Serum osteopontin negatively impacts on intima-media thickness in patients with systemic lupus erythematosus

Carbone, Federico ; Dallegri, Franco ; Montecucco, Fabrizio ; Poggi, Alessandro ; Nobili, Flavio Mariano ; Cacciapaglia, Fabio ; Afeltra, Antonella ; Moccetti, Tiziano ; Colombo, Barbara M

DOI: https://doi.org/10.1111/eci.13089

PROF. FEDERICO CARBONE (Orcid ID : 0000-0003-2957-4078)

PROF. FABRIZIO MONTECUCCO (Orcid ID : 0000-0003-0823-8729)

Article type : Original Paper

Serum osteopontin negatively impacts on intima media thickness in patients with SLE

Federico Carbone[a], Franco Dallegri[a,b], Fabrizio Montecucco[b,c,], Alessandro Poggi[d], Flavio Mariano Nobili[b,e], Fabio Cacciapaglia[f], Antonella Afeltra[g], Tiziano Moccetti[h], Barbara Maria Colombo[b]

[a] First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, 6 viale Benedetto XV, 16132 Genoa, Italy.

[b] IRCCS Ospedale Policlinico San Martino Genoa – Italian Cardiovascolar Network, 10 largo Benzi, 16132 Genoa, Italy.

[c] First Clinic of Internal Medicine, Department of Internal Medicine and Centre of Excellence for Biomedical Research (CEBR), University of Genoa, 6 viale Benedetto XV, 16132 Genoa, Italy.

[d] Molecular Oncology and Angiogenesis Unit, Policlinico San Martino, Genoa, Italy.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/eci.13089

[e] Clinical Neurology, Department of Neuroscience (DINOGMI), University of Genoa, Ospedale Policlinico San Martino, Genoa, Italy.

[f] Rheumatology Unit Department of Emergency and Organs Transplantation (DETO) University of Bari, Italy.

[g] Unit of Allergology, Immunology, Rheumatology, Department of Medicine, University Campus Bio-Medico, Rome, Italy.

[h] Cellular and Molecular Cardiology Laboratory, Cardiocentro Ticino Foundation and Swiss Institute for Regenerative Medicine (SIRM), Lugano, Switzerland.

Running title: OPN levels are associated with cIMT in SLE.

Correspondence to: Prof. Fabrizio Montecucco, MD, PhD. First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, Genoa, Italy. Address: 6 viale Benedetto XV, 16132 Genoa, Italy. Tel: +39 010 33 51054; Fax: +39 010 353 86 86; E-mail: fabrizio.montecucco@unige.it

Abstract

Background: Ultrasound evaluation of carotid intima media thickness (cIMT) has been extensively used for potentially improving cardiovascular (CV) risk stratification in several patients' categories. Subjects with systemic lupus erythematosus (SLE) have been investigated by both imaging and molecular biomarker approaches with contrasting results. Here, we

focused on the role of osteopontin (OPN) as biomarker of subclinical atherosclerosis associated with SLE.

Materials and Methods: Eighty females (age 18–65 years) affected by SLE and eighty age-matched healthy female controls without a clinical history of CV disease underwent ultrasound evaluation of cIMT and blood sample assay of high-sensitivity C-reactive protein (hs-CRP) and osteopontin (OPN).

Results: Healthy controls and SLE patients significantly differed for CV risk factors (i.e. waist circumference, hypertension and dyslipidemia) and the inflammatory status. Noteworthy, an opposite association between cIMT and OPN was observed in the two study groups. Whereas OPN was positively associated with mean cIMT (r=0.364; p=0.001) in SLE patients, a negative correlation was found in healthy controls. Furthermore, in SLE patients increased circulating levels of OPN were associated with the use of hydroxychloroquine and the positivity for the anti dsDNA autoantibodies. At linear regression analysis, only OPN remained independently associated with cIMT also after adjustment for age, smoking pack-year, Heart SCORE, disease length and steroid therapy length.

Conclusions: These results indicate that serum OPN levels were strongly associated with subclinical atherosclerosis in patients with LES and it might be a useful CV biomarker that requires additional validation in larger trials.

Keywords: systemic lupus erythematosus, carotid intima media thickness, C-reactive protein, osteopontin, and atherosclerosis

.

Introduction

Dysregulation of immune response have been described in all stages of atherosclerosis, from early fatty streak development to vulnerable plaques erosion/rupture [1]. Systemic inflammatory diseases, such as systemic lupus erythematosus (SLE), are closely associated with an accelerated atherosclerosis and substantial epidemiological data attribute to cardiovascular (CV) diseases the excess mortality observed in those patients [2]. Nevertheless, this association is only partially due to SLE itself. Both immunosuppressive treatments and different classes of autoantibodies (e.g. antiphospholipid antibodies) might actively contribute to the increased CV risk [3]. SLE should be considered as an independent CV risk factor and the rate of acute events in those patients is two-fold higher than estimated by traditional risk models (i.e. Framingham and ACC/AHA scores) [4, 5]. On this basis, patients with SLE would generally benefit from additional CV screening and more intensive prevention strategies [6, 7]. To estimate subclinical atherosclerosis, different non-invasive approaches have been used, including carotid intima media thickness (cIMT), pulse wave velocity, and myocardial perfusion strategies [8]. Among those approaches, ultrasound evaluation of cIMT is one of the most used for risk stratification in SLE patients, being characterized by a high sensitivity [9] and with a high predictive value for adverse CV events [10, 11]. Conversely, the majority of biomarkers are burdened by low predictive value. Although biomarkers still require validation and their measurement might be quite expensive, these molecules have been shown to improve the longitudinal prediction of atherosclerotic plaques in SLE patients [12]. Traditionally known for the role in bone homeostasis, osteopontin (OPN) is increasingly emerging as biomarker of vascular inflammation [13]. With this aim, the present study was designed to test the potential role of OPN as a promising biomarker of subclinical atherosclerosis associated with SLE in a previously published cohort [14].

Material and methods

Patients and clinical assessment

Eighty females (aged 18–65 years) affected by SLE and followed as outpatients were enrolled in the present sub-study, as previously described [14]. All subjects fulfilled the diagnostic criteria of the American College of Rheumatology for diagnosis of SLE [15] and were in inactive stage of the disease defined as SLE disease activity index score ≤4 [16]. Eighty age-matched healthy females were the enrolled as controls. None of subjects (both cases and controls) had an history of clinical CV or cerebrovascular events defined as angina, myocardial infarction, transient ischemic attack or ischemic stroke. For all subjects, physical examination, laboratory tests and structured interview were performed. In addition, traditional CV risk factors and family history for CV events were recorded, as previously described [14]. The Ethics Committee of Ospedale Policlinico San Martino (Genoa, Italy) approved this protocol, performed in accordance to the guidelines of the Declaration of Helsinki. Patients gave informed consent before entering in the study.

Study endpoints and power estimation

The primary outcome of the study is to determine whether circulating OPN values independently correlate with the extent of cIMT in SLE patients. Due to the small sample size and the absence of previous studies investigating the subset of patients with SLE, the results concerning the primary outcome should be considered as part of "pilot" study. Nevertheless, the sample size is in line with previous studies investigating the relationship between OPN and cIMT in other autoimmune systemic inflammatory diseases [17-19]).

Ultrasonography measure

All subjects underwent B-mode ultrasonography of extracranial carotid arteries using a 7.5 MHz probe which provides a direct and non-invasive assessment of subclinical atherosclerosis through IMT measurement, as previously described [14]. Right and left common carotid arteries were scanned longitudinally and the common carotid artery IMT (CCA-IMT) measurement was taken 1.0 cm proximal to the carotid bulb, as in this point we have the maximum distance between the intima-lumen and the adventitia-media interfaces in areas without carotid plaque [20]. The cIMT image was then "frozen," and stored for offline analysis. Offline cIMT measurements were performed with the M Ath Demo software (Version 2.0.1.0, by Metris, Argenteuil, France).

Serum biomarker measurement

Serum levels of OPN and high-sensitivity C-reactive protein (hs-CRP) were measured by colorimetric enzyme-linked immunosorbent assay, following manufacturer's instructions (R&D Systems, Minneapolis, MN). The limits of detection were 62.5 pg/mL for OPN and 15.63 pg/mL for hs-CRP. Mean intra- and inter-assay coefficients of variation were below 8% for both [13, 21, 22].

Statistical analysis

Analyses were performed with IBM SPSS Statistics for Windows, Version 23.0 (IBM CO., Armonk, NY). Categorical data are presented as relative and absolute frequencies and analysed by Fisher's exact test. Continuous variables were expressed as median (interquartile range

[IQR] and analysed with Mann-Whitney test as the normality assumption was not demonstrated. Correlations were analysed through the Spearman Rank correlation test. Finally, multiple linear regressions were then performed to model the CCA-IMT with the serum levels of OPN. For all statistical analyses a 2-sided *p*-value <0.05 was considered as statistically significant.

Results

Characteristics of the overall cohort

Baseline characteristics of the whole cohort (n=160) and the two study groups were listed in Supplementary Table 1 and 2. These characteristics implement the already published clinical variables of the cohort [14]. As expected given the young median age (41 years), patients showed low CV risk as calculated by the Heart Score. However, patients with SLE were characterized by increased waist circumference, blood pressure and dyslipidemia. In line with the high levels of classical inflammatory biomarkers (i.e. ESR and hs-CRP), also circulating value of OPN were increased in SLE patients (median value 16.9 *vs.* 25.0 ng/mL, *p*<0.001) (Supplementary Table 1, Figure 1A and 1B).

OPN is differently associated with CCA-IMT in patients with SLE and healthy controls

CCA-IMT was detectable in both right and left common carotid arteries, despite the low CV risk profile (young women). As previously shown in the same cohort for mean CCA-IMT [14], patients with SLE were characterized by an increased right and left values CCA-IMT as compared to healthy controls (*p*<0.001 for all) (Figure 1C-1D). Nevertheless, a positive

correlation between CCA-IMT and traditional CV risk factors (i.e. age, BMI, waist circumference, systolic blood pressure and SCORE Risk) was observed in the both study groups ($p<0.05$ for all) (Table 1).

Unexpectedly, opposite associations between CCA-IMT and OPN was observed in the two study groups. Serum OPN was negatively associated with right (p=0.002), left (p=0.003) and mean (p=0.001) CCA-IMT in healthy controls (Figure 2A, 2C and 2E). Conversely, a positive correlation in SLE patients was observed for right (p=0.004), left (p=0.008) and mean (p=0.014) CCA-IMT (Figure 2B, 2D and 2F). Instead, hs-CRP failed to show any significant correlation with CCA-IMT in the two study groups. (Supplementary Figure 1A-1F).

Serum OPN is positively associated with the inflammatory status and CCA-IMT in SLE patients

In SLE patients, circulating levels of OPN were positively correlated with blood press values and inflammatory status, assessed by ESR and fibrinogen (Table 2). Furthermore, high circulating levels of OPN were associated with the use of hydroxychloroquine and the positivity for the anti dsDNA autoantibodies (Supplementary Table 3). When linear regression analysis was performed, serum OPN was significantly associated with right, left and mean CCA-IMT alongside with age and CV risk assessed by the Heart Score risk (Table 3). However, OPN remained the only variable independently associated with CCA-IMT in the adjusted analysis for age, smoking pack-year, Heart SCORE, disease length and steroid therapy length (Table 3).

Discussion

We showed that serum levels of OPN, but not hs-CRP, were associated to CCA-IMT (a marker of subclinical atherosclerosis) in patients with SLE. Furthermore, the association between serum OPN and cIMT remained significant also after adjustment for age, smoking, Heart

SCORE, disease length and steroid therapy length. Except for this latter, no other classical CV risk factor demonstrated a relevant association with cIMT in SLE patients. The young age of the cohort of women should be taken into account to potentially explain this finding [14]. Furthermore, the characteristics of the study cohort we investigated young women (with SLE or healthy controls) might potentially explain the opposite association between OPN and CCA-IMT observed in the two study groups. Despite speculative, it is likely that in healthy young women OPN does not reflect an increased CV risk (atherosclerosis is expected to be very initial), but rather a response to hormonal or environmental factors [23-26]. Conversely, the strong positive association between OPN and CCA-IMT observed in patients further indicates SLE as a surrogate of a more advanced CV disease independently of age [27]. In this context, OPN would act as an active pro-atherosclerotic factor, as previously suggested [13].

On this basis, OPN emerges as potential biomarker of atherosclerosis in SLE. Traditionally known for the role in bone homeostasis, OPN is emerging as promising biomarker of vascular inflammation and atherogenesis [28]. Also, OPN has been widely recognized as predictor of atherosclerotic plaque rupture in cohorts of patients with severe carotid atherosclerosis [13, 29, 30]. OPN is constitutively expressed by macrophages/foam cells and sustains pro-inflammatory environment within atherosclerotic plaque by exerting an autocrine/paracrine activity [31]. Specifically, OPN contributes to macrophage chemotaxis, activation, survival and pro-inflammatory M1 polarization, in addition to promote neutrophil recruitment and activation [13, 32]. As an additional mechanism, OPN has been demonstrated to regulate interferon-I response, which has a role in both pathophysiology of SLE and subclinical atherosclerosis [33, 34]. Despite highly speculative, available data indicate OPN not only as an innocent biomarker, but rather an active mediator of plaque vulnerability. Similarly, both circulating and intraplaque expression of hs-CRP have been associated with atherosclerotic plaque rupture and the occurrence of adverse CV events [35, 36]. However, clinical studies

failed to clearly demonstrate hs-CRP as a useful biomarker of CV risk in patients with SLE [37-40]. Even, abnormally low levels of CRP have been observed in active SLE [41]. This partial discrepancies as compared to the general population may be due to formation of anti-CRP autoantibodies, as reported in about 35 to 40% of patients with SLE [42]. In addition, a role of CRP polymorphisms has been hypothesized [43]. Conversely, OPN has been already described as reliable biomarker of subclinical atherosclerosis in other systemic inflammatory diseases, such as rheumatoid arthritis [17] and psoriasis [18, 19]. Growing data also indicate an active role of OPN in SLE pathophysiology. Genetic polymorphisms of OPN have been associated with disease activity [44, 45]. This may due to a potential role of OPN variants in developing autoimmune response by inducing Th1 responses and potentiating polyclonal activation of B cells [46]. Future studies may establish whether targeting OPN might modify the natural history of both SLE and related atherosclerosis. We acknowledge that the present study have some limitations. Due to the small sample size of this cohort, a result overestimation has to be carefully considered and no definite conclusions can be stated. However, our cohort was already validated [14] and the sample size is in line with previous studies [17-19]. Similarly, the ultrasonography method to measure cIMT has been widely published and known to be partially dependent on the operator and the methods [14]. Nevertheless, meta-analyses clearly established the increase of cIMT in SLE patients, independent of ultrasonography method [9, 47].

Conclusions

The present study indicates circulating OPN as a sensible biomarker of subclinical atherosclerosis in patients with SLE. As compared with serum levels of hs-CRP, OPN correlates with cIMT independently of traditional CV risk factors or SLE-related variables. Although no

conclusive statements can be formulated, the assessment of serum OPN (alone, or eventually included in a panel of combined biomarkers) might be suggested as a useful tool to implement CV risk stratification in patients with SLE

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

Acknowledgements

This study was supported by a grant from the European Commission (FP7-INNOVATION I HEALTH-F2-2013-602114; Athero-B-Cell: Targeting and exploiting B cell function for treatment in cardiovascular disease).

References

1 Montecucco F, Liberale L, Bonaventura A, Vecchie A, Dallegri F and Carbone F. The Role of Inflammation in Cardiovascular Outcome. *Curr Atheroscler Rep* 2017;**19**:11.

2 Fasano S, Margiotta DP, Gualtierotti R, Corrado A, Berardicurti O, Iacono D *et al.* The incidence of cardiovascular events in Italian patients with systemic lupus erythematosus is lower than in North European and American cohorts: implication of disease-associated and traditional risk factors as emerged by a 16-year retrospective GIRRCS study: GIRRCS=Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale. *Medicine (Baltimore)* 2018;**97**:e0370.

3 Ballocca F, D'Ascenzo F, Moretti C, Omede P, Cerrato E, Barbero U *et al.* Predictors of cardiovascular events in patients with systemic lupus erythematosus (SLE): a systematic review and meta-analysis. *Eur J Prev Cardiol* 2015;**22**:1435-41.

4 Magder LS and Petri M. Incidence of and risk factors for adverse cardiovascular events among patients with systemic lupus erythematosus. *Am J Epidemiol* 2012;**176**:708-19.

5 Jafri K, Ogdie A, Qasim A, Patterson SL, Gianfrancesco M, Izadi Z *et al.* Discordance of the Framingham cardiovascular risk score and the 2013 American College of Cardiology/American Heart Association risk score in systemic lupus erythematosus and rheumatoid arthritis. *Clin Rheumatol* 2018;**37**:467-74.

6 Andrades C, Fuego C, Manrique-Arija S and Fernandez-Nebro A. Management of cardiovascular risk in systemic lupus erythematosus: a systematic review. *Lupus* 2017;**26**:1407-19.

7 Hallajzadeh J, Khoramdad M, Izadi N, Karamzad N, Almasi-Hashiani A, Ayubi E *et al.* The association between metabolic syndrome and its components with systemic lupus erythematosus: a comprehensive systematic review and meta-analysis of observational studies. *Lupus* 2018:961203317751047.

8 Teixeira V and Tam LS. Novel Insights in Systemic Lupus Erythematosus and Atherosclerosis. *Front Med (Lausanne)* 2017;**4**:262.

9 Henrot P, Foret J, Barnetche T, Lazaro E, Duffau P, Seneschal J *et al.* Assessment of subclinical atherosclerosis in systemic lupus erythematosus: A systematic review and meta-analysis. *Joint Bone Spine* 2018;**85**:155-63.

10 Tyrrell PN, Beyene J, Feldman BM, McCrindle BW, Silverman ED and Bradley TJ. Rheumatic disease and carotid intima-media thickness: a systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol* 2010;**30**:1014-26.

11 Kao AH, Lertratanakul A, Elliott JR, Sattar A, Santelices L, Shaw P *et al.* Relation of carotid intima-media thickness and plaque with incident cardiovascular events in women with systemic lupus erythematosus. *Am J Cardiol* 2013;**112**:1025-32.

12 McMahon M, Skaggs BJ, Grossman JM, Sahakian L, Fitzgerald J, Wong WK *et al.* A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus. *Arthritis Rheumatol* 2014;**66**:130-9.

13 Carbone F, Rigamonti F, Burger F, Roth A, Bertolotto M, Spinella G *et al.* Serum levels of osteopontin predict major adverse cardiovascular events in patients with severe carotid artery stenosis. *Int J Cardiol* 2018;**255**:195-9.

14 Colombo BM, Cacciapaglia F, Puntoni M, Murdaca G, Rossi E, Rodriguez G *et al.* Traditional and non traditional risk factors in accelerated atherosclerosis in systemic lupus erythematosus: role of vascular endothelial growth factor (VEGATS Study). *Autoimmun Rev* 2009;**8**:309-15.

15 Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;**25**:1271-7.

16 Bombardier C, Gladman DD, Urowitz MB, Caron D and Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992;**35**:630-40.

17 Bazzichi L, Ghiadoni L, Rossi A, Bernardini M, Lanza M, De Feo F *et al.* Osteopontin is associated with increased arterial stiffness in rheumatoid arthritis. *Mol Med* 2009;**15**:402-6.

18 Chen YJ, Shen JL, Wu CY, Chang YT, Chen CM and Lee FY. Elevated plasma osteopontin level is associated with occurrence of psoriasis and is an unfavorable cardiovascular risk factor in patients with psoriasis. *J Am Acad Dermatol* 2009;**60**:225-30.

19 Robati RM, Partovi-Kia M, Sadat-Amini H, Haghighatkhah HR, Younespour S and Toossi P. Serum osteopontin level and common carotid artery intima-media wall thickness in psoriasis. *Int J Dermatol* 2016;**55**:e262-7.

20 Sidhu PS and Desai SR. A simple and reproducible method for assessing intimal-medial thickness of the common carotid artery. *Br J Radiol* 1997;**70**:85-9.

21 Carbone F, Vuilleumier N, Burger F, Roversi G, Tamborino C, Casetta I *et al.* Serum osteopontin levels are upregulated and predict disability after an ischaemic stroke. *Eur J Clin Invest* 2015;**45**:579-86.

22 Carbone F, Nulli Migliola E, Bonaventura A, Vecchie A, De Vuono S, Ricci MA *et al.* High serum levels of C-reactive protein (CRP) predict beneficial decrease of visceral fat in obese females after sleeve gastrectomy. *Nutr Metab Cardiovasc Dis* 2018;**28**:494-500.

23 Humphries B, Fenning A, Dugan E, Guinane J and MacRae K. Whole-body vibration effects on bone mineral density in women with or without resistance training. *Aviat Space Environ Med* 2009;**80**:1025-31.

24 Miyajima J, Hayashi T, Saito K, Iida S and Matsuoka K. The Interaction between female sex hormone receptors and osteopontin in a rat hyperoxaluric model. *Kurume Med J* 2010;**57**:73-80.

25 Farr JN, Charkoudian N, Barnes JN, Monroe DG, McCready LK, Atkinson EJ *et al.* Relationship of sympathetic activity to bone microstructure, turnover, and plasma osteopontin levels in women. *J Clin Endocrinol Metab* 2012;**97**:4219-27.

26 Saklamaz A, Calan M, Yilmaz O, Kume T, Temur M, Yildiz N *et al.* Polycystic ovary syndrome is associated with increased osteopontin levels. *Eur J Endocrinol* 2016;**174**:415-23.

27 Carbone F, Nencioni A, Mach F, Vuilleumier N and Montecucco F. Evidence on the pathogenic role of auto-antibodies in acute cardiovascular diseases. *Thromb Haemost* 2013;**109**:854-68.

28 Wolak T. Osteopontin - a multi-modal marker and mediator in atherosclerotic vascular disease. *Atherosclerosis* 2014;**236**:327-37.

29 Golledge J, McCann M, Mangan S, Lam A and Karan M. Osteoprotegerin and osteopontin are expressed at high concentrations within symptomatic carotid atherosclerosis. *Stroke* 2004;**35**:1636-41.

30 de Kleijn DP, Moll FL, Hellings WE, Ozsarlak-Sozer G, de Bruin P, Doevendans PA *et al.* Local atherosclerotic plaques are a source of prognostic biomarkers for adverse cardiovascular events. *Arterioscler Thromb Vasc Biol* 2010;**30**:612-9.

31 Nystrom T, Duner P and Hultgardh-Nilsson A. A constitutive endogenous osteopontin production is important for macrophage function and differentiation. *Exp Cell Res* 2007;**313**:1149-60.

32 Singh R, Hui T, Matsui A, Allahem Z, Johnston CD, Ruiz-Torruella M *et al.* Modulation of infection-mediated migration of neutrophils and CXCR2 trafficking by osteopontin. *Immunology* 2017;**150**:74-86.

33 Somers EC, Zhao W, Lewis EE, Wang L, Wing JJ, Sundaram B *et al.* Type I interferons are associated with subclinical markers of cardiovascular disease in a cohort of systemic lupus erythematosus patients. *PLoS One* 2012;**7**:e37000.

34 Crow MK. Type I interferon in the pathogenesis of lupus. *J Immunol* 2014;**192**:5459-68.

35 Schlager O, Exner M, Mlekusch W, Sabeti S, Amighi J, Dick P *et al.* C-reactive protein predicts future cardiovascular events in patients with carotid stenosis. *Stroke* 2007;**38**:1263-8.

36 Bonaventura A, Mach F, Roth A, Lenglet S, Burger F, Brandt KJ *et al.* Intraplaque Expression of C-Reactive Protein Predicts Cardiovascular Events in Patients with Severe Atherosclerotic Carotid Artery Stenosis. *Mediators Inflamm* 2016;**2016**:9153673.

37 Doria A, Shoenfeld Y, Wu R, Gambari PF, Puato M, Ghirardello A *et al.* Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. *Ann Rheum Dis* 2003;**62**:1071-7.

38 Maksimowicz-McKinnon K, Magder LS and Petri M. Predictors of carotid atherosclerosis in systemic lupus erythematosus. *J Rheumatol* 2006;**33**:2458-63.

39 de Leeuw K, Smit AJ, de Groot E, van Roon AM, Kallenberg CG and Bijl M. Longitudinal study on premature atherosclerosis in patients with systemic lupus erythematosus. *Atherosclerosis* 2009;**206**:546-50.

40 Rua-Figueroa I, Arencibia-Mireles O, Elvira M, Erausquin C, Ojeda S, Francisco F *et al.* Factors involved in the progress of preclinical atherosclerosis associated with systemic lupus erythematosus: a 2-year longitudinal study. *Ann Rheum Dis* 2010;**69**:1136-9.

41 Dima A, Opris D, Jurcut C and Baicus C. Is there still a place for erythrocyte sedimentation rate and C-reactive protein in systemic lupus erythematosus? *Lupus* 2016;**25**:1173-9.

42 Meyer O. Anti-CRP antibodies in systemic lupus erythematosus. *Joint Bone Spine* 2010;**77**:384-9.

43 Kim HA, Chun HY, Kim SH, Park HS and Suh CH. C-reactive protein gene polymorphisms in disease susceptibility and clinical manifestations of Korean systemic lupus erythematosus. *J Rheumatol* 2009;**36**:2238-43.

44 Rullo OJ, Woo JM, Parsa MF, Hoftman AD, Maranian P, Elashoff DA *et al.* Plasma levels of osteopontin identify patients at risk for organ damage in systemic lupus erythematosus. *Arthritis Res Ther* 2013;**15**:R18.

45 Lee YH and Song GG. Correlation between circulating osteopontin level in systemic lupus erythematosus and disease activity and associations between osteopontin polymorphisms and disease susceptibility: A meta-analysis. *Lupus* 2017;**26**:132-8.

46 Kaleta B. Role of osteopontin in systemic lupus erythematosus. *Arch Immunol Ther Exp (Warsz)* 2014;**62**:475-82.

47 Wu GC, Liu HR, Leng RX, Li XP, Li XM, Pan HF *et al.* Subclinical atherosclerosis in patients with systemic lupus erythematosus: A systemic review and meta-analysis. *Autoimmun Rev* 2016;**15**:22-37.

Figure legend

Figure 1. Box plot comparing healthy controls and patients with systemic lupus erythematosus (SLE). Mann-Whitney test comparing high-sensitivity C-reactive protein (hs-CRP) (**A**), osteopontin (OPN) (**B**) and right (**C**), and left (**D**) of common carotid artery-intima media thickness (CCA-IMT) in healthy controls and patients with SLE.

Figure 2. Spearman's Rank correlation. Serum concentrations of osteopontin (OPN) correlate with right (**A** and **B**), left (**C** and **D**) and mean values (**E** and **F**) of common carotid artery-intima media thickness (CCA-IMT), negatively in healthy controls and positively in SLE patients.

Table 1. Correlation of common carotid artery intima media thickness (CCA IMT) in the two study groups.

	Right CCA IMT			**Left CCA IMT**			**Mean CCA IMT**	
Healthy controls								
Age	0.539	**<0.001**		0.512	**<0.001**		0.574	**<0.001**
Weight	0.189	0.101		0.207	0.069		0.213	0.063
BMI	0.238	**0.037**		0.248	**0.028**		0.260	**0.022**
Waist circumference	0.321	**0.004**		0.325	**0.004**		0.343	**0.002**
sBP	0.303	**0.007**		0.341	**0.002**		0.322	**0.004**
dBP	0.124	0.283		0.187	0.102		0.163	0.156
Smoking pack-year	0.004	0.973		0.122	0.288		0.069	0.554
SCORE Risk Chart	0.274	**0.016**		0.287	**0.011**		0.301	**0.008**
Fasting glycaemia	0.072	0.531		0.217	0.057		0.160	0.166
Total cholesterol	0.095	0.410		0.195	0.087		0.151	0.189
HDL	0.094	0.414		0.014	0.902		0.057	0.625
LDL	-0.096	0.406		0.036	0.757		-0.034	0.767
TAG	0.069	0.549		0.229	0.044		0.155	0.177
SLE								
Age	0.394	**<0.001**		0.414	**<0.001**		0.422	**<0.001**
Weight	0.013	0.912		0.113	0.330		0.092	0.424
BMI	0.125	0.276		0.195	0.089		0.177	0.123
Waist circumference	0.115	0.316		0.164	0.154		0.166	0.148
sBP	0.166	0.147		0.224	0.050		0.29	0.056
dBP	0.153	0.182		0.206	0.072		0.185	0.108
Smoking pack-year	0.215	0.059		0.211	0.066		0.239	**0.036**
SCORE Risk Chart	0.290	**0.010**		0.303	**0.007**		0.328	**0.004**
SLE duration	0.260	**0.021**		0.144	0.210		0.212	0.064
Steroid therapy length	0.205	0.072		0.171	0.137		0.200	0.081

Fasting glycaemia	-0.115	0.325		-0.223	0.056		-0.179	0.127
Total cholesterol	0.042	0.717		-0.007	0.953		-0.004	0.972
HDL	0.100	0.382		-0.044	0.706		-0.023	0.840
LDL	-0.028	0.756		0.107	0.354		0.091	0.429
TAG	-0.036	0.756		0.107	0.354		0.091	0.429

Comparisons were drawn by Spearman Rank correlation test.

BMI: body mass index; sBP: systolic blood pressure; dBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TAG: triglyceride.

Table 2. Correlation between osteopontin (OPN) and clinical parameters in the subgroups of SLE patients.

	OPN	
	r	***p*-value**
Age	0.108	0.186
Weight	-0.060	0.465
BMI	0.059	0.475
Waist circumference	0.070	0.392
sBP	0.156	0.055
dBP	0.243	**0.003**
Smoking pack-year	0.052	0.647
SCORE Risk Chart	0.136	0.097
Disease length	0.085	0.453
Steroid therapy length	0.151	0.180
ESR	0.398	**<0.001**
Fibrinogen	0.355	**<0.001**
Fasting glycaemia	0.159	0.054
Total cholesterol	0.009	0.913
HDL	0.094	0.253
LDL	-0.053	0.520
TAG	0.106	0.194

Comparisons were drawn by Spearman Rank correlation test.

BMI: body mass index; sBP: systolic blood pressure; dBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TAG: triglyceride.

Table 3. Linear regression analysis in the subgroup of patients with SLE.

	Univariate		**Adjusted**	
	β	***p*-value**	β	***p*-value**
Right CCA IMT				
OPN	0.323	**0.004**	0.291	**0.008**
Age	0.278	**0.014**	0.240	**0.028**
Smoking pack-year	0.160	0.163	-	-
Heart SCORE	0.218	0.055	-	-
Disease length	0.181	0.113	-	-
Steroid therapy length	0.140	0.220	-	-
Left CCA IMT				
OPN	0.299	**0.008**	0.256	**0.017**
Age	0.370	**0.001**	0.266	0.044
Smoking pack-year	0.159	0.166	-	-
Heart SCORE	0.317	**0.005**	0.127	0.332
Disease length	0.108	0.349	-	-
Steroid therapy length	0.178	0.122	-	-
Mean CCA IMT				
OPN	0.280	**0.014**	0.240	**0.027**
Age	0.342	**0.002**	0.232	0.083
Smoking pack-year	0.190	0.097	0.084	0.503
Heart SCORE	0.307	**0.007**	0.141	0.291
Disease length	0.149	0.196	-	-
Steroid therapy length	0.165	0.151	-	-

CCA IMT: common carotid artery intima media thickness; OPN: osteopontin.

Figure 1

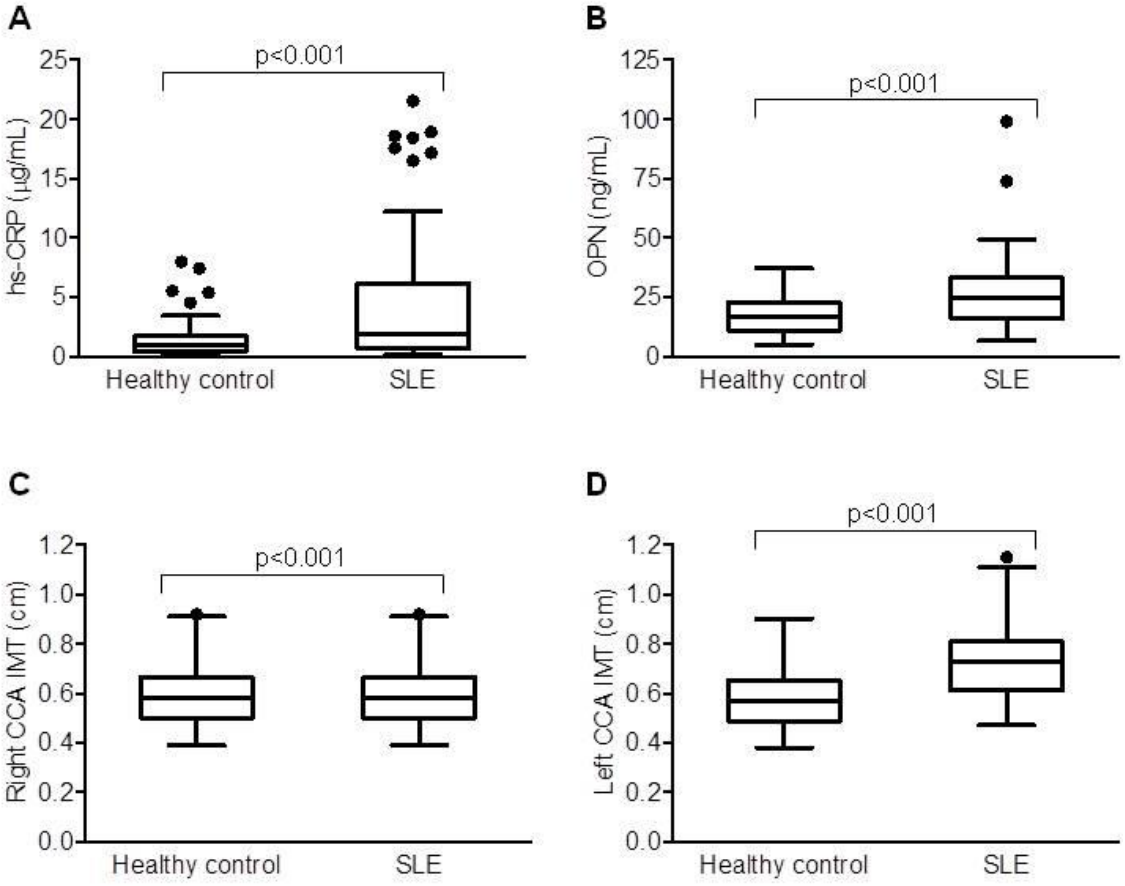

Figure 2