Serum calprotectin is a biomarker of carotid atherosclerosis in patients with primary Sjögren's syndrome

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Abstract

Objective

We aimed to identify the association of carotid atherosclerosis with the traditional risk factors, disease features, cytokine profile, and calprotectin in patients with primary Sjögren's syndrome (pSS).

Methods

63 primary pSS patients and 63 age- and sex-matched healthy controls underwent carotid ultrasound, clinical and laboratory examination. The presence of carotid plaques was taken as carotid atherosclerosis. The covariates of carotid atherosclerosis were identified in univariate and multivariate regressions.

Results

Patients with pSS had higher prevalence of carotid atherosclerosis (13% vs. 2%, $p<0.05$) and higher serum levels of calprotectin, tumour necrosis factor receptor 2 (TNF-R2), hepatocyte growth factor (HGF), and monocyte chemoattractant protein-1 (MCP-1) than controls. Sex, menopause, and the prevalence of traditional cardiovascular did not differ between groups (all $p>0.05$). In univariate analyses, serum calprotectin, most traditional cardiovascular (age, male sex, metabolic syndrome, hypertension, hypertriglyceridaemia, and serum creatinine), and some disease-associated risk factors (glucocorticoid or saliva substitute use, constitutional domain of Eular-Sjögren's syndrome disease activity index - EULAR) were associated with a higher risk for plaque. In a multivariate analysis, having pSS and higher serum calprotectin were associated with carotid atherosclerosis independent of traditional risk factors.

Conclusion

pSS have a higher prevalence of carotid atherosclerosis, which is associated with higher serum calprotectin level independent of traditional cardiovascular risk factors. Our findings suggest calprotectin as a biomarker of subclinical atherosclerosis in pSS.

Key words

Sjögren's syndrome, subclinical cardiovascular disease, atherosclerosis, risk factors, biomarkers, calprotectin

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Introduction

Cardiovascular disease is a frequent and potentially severe complication of autoimmune disease, well described in systemic lupus erythematosus (SLE) (1) and rheumatoid arthritis (RA) (2-4), while the literature on primary Sjögren's syndrome (pSS) is scarce. However, recent studies from different countries has shown that the risk for a cardiovascular event seems to be increased also in pSS (5-9). pSS is characterised by glandular and systemic inflammation with slow progression, contributing to higher risk for cardiovascular risk (10).

Atherosclerosis is accelerated in autoimmune diseases, such as SLE and RA, and has been associated with a higher burden of traditional risk factors, production of atherogenic pro-inflammatory cytokines, imbalance between damage and endothelium repair, higher disease activity, and treatment-associated vascular damage (2). Previous research has demonstrated that traditional risk factors like hypertension, hypertriglyceridaemia, and low-HDL are also more prevalent in pSS (11-13), but the relationship between atherosclerosis, traditional risk factors, and disease activity is not well explored (7, 14).

A few circulating biomarkers of atherosclerosis have been evaluated in pSS (15-17). Calprotectin may be of potential interest as a biomarker of atherosclerosis (18). It binds receptors of advanced glycation end products (RAGE), thereby activating the innate system and stimulating pro-inflammatory cytokine production by macrophages in the atherosclerotic plaque (19, 20).

So far, only a few studies evaluating the relationship between inflammatory biomarkers, traditional cardiovascular risk factors, and carotid atherosclerosis have been published from pSS patients and with conflicting results (15, 21-23). Thus, the objectives of this study were to evaluate if carotid atherosclerotic plaque prevalence was higher in pSS compared to healthy controls and to identify the association of carotid atherosclerosis with traditional risk factors, disease features, cytokine profile, and with calprotectin.

Patients and methods

Design and study population

In this transversal study, 63 pSS patients classified according to the American-European Consensus Group (24) and 63 age- and sex-matched controls were included. The patients and controls were recruited from two university hospitals (The Federal University of Espírito Santo and Federal University of Minas Gerais). The controls were volunteers, mainly workers at the university and were recruited through posters distributed in the hospital. The exclusion criteria for both groups were any other autoimmune inflammatory disease, concomitant infection or inflammatory condition, chronic infection, previous cardiovascular event, and cardiac, renal, respiratory, and/or liver disease or insufficiency, and age ≤18 or ≥70 years. The study was approved by the Ethics Committee of the University, Hospital Cassiano Antônio de Moraes (protocol number 407.199/2013), and all participants gave written informed consent.

Clinical parameters

Self-reported information on age, sex, ethnicity, family history of premature cardiovascular disease, history of cardiovascular risk-factors, and use of medication were collected using a structured interview facilitated by the same physician (25). Disease activity was measured by EULAR Sjögren's syndrome disease activity index (ESSDAI). Blood pressure measurement was performed in accordance with guidelines of the Brazilian Society of Hypertension (27), which was considered hypertension if the clinical systolic blood pressure (SBP) ≥140 mmHg, or diastolic blood pressure (DBP) ≥90 mmHg, or when using antihypertensive drug treatment.

Body height and weight were measured, and body mass index (BMI) was calculated. Obesity was considered present if the BMI ≥30 kg/m^2. Waist circumference (WC) was measured at the level midway between the 12th costal arch and the anterior superior iliac crest and considered increased (abdominal obesity) if WC ≥80cm for women and ≥94cm for men, in accord-

ance with the Brazilian Guidelines (28). Diabetes was considered present if fasting glucose ≥126 mg/dl or use of hypoglycaemic drug treatment.

Dyslipidaemia was considered present when total cholesterol ≥200 mg/dl or high-density lipoprotein (HDL) cholesterol<40 mg/dl or low density lipoprotein (LDL) cholesterol ≥130 mg/dl or statin treatment or triglycerides ≥ 50 mg/dl (28).

Metabolic syndrome (MetS) was identified if ≥3 of the following 5 criteria were present (28): WC (≥94 cm for men and ≥80 cm for women), HDL-cholesterol (<40 mg/dl for men, and <50 mg/dl for women), hypertension (SBP ≥130 mmHg or DBP ≥85 mmHg or anti hypertensive drug treatment), hypertriglyceridaemia (≥150 mg/dl) or fasting glucose ≥100 mg/dl. We calculated the Global risk score taking sex, age, total and HDL-cholesterol, SBP, smoking and diabetes into account (29, 30).

Carotid ultrasound

All individuals underwent carotid ultrasound examination performed by the same experienced radiologist (CCD), using B-mode ultrasound (My Lab 70 Xvision, Esaote) and a 6-18 MHZ linear transducer (LA435). The common, external and internal carotid arteries were evaluated on both sides, following a standardised protocol (32, 33). Carotid atherosclerosis was defined as presence of atherosclerotic plaque, *i.e.* identification of either a protrusion into the lumen >0.5 mm, or a protrusion into the lumen >50% of intima-media thickness (IMT) at adjacent area, or IMT ≥1.5mm.

Blood collection and serum storing

Serum samples were obtained by peripheral blood collection in BD Vacutainer® SST™ II Advanced plus blood collection tubes. To allow clotting, the tubes were incubated at room temperature for 30-60 min. Following centrifugation at 1800 x g for 10 min, the serum layer was extracted, aliquoted and stored at -70°C until analysis.

High-sensitive C-reactive protein and cytokines analyses

Some biomarkers associated with inflammation and cardiovascular disease were analysed. High-sensitivity C-reactive protein (hs-CRP), and interleukins (IL) were analysed by Multiplex system, using commercial kits Milliplex Map®. Beyond hs-CRP, it was included IL-1β, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), hepatocyte growth factor (HGF), TNF-α and receptors I/II, leptin, resistin, and adiponectin.

Calprotectin

Calprotectin was investigated by commercial kits (CALPROLAB™, Lysaker, Norway).

Table I. Baseline characteristics of study population.

	pSS (nn=63)	Controls (n=63)	*p*-value
Age (years)	50 ± 11	50 ± 10	0.930
Female (%)	93.7	95.2	0.687
Ethnicity			0.053
Caucasian (%)	19	33.3	
Mixed ethnicity (%)	74.6	54	
Black (%)	6.3	12.7	
Weight (47)	67.42 ± 12.93	70.1 ± 12.25	0.231
BMI (kg/m^2)	26.52 ± 4.43	28.03 ± 5.04	0.072
Abdominal circumference (cm)	88.8 ± 30	90.9 ± 12.3	0.355
Metabolic syndrome (%)	44	49	0.581
Hypertension (%)	40	43	0.717
Diabetes (%)	10	10	0.991
SBP (mmHg)	129 ± 19	137 ± 22	**0.021**
DBP (mmHg)	81 ± 11	90 ± 10	**<0.001**
Total cholesterol (mg/dl)	181 ± 30	244 ± 267	**<0.001**
LDL cholesterol (mg/dl)	101.77 ± 26	134.63 ± 30.98	**<0.001**
HDL cholesterol (mg/dl)	51 ± 11	51 ± 10	0.988
Triglycerides (mg/dl)	135 ± 77	149 ± 207	0.752
Tobacco (%)	3	3	0.894
Menopause (%)	55	45	0.180
Global risk score	8 ± 6	11 ± 6	**0.04**
Global risk rate (%)	7.24 ± 6.72	9.12 ± 7.45	0.074
Fasting glucose (mg/dL)	91 ± 16	99 ± 29	**0.013**
Haemoglobin (g/dL)	13.1 ± 1.2	13.3 ± 0.9	0.136
Leucocyte	5869 ± 2287	6092 ± 1625	0.530
Lymphocyte	1598 ± 828	2008 ± 473	**0.001**
Platelets (units/1,000)	254.5 ± 73.9	248.9 ± 61.9	0.646
ESR (mm/1h)	31 ± 18	23 ± 18	0.219
Urea (mg/dl)	29.18 ± 8.85	24 ± 8	**0.001**
Serum creatinine (mg/dl)	0.71 ± 0.13	0.61 ± 0.12	**<0.001**
ESSPRI	5.53 ± 2.78		
ESSDAI	5.16 ± 5.96		
Disease duration (months)	111 ± 76		
Current systemic manifestation (%)	71.4		
Anti-Ro-SSA (U/ml) (%)	69.8		
Anti-La-SSB (U/ml) (%)	30.2		
ANA (%)	90.3		
RF (UI/ml) (%)	19.1		
Gamma globulins (g/dl)	1.47 ± 0.54		
IgG (mg/dl)	1475.96 ± 593.42		
IgM (mg/dl)	154.99 ± 141.71		
IgA (mg/dl)	317.87 ± 144.66		
C3 (mg/dl)	152.90 ± 33.34		
C4 (mg/dl)	32.80 ± 11.77		
hsCRP (mg/dl)	4.09 ± 8.67		
Glucocorticoid (%)	32		
Hydroxychloroquine (%)	40		

pSS: primary Sjögren's syndrome; BMC: body mass index; LDL: low density lipoprotein; HDL: high density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; ERS: erythrocyte rate sedimentation; *t*-test, Mann-Whitney and chi-square tests were used. ESSDAI: EULAR SS Disease Activity; ESSPRI: EULAR SS Patient Report Index; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IgA: Immunoglobulin A; C3: Complement 3; C4: Complement 4; hsCRP: high sensitive C-reactive protein; Anti-Ro-SSA: anti-Ro-SSA antibodies; Anti-La-SSB: anti-La-SSB antibodies; RF: rheumatoid factor; ANA: anti-nuclear autoantibody.

Statistical analysis

Statistical analysis was performed using the SPSS v. 21 program (IBM, Almond, USA). The Kolmogorov-Smirnov test was used to identify Gaussian distribution. Mann-Whitney and *t*-test were used to compare quantitative variables, as appropriate, and Chi-square test to qualitative parameters. Univariate and multivariate logistic regression analyses were used to identify factors associated with carotid atherosclerosis (plaque). Criteria for including variables in the multivariate models was reaching *p*-value <0.1 in univariate analysis. For all analyses, $p<0.05$ was considered statistically significant.

Results

Traditional cardiovascular risk factors like hypertension, diabetes, smoking, hypertrygliceridaemia, BMI, WC, and MetS did not differ between patients and controls (Table I). However, the control group showed higher blood pressure, fasting glucose, and cholesterol levels, indicating that knowledge of elevated traditional cardiovascular risk factors may have been a driving force for participation in the project (Table I). Despite that, pSS patients had higher prevalence of carotid atherosclerosis (13% *vs*. 2%, $p=0.015$) (Table II). In univariate analyses, most traditional cardiovascular risk factors were associated with higher risk for presence of carotid atherosclerosis in the total study population (Table II). In multivariate analysis, having pSS predicted higher risk for presence of carotid atherosclerosis independent of traditional cardiovascular risk factors (Table III).

Among pSS patients, disease-associated risk factors including glucocorticoid use, constitutional ESSDAI-domain and use of saliva substitute were associated with carotid atherosclerosis plaque (all $p<0.05$) (Table IV). In contrast, presence of carotid atherosclerosis was not associated with ESSDAI total score or any other domain of ESSDAI, or with presence of auto-antibodies or leucocyte or lymphocyte count (Table I). Furthernore, TNF-R2 and some cytokines (MCP-1 and HGF) potentially important in the pathogenesis of atherosclerosis were higher in pSS (Table V).

Table II. Cardiovascular risk factors for carotid atherosclerosis in pSS and controls.

Risk Factor	Group	Plaque		OR	OR 95%CI	*p*-value
		Yes (n=9)	No (n=117)			
pSS disease	pSS	8 (88%)	55 (47%)	9.02	1.09-94.41	0.040
	Controls	1 (11%)	62 (53%)			
Gender	male	2 (22%)	5 (4%)	6.4	1.05-39.86	
	female	7 (78%)	112 (96%)			0.040
MetS	Yes	7 (88%)	51 (44%)	8.92	1.06-74.86	0.040
	No	1 (13%)	65 (56%)			
Hypertension	Yes	7 (78%)	45 (38%)	5.6	1.11-28.16	0.044
	No	2 (22%)	72 (62%)			
Hypertriglyceridaemia	Yes	6 (67%)	30 (26%)	5.8	1.37-24.64	0.017
	No	3 (33%)	87 (74%)			
Familiar history of myocardial infarct	Yes	7 (88%)	45 (41%)	10.27	1.22-86.33	0.002
	No	1 (13%)	66 (59%)			
Glucocorticoid use	Yes	5 (63%)	15 (27%)	11.76	2.68-51.59	0.001
	No	3 (38%)	40 (73%)			
Global risk score	Yes	15 ± 2.92	9 ± 6.21	0.84	0.73-0.93	0.009
	No					
Serum creatinine (mg/dL)		0.89 ± 0.25	0.67 ± 0.18	0.04	0.01-0.55	0.016
Age (years)		61.22 ± 5.19	48.72 ± 10.85	0.86	0.78-0.95	0.002

CI: confidence interval; OR: odds ratio; pSS: primary Sjögren's syndrome; MetS: metabolic syndrome.

Table III. Sjögren's syndrome disease-risk for carotid atherosclerosis after adjusting for age, hypertriglyceridaemia, hypertension and creatinin level.

Risk factor	Model 1				Model 2			
	OR	95% CI Lower Limit	95% CI Upper Limit	*p*-value	OR	95% CI Lower Limit	95% CI Upper Limit	*p*-value
Sjögren's syndrome	28.76	1.69	490.19	0.020	-	-	-	-
Age	0.82	0.72	0.94	0.004	-	-	-	-
Hypertension	1.89	0.27	13.05	0.519	1.04	0.99	1.09	0.004
Hypertriglyceridaemia	10.07	1.16	87.33	0.036	-	-	-	-
Serum creatinine	1.07	0.01	23.06	0.965	10.20	0.60	166.67	0.055
Calprotectin	-	-	-	-	1.001	1.000	1.001	0.023

CI: confidence interval; OR: odds ratio.

Interesting, among all measured circulating biomarkers, only calprotectin was independently associated with presence of carotid atherosclerosis in the patient group (Table III). Of note, this association was independent of serum creatinine and presence of hypertension (Table III). Calprotectin was also associated with inflammation (CRP) and with cytokines involved with MetS (resistin and adiponectin) and with atherogenesis (HGF and TNF receptors) (Table V).

Discussion

The present study demonstrates that having pSS is associated with increased prevalence of carotid atherosclerosis independent of traditional cardiovascular risk factors. In particular calprotectin was higher in pSS patients and independently associated with presence of carotid plaque, indicating that calprotectin may be used as a biomarker of subclinical atherosclerosis in pSS.

It is well known from other populations that subclinical atherosclerosis evaluated by presence of carotid atherosclerotic plaque by ultrasound is predictive of cardiovascular death, myocardial infarction and ischaemic stroke, and significantly improves risk prediction beyond that of clinical risk factor assessment

Table IV. Disease-factors associated with carotid atherosclerosis plaque in pSS.

Risk factor	Group	Plaque		*p*-value
		Yes (n=8)	No (n=55)	
Glucocorticoid use	yes	6 (75%)	17 (31%)	**0.016**
Saliva substitute	yes	3 (38%)	6 (11%)	**0.045**
Menopause	yes	6 (100%)	29 (55%)	**0.032**
Anti-Ro-SSA	yes	7 (88%)	37 (69%)	0.270
ESSDAI constitutional	yes	1 (13%)	0 (0%)	**0.046**
ESSDAI		5.38 ± 5.88	5.13 ± 6.03	0.967
Lag time		88 ± 71.47	57.84 ± 63.93	0.265
Disease duration		138 ± 96.55	107.16 ± 72.18	0.451
Calprotectin (ng/mL)		3035 ± 2825.40	1681.89 ± 1341.55	**0.025**

pSS: primary Sjögren's syndrome; ESSDAI: EULAR SS Disease Activity; Anti-Ro-SSA: anti-Ro-SSA antibodies.

Table V. Comparison of inflammation and cytokines between patients and controls and their correlation with calprotectin.

Biomarker	pSS (n=63)	Controls (n=63)	*p*-value*	Spearman	*p*-value**
Calprotectin	1878.8 ± 1501.1	1215 ± 520	**0.039**	-	-
IL-1B (pg/ml)	1.9 ± 5.45	0.49 ± 0.7	0.732	0.46	0.622
IL-6 (pg/ml)	11.65 ± 23.38	5.21 ± 9.23	0.229	0.019	0.839
TNF-a (pg/ml)	4.7 ± 5.0	3.6 ± 3.4	0.239	-0.138	0.136
TNF-R1 (pg/ml)	1113.65 ± 691.39	919.01 ± 408.53	0.435	0.277	**0.002**
TNF-R2 (pg/ml)	8144.32 ± 4106.39	5914.74 ± 2458.23	**0.002**	0.269	**0.003**
MCP-1 (pg/ml)	251.8 ± 139.5	208.3 ± 83.8	0.186	0.081	0.382
Leptin (pg/ml)	3056.8 ± 2295.3	2786.6 ± 1881.1	0.704	-0.83	0.373
Resistin (pg/ml)	12031.8 ± 4923.6	12902.2 ± 4226	0.241	0.379	**0.000**
Adiponectin (pg/ml)	152558.2 ± 28456.5	40875.65 ± 38121.54	**0.000**	0.100	0.280
HGF (pg/ml)	266.1 ± 189.5	206.6 ± 150.7	**0.047**	0.182	**0.049**
CRP (mg/dL)	4.09 ± 8.67	3.36 ± 7.09	0.115	0.391	**0.000**

pSS: primary Sjögren's syndrome; IL: interleukin; TNF: tumour necrosis fator; TNF-RI: tumour necrosis fator receptor 1; TNF-RII: tumour necrosis factor receptor II; MCP-1: monocyte chemo attractant protein-1; HGF: hepatocyte growth factor; CRP: C-reactive protein. *Comparison between controls and patients, *p*-value ≤0.05, *Comparison between controls and pSS by Mann-Whitney, **Spearman correlation between calprotectin and cytokines.

(31). Our results demonstrate that presence of pSS is associated with increased risk for subclinical atherosclerosis, adding to smaller previous studies reporting higher carotid intima-media thickness by ultrasound and higher aortic stiffness measured by pulse wave velocity in pSS patients compared to healthy controls (14, 15, 22, 23). Of note, although most traditional cardiovascular risk factors including age, male gender, MetS, hypertrigliceridaemia, hypertension, and renal function correlated with carotid atherosclerosis in the present study, having pSS predicted presence of carotid atherosclerosis independent of presence of these risk factors. However, our findings also point to the importance of identification and control of traditional risk factors to prevent subclinical atherosclerosis in pSS.

Traditional risk factors like hypertension and dyslipidaemia are more prevalent in pSS as demonstrated in three large cohorts from Spain, United Kingdom and Italy (7, 12, 13). Our study found the same high prevalence of hypertension as demonstrated by these studies, 2-fold higher that in the Brazilian general population (35). Almost 50% of patients in the present population had MetS, pointing to clustering of cardiovascular risk factors in pSS. The finding that controls had higher blood pressure and higher levels of serum lipids and fasting glucose indicate that participation in the project was motivated by knowledge of increased cardiovascular risk among the controls. In spite of this confounding factor, carotid atherosclerosis was more common among pSS patients, confirming that disease itself is a risk for atherosclerosis.

Different studies have shown different lipid profile in pSS. In particular lower HDL (11, 16, 36) and hypertriglyceridaemia (12, 13), more than hypercholesterolaemia (7) have been reported from different pSS cohort studies. The present study demonstrates that hypertriglyceridaemia was particularly associated with carotid atherosclerosis in multivariate analysis.

As demonstrated, we found that postmenopausal status, use of glucocorticoid, renal dysfunction, constitutional systemic manifestation, and glandular manifestation indicated by use of saliva substitutes, were associated with presence of carotid atherosclerosis plaque. Constitutional domain represents systemic constitutional symptoms and more inflammation. Recently, Bartoloni *et al*. demonstrated that pSS patients with visceral and central involvement had higher risk for cardiovascular events in a retrospective cohort study, and those with leukopenia had more angina pectoris (7). In the present study, we did not find an association of disease activity evaluated by total ESSDAI and presence of carotid atherosclerosis. Others have reported that Sjögren's syndrome damage index (SSDI) but not ESSDAI was associated with higher aortic stiffness measured by pulse wave velocity (14). Subclinical atherosclerosis indicates the presence of structural damage in the vessel, and it is more logical to have association with an intense inflammatory process for many years, as represented by SSDI. We did not evaluate SSDI, but even matched for aging, creatinine was higher in the patients group and the renal involvement with chronic renal disease was associated with plaque.

Glucocorticoid is a known disease-risk factor that leads to abdominal obesity, hypertension, dyslipidaemia and diabetes, all factors predisposing for subclinical atherosclerosis. In pSS, glucocorticoids therapy was associated with higher cardiovascular event rate in an Italian cohort (7). In the present population, use of glucocorticoids was independently associated with presence of carotid atherosclerosis in multivari-

ate analysis, after adjusting for hypertension and dyslipidaemia (data not shown). It points that glucocorticoid should use as less as possible in pSS to avoid cardiovascular risk increase.

Several biomarkers are being described in subclinical atherosclerosis and could be implicated in the endothelial dysfunction and plaque formation in rheumatic disease. Carotid atherosclerosis was previously associated with leptin, soluble tumour necrosis factor-like weak induced of apoptosis (sTWEAK), homocystein, and osteoprotegerin (OPG) (3, 4, 37, 38) in patients with SLE. In this disease population, the combination of at least 3 biomarkers (pro-inflammatory high-density lipoprotein (piHDL), leptin, sTWEAK, and homocysteine) or 1 biomarker plus diabetes confers 28-fold increased odds for the presence, progression, or occurrence of new carotid plaque in both SLE and controls (38).

OPG may contribute to the link between systemic inflammation and increased cardiovascular risk. OPG concentrations are independently associated with endothelial activation and carotid atherosclerosis in RA, and it decreases upon infliximab administration (39). In non-diabetic Ankylosing Spondylitis (AS) patients undergoing TNF-α antagonist therapy, OPG shows a correlation with markers of disease activity and endothelial activation (40).

We showed normal CRP (lower than 6 mg/dL) and similar to controls. CRP is usually normal or not too high in pSS and few studies evaluate association with atherosclerosis, with controversial results. In a Spanish cohort, CRP was associated with more cardiovascular risk factors (12). We did not find any association with CRP and it could be explained by non-inflammatory levels and low disease activity.

Endothelial dysfunction is the first reversible stage of the CVD, followed by subclinical atherosclerosis characterised by inflammatory infiltration and plaque formation. Preliminary few studies show that pSS has impaired endothelial function (16, 41). Some biomarkers to endothelial cell damage were higher in pSS, such as anti-endothelial cell antibody (AECA) (15), soluble trombomodulin (sTM) (15), adhesion molecules (VCAM-1 and ICAM-1), nytrosine (16), and asymmetric dimethylarginine (ADMA) (22) and could indicate precocious endothelial dysfunction in pSS. It would be interesting to know if the very precocious biomarker of endothelial lesion, the endothelial progenitor cells, is related to endothelial dysfunction measure in pSS likely SLE (42). Osteopontin and angiopoietin-2 are endothelial biomarkers associated with disease activity and that improved after infliximab treatment in AS (43). Future studies should evaluate laboratorial biomarkers for endothlial dysfunction, its association with cardiovascular risk, disease activity and treatment.

As demonstrated, serum levels of calprotectin, TNF-R2, MCP-1, and HGF were higher in pSS than controls, and could potentially contribute to the higher prevalence of carotid atherosclerosis. However, in multivariate analysis, only calprotectin was associated with presence of carotid atherosclerosis, while no independent association was found with serum levels of pro-inflammatory cytokines despite the higher levels in pSS patients compared to controls. Calprotectin is a protein complex compounded by the S100A8 and S100A9 proteins, also referred to as myeloid-related proteins (MRP)-8 and MRP-14, which is secreted by activated neutrophils and monocytes and binds to receptors on advanced glycation end-products and has been associated with enhanced atherosclerosis in animal studies. Calprotectin represents an interesting peptide known to be involved in the pathophysiology of various inflammatory processes. Recent studies, however, suggest that calprotectin could serve as an important prognostic factor for cardiovascular and cardiometabolic diseases, since these are occurring on the basis of low-grade chronic inflammation (44). Calprotectin is therefore an interesting biomarker of integrated arterial inflammation and cardiovascular risk factor burden. As proof of concept, anti-inflammatory treatment with tumour necrosis factor alpha (TNF-α) inhibitor in rheumatoid arthritis was associated with reduction in aortic stiffness, carotid atherosclerosis and circulating calprotectin (45). In pSS patients, higher serum calprotectin has been associated with fatigue (46), potentially leading to sedentary lifestyle and worsening of metabolic risk profile. The present study adds to this knowledge by demonstrating that serum calprotectin is associated with subclinical atherosclerosis in pSS. Calprotectin is a biomarker of inflammation and it was associated with CRP in our studied population. Interestingly it was associated with abnormal profile of adipocytokines and with biomarkers of atherosclerosis such as HGF and TNF receptors, suggesting that the presence of calprotectin indicates a cluster of patients with higher risk, confirming our hypothesis that calprotectin can be involved in the pathogenesis of atherosclerosis in pSS and other rheumatic disease as well. Any biomarker was previously associated to subclinical atherosclerosis in pSS and our study found for the first time the calprotectin as a biomarker in that sub-population.

In conclusion, the study demonstrates that patients with pSS have a higher prevalence of carotid atherosclerosis, which is associated with higher serum calprotectin level independent of traditional cardiovascular risk factors. Our findings suggest calprotectin as a biomarker of subclinical atherosclerosis in pSS.

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