclasses and the APOE Genotype Influence Carotid Atherosclerosis in Patients with Systemic Lupus Erythematosus.J Rheumatol. 2010;37:2259-2267

[41] Wolbink GJ, Brouwer MC, Buysmann S, ten Berge IJ, Hack CE. CRP-mediated activation of complement in vivo: assessment by measuring circulating complement-C-reactive protein complexes. J Immunol.1996;157:473-479

[42] Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B; ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med. 2007; 357:2109-22.

Legend to the figures and tables

Figure 1. Bivariate correlations between complement compounds and cIMT in the different territories.
* *P<0.05*

Figure 2. Bivariate correlations between complement compounds and total and small HDL particles.
* *P<0.05*

Figure 3. Mean differences regarding levels of HDL particles and the tertiles of cIMT values in the three territories and the mean from the three territories.
* P < 0.05

Table 1. General characteristics of SLE population.

Table 2. Bivariate correlations between complement components and cardiovascular risk factors, inflammatory factors and disease activity markers.

Table 3: Multivariate analyses. Linear regression analyses.