Characterization of circulating endothelial microparticles and endothelial progenitor cells in primary Sjögren’s syndrome: new markers of chronic endothelial damage?

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Abstract

Objective. Chronic autoimmune diseases are associated with increased risk of cardiovascular death. Endothelial dysfunction represents the first stage of subclinical atherosclerosis and multiple factors contribute to endothelial injury. Among these, an altered balance between endothelial microparticle (EMP) release and endothelial progenitor cell (EPC) generation promotes endothelial dysfunction. The role of EMPs and EPCs in promoting endothelial damage in primary SS (pSS) has never been investigated. Our aim was to evaluate the role of EMPs and EPCs as markers of endothelial damage in pSS and their correlation with disease clinical and immunological features.

Methods. Circulating EMPs (CD31+/CD42b), true EPCs (CD34+/KDR+/CD133+) and mature EPCs (CD34+/KDR+/CD133−) were quantified by FACS analysis in 34 pSS patients and 18 age- and sex-matched controls. Correlation between EMP and EPC levels and parameters of disease activity and damage, clinical features and markers of immunological dysfunction was performed.

Results. Patients displayed higher EMP numbers with respect to healthy controls [HCs; mean 450 n/ml (S.D. 155) vs 231 (110), P < 0.0001]. EPC and mature EPC levels were higher in patients compared with HCs [mean 226 n/ml (S.D. 181) vs 69 (53), P < 0.001 and 166 (161) vs 36 (32), P < 0.0001, respectively]. EMP levels directly correlated with disease duration from symptoms and diagnosis (r = 0.5, P < 0.01). Early EPCs inversely correlated with disease duration from symptoms (r = −0.5, P < 0.01) and diagnosis (r = −0.4, P < 0.05).

Conclusion. This is the first demonstration of chronic endothelial fragmentation characterizing pSS. The reparative potentiality of the endothelial layer appears to be preserved in the earliest stages of disease. During the course of the disease, progressive exhaustion of the precursor endothelial pool may be hypothesized, leading to defective vascular layer restoration and endothelial dysfunction.

Key words: Sjögren’s syndrome, endothelial microparticles, endothelial progenitor cells, subclinical atherosclerosis, endothelial dysfunction.

Introduction

Subclinical atherosclerosis can be now considered a systemic condition in patients with chronic inflammatory joint and CTDs [1]. Multiple and as yet unexplored mechanisms have been postulated as contributing to accelerated endothelial damage. In particular, close interplay between traditional cardiovascular (CV) risk factors, chronic inflammation, autoimmune system dysregulation...
and imbalance of the subtle equilibrium between endothelial injury and repair may be advocated as plausible mechanisms involved in the induction and progression of atherosclerotic vascular damage [2].

Endothelial dysfunction represents an early and potentially reversible stage of vascular disease and can be considered a reliable predictor of CV morbidity and mortality in a number of conditions, including arterial hypertension, diabetes mellitus (DM), chronic end-stage renal disease and inflammatory and autoimmune rheumatic disorders. In particular, there is evidence that young patients with chronic inflammatory and systemic autoimmune diseases, free from traditional CV risk factors, display signs of endothelial damage while still in the earliest stage of the disease [3–5].

Given the early documentation of endothelial dysfunction in different diseases, attention has been given to the investigation of mechanisms involved in the condition. Among these, recent studies have focused on the intriguing role played by the altered balance between endothelial microparticle (EMP) release and endothelial progenitor cell (EPC) generation in the disruption of endothelial wall integrity and subsequent increased endothelial thrombogenicity and dysfunction [6, 7]. Indeed, an increase in microparticles (MPs) of endothelial as well as platelet and leucocyte origin has been demonstrated in conditions associated with endothelial damage, including DM, CV diseases and systemic arterial hypertension [6, 8]. Similarly, decreased absolute number and/or functional capacity of EPCs, predictive of major adverse cardiac events in patients with coronary artery disease, has been shown to be reduced in the same disease states. Thus an increased ratio of EMPs:EPCs has been proposed as a reliable marker of imbalance between endothelial injury and repair and thus an indirect indicator of CV risk [6].

Recent studies have provided evidence that patients affected by chronic inflammatory and autoimmune rheumatic disorders display significantly increased levels of circulating platelet, leucocyte and endothelial MPs with respect to control populations [9–12]. Depending on their specific origin, circulating MPs have been demonstrated to exert multiple effects in rheumatic diseases [13]. Indeed, they act as pro-inflammatory and pro-thrombotic mediators, mediate intracellular signalling and may contribute to rheumatic disease pathogenesis [13]. Interestingly, EMP levels have been demonstrated to correlate inversely with indirect measures of subclinical atherosclerotic damage in SLE patients, thus postulating their role as biomarkers of endothelial damage and CV risk in these disorders [14]. It is noteworthy that suppression of inflammatory disease activity and improvement of endothelial damage measures are associated with a significant reduction of circulating EMP levels, suggesting a dynamic role of these molecules in endothelial fragmentation and repair [10, 14].

In addition, endothelial anatomical and functional damage may be restored by the ability of EPCs to home to sites of vascular injury and to differentiate into mature endothelial cells, promoting arterial wall repair [15].

Circulating EPC levels and function have been demonstrated to be reduced in patients with systemic rheumatic diseases, particularly in the earliest stage of the disease [16]. Through multiple mechanisms, a reduction in EPC levels and altered function have been correlated with the increased presence of traditional CV risk factors, higher rheumatic disease activity and endothelial dysfunction, suggesting their role as sensitive biomarkers of CV involvement in inflammatory and systemic autoimmune diseases [16].

Across the wide spectrum of systemic autoimmune disorders, primary SS (pSS) represents an ideal model to explore the interaction between autoimmune dysfunc-

tion, chronic inflammation and accelerated atherosclerosis. Indeed, it often has an indolent course, not requiring immunosuppressive therapy, and affects young women who are free of traditional CV risks or events. There is evidence that pSS patients, free of previous CV manifestations, display signs of endothelial dysfunction and extensive organic artery wall damage [17–20]. The pathogenic mechanisms underlying subclinical endothelial damage in pSS are still under investigation. However, some disease-related clinical and immunological features, including articular involvement, parotid swelling, RP, leucopenia and anti-SSA/SSB antibodies, have been identified as contributing to endothelial atherosclerotic involvement [17–20]. On the other hand, to date there are no studies that have examined the number and function of EMPs, EPCs and mature EPCs in pSS patients.

In the present study we investigated whether pSS is associated with endothelial damage by quantification of circulating EMPs. We subsequently evaluated endothelial repair capacity by assessing circulating EPC numbers and their maturation potential in more mature EPCs. Finally, we examined the relationship of these biomarkers with specific disease-related clinical and/or immunological features to test the hypothesis that the immune system dysfunction and chronic inflammation that characterize disease pathogenesis contribute to endothelial wall damage in these patients.

**Methods**

**Patients**

We consecutively included 34 pSS patients who fulfilled the classification criteria proposed by the American-European Consensus Group [21]. Eighteen age-matched healthy females were enrolled as a control population (HCs). Subjects with recent acute infection (≤1 month), recent CV event (≤3 months), any chronic infection, chronic kidney disease or who were pregnant or lactating were excluded. Current or past immunosuppressive therapies represented an exclusion criterion and only treatment with HCQ and/or low-dose corticosteroid (CS) therapy (prednisone ≤10 mg/day) at a stable dosage in the previous 3 months were permitted. Antihypertensive therapy, in particular angiotensin-converting enzyme (ACE) inhibitors and angiotensin type 2 receptor blockers (A2RBs), had to be withdrawn at least 3 days before inclusion. Subjects fasted for 12 h before sample collection and were asked to abstain from smoking the same day as

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EMP assay

EMPs were assayed immediately after venipuncture, as previously described [10]. Briefly, blood samples were drawn into citrated Vacutainer tubes (5 ml) and were centrifuged for 10 min at 160 g to prepare platelet-rich plasma (PRP). The PRP was then centrifuged for 6 min at 1000 g to prepare platelet-poor plasma (PPP). The PPP (50 μl) in a 12 × 75 mm polypropylene tube was incubated with 5 μl of phycocyanin (PE) anti-CD31 (BD, Franklin Lakes, NJ, USA) plus 5 μl of FITC anti-CD42 (BD) for 20 min with gentle (100 rpm) orbital shaking. Then 940 μl of 0.2 μm filtered PBS was added and the sample was ready for flow cytometry on a FACSCalibur flow cytometer using CellQuest Pro software (BD). EMPs were defined as CD31+/CD42− particles with a diameter of <1.5 μm (calibrated with flow cytometry size calibrations beads; Invitrogen, Eugene, OR, USA). The number of CD31+/CD42− microparticles (per microlitre of PPP) was calculated by dividing the number of CD31+/CD42− events in the final volume of the sample (50 μl of PPP, 10 μl of antibody suspension, 940 μl of PBS) by the volume of PPP in the test sample (50 μl). To assess the reproducibility of CD31+/CD42− EMP measurements, circulating EMPs were measured in two separate blood samples from the same participants (in 30 HCs). A very close correlation between the two measurements of CD31+/CD42− EMPs was obtained (r = 0.89, P < 0.001).

**EPC assay**

Heparinized venous blood was separated by density gradient (Lymphoprep) and peripheral blood mononuclear cells were stained with a cocktail of FITC, PE-Cy7 and human allophycocyanin (APC)-labelled anti-CD34, CD309 and CD133 antibodies (BD and Miltenyi Biotech, Cologne, Germany). Cells stained with isotopic controls for IgG1-FITC, PE-Cy7 or APC were used as negative controls. EPCs were defined as CD34+/CD309+/CD133− in accordance with the EULAR Scleroderma Trials and Research Group recommendations [24]. Accordingly, more mature EPCs were defined as CD34+CD309−CD133−. At least 500,000 events per sample were acquired. Samples were analysed with a FACSCalibur flow cytometer and CellQuest Pro software.

### Statistical methods

All data analysis was performed using SPSS version 13.0 (IBM, Armonk, NY, USA). The Mann-Whitney U test was employed to compare pSS and control groups. Spearman’s correlation coefficient and binary logistic regression were used to identify a possible association between clinical/serological variables and either EMPs or EPCs. The significance level was two-sided and set at P < 0.05.

### Results

As depicted in Table 1, epidemiological features and traditional CV risk factors were equally distributed between patients and controls. In particular, factors potentially affecting EMP and EPC concentration, including age, menopausal status, hypertension, cholesterol levels and smoking, were matched between the two groups.

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**TABLE 1** Characteristics and traditional CV risk factors of primary SS patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 34)</th>
<th>Controls (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (a.o.), years</td>
<td>59 (10)</td>
<td>56 (4)</td>
<td>0.61</td>
</tr>
<tr>
<td>Menopause, n (%)</td>
<td>23 (68)</td>
<td>13 (72)</td>
<td>0.36</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>10 (29)</td>
<td>6 (33)</td>
<td>0.45</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidaemia, n (%)</td>
<td>4 (12)</td>
<td>2 (11)</td>
<td>0.58</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>2 (6)</td>
<td>1 (5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Total cholesterol, mean (a.o.), mg/dl</td>
<td>193 (36)</td>
<td>190 (30)</td>
<td>0.54</td>
</tr>
<tr>
<td>HDL-c, mean (a.o.), mg/dl</td>
<td>54 (18)</td>
<td>50 (21)</td>
<td>0.47</td>
</tr>
<tr>
<td>LDL-c, mean (a.o.), mg/dl</td>
<td>123 (32)</td>
<td>125 (29)</td>
<td>0.37</td>
</tr>
<tr>
<td>Triglyceride, mean (a.o.), mg/dl</td>
<td>116 (77)</td>
<td>110 (71)</td>
<td>0.10</td>
</tr>
<tr>
<td>Anti-hypertensive therapy, n (%)</td>
<td>9 (26)</td>
<td>6 (33)</td>
<td>0.39</td>
</tr>
<tr>
<td>β-blockers, n</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A2RBs, n</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Diuretics, n</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Combination therapy, n</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fibrates, n</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Omega-3 fatty acids, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; A2RBs: angiotensin type 2 receptor blockers.
Table 2: Disease-specific clinical and immunological features of primary SS patients (n=34)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
<th>Median (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration, mean (s.d.), years</td>
<td>5 (6)</td>
<td></td>
</tr>
<tr>
<td>Symptom duration, mean (s.d.), years</td>
<td>8 (6)</td>
<td></td>
</tr>
<tr>
<td>ESSDAI (0-15), median</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>SSDDI (0-5), median</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CRP, mean (s.d.), mg/dl</td>
<td>0.3 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Parotid swelling, n (%)</td>
<td>10 (29)</td>
<td></td>
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<tr>
<td>Articular, n (%)</td>
<td>10 (29)</td>
<td></td>
</tr>
<tr>
<td>RP, n (%)</td>
<td>5 (15)</td>
<td></td>
</tr>
<tr>
<td>Cutaneous involvement, n (%)</td>
<td>4 (12)</td>
<td></td>
</tr>
<tr>
<td>Pericarditis, n (%)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td>Lung, n (%)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Kidney, n (%)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma, n (%)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Patients on HCO ± CS therapy, n (%)</td>
<td>11 (32)</td>
<td></td>
</tr>
<tr>
<td>Anti-Ro/La, n (%)</td>
<td>15 (44)</td>
<td></td>
</tr>
<tr>
<td>Anti-Ro, n (%)</td>
<td>12 (35)</td>
<td></td>
</tr>
</tbody>
</table>

ESSDAI: EULAR Sjögren’s Syndrome Disease Activity Index; SSDDI: SS Disease Damage Index; CS: corticosteroid.

Disease-related clinical and immunological features are illustrated in Table 2. Articular involvement was the most common extraglandular manifestation, followed by Raynaud’s phenomenon and cutaneous vasculitic involvement. Myositis and peripheral or central nervous system involvement were not shown. Fifteen patients (44%) were positive for anti-Ro/SSA and/or anti-La/SSB antibodies, while 11 (35%) were positive only for anti-Ro/SSA. Eleven patients (32%) were being treated with HCO and/or low-dose CS therapy (nine on HCO monotherapy and two on HCO in association with low-dose CS).

A statistically significant increased number of circulating EMPs was detected in pSS patients with respect to HCs [mean 450 n/μl (s.d. 155) vs 231 (110), P < 0.0001; Fig. 1a]. Also, EPC and mature EPC mean concentrations were significantly higher in patients compared with HCs [mean 226 n/ml (s.d. 181) vs 69 (53), P < 0.001 and 166 (161) vs 36 (32), P < 0.0001, respectively] (Fig. 1b and c). In order to assess the EPC and mature EPC relationship and reproducibility, a direct comparison between the two populations was performed. EPC concentration directly correlated with mature EPC levels (r = 0.7, P < 0.0001) (data not shown). Interestingly, pSS patients exhibited a significantly lower EPC: mature EPC ratio compared with controls (P < 0.01) (Fig. 2). A significant direct correlation was observed between EMP levels and disease duration as assessed from both symptom appearance and diagnosis (r = 0.5, P < 0.01 for both) (Fig. 3a and b). Moreover, there was a close inverse correlation between circulating EPCs and disease duration from symptoms (r = −0.5, P < 0.01) and diagnosis (r = −0.4, P < 0.05) (Fig. 4a and b). In the logistic regression analysis it was found that EMP, EPC and the EPC: mature EPC ratio were not correlated with the ESSDAI, SSDDI or other clinical and/or immunological disease-specific parameters.

**Conclusion**

To the best of our knowledge, the present results provide the first evidence that pSS patients display an increased level of circulating EMPs in comparison with HCs. In this context, increased levels of MPs, but of leucocyte origin,
have been demonstrated in a cohort of pSS patients, likely reflecting the activation of this cell population as part of the inflammatory and autoimmune mechanisms characterizing disease pathogenesis [9]. Moreover, a higher concentration of MPs of platelet origin compared with HCs has been demonstrated in the same cohort of patients [9]. Platelet-derived MPs have been detected in rheumatic and non-rheumatic conditions associated with vascular endothelial damage and current evidence supports their ability to activate endothelium and promote thrombosis [13]. In this setting, our results showing increased EMP concentration in pSS patients may further support a condition of abnormal endothelial fragmentation and damage irrespective of disease activity. To date, published results of circulating EMPs in patients with systemic rheumatic diseases, including SLE and SSc, are quite contradictory. Differences in techniques employed to quantify these molecules represent the most plausible explanation for this discrepancy [13]. In this study we used a standard combination of markers to identify EMPs (CD31+/CD42b-). Indeed, CD31, a constitutive marker expressed on endothelial cells and, at low level, on platelets, has been demonstrated to characterize EMPs released during endothelial cell apoptosis, whereas inducible markers such as CD62E are increased on EMPs.
released during endothelial cell activation [25]. As CD42 is a platelet-specific molecule, combined CD31+CD42+ expression is considered the most reliable marker of EMPs, although other CD markers have been employed to identify EMPs [8].

Platelet and leucocyte MP levels seem to fluctuate according to disease activity in some autoimmune disorders, including SS [9, 26]. In particular, MP plasma concentration inversely correlated with disease activity in RA and SLE patients, suggesting molecule confinement in the tissue target of the autoimmune process, and with disease severity in SSC patients, thus postulating a direct role in the pathogenesis of clinical manifestations reflecting disease-related chronic damage [9, 27]. In contrast, we did not find a significant relationship between EMP level and disease activity, severity index or peculiar clinical features. The low-grade disease activity and the mild disease severity characterizing our cohort, almost exclusively associated with oral and ocular damage, may explain, at least in part, such conflicting results.

Mechanisms regulating EMP release in pSS are unknown. In vitro, a variety of prolonged stimuli have been demonstrated to induce EMP vesiculation from cultured endothelial cells both in physiological and pathological conditions [28]. Among these, high-grade systemic inflammation is one of the major stimuli able to induce increased circulating EMP release in vitro and in vivo [10, 29]. However, pSS is an autoimmune disease generally characterized by low-grade systemic inflammation, as also demonstrated by the normal CRP mean value characterizing our cohort. Thus other still unexplored mechanisms can be reasonably associated with endothelial damage and enhanced release of EMPs in pSS. In this context, the significantly higher EPC concentration detected in patients with longer disease duration may suggest a condition of chronic endothelial layer damage leading to endothelial cell membrane shedding and subsequent EMP release.

Our findings also demonstrate for the first time that pSS patients display an increased absolute number of circulating EPCs, suggesting enhanced cell mobilization from the bone marrow and thus a potential early reparative attempt of the damaged endothelium. This is in line with recent findings showing an increased positive EPC count in patients with other rheumatic diseases, including RA, SLE and SSc, which are all characterized by systemic vascular damage [30–34]. In fact, EPCs are released in response to vascular injury and contribute to endothelial layer repair through differentiation in mature EPCs and, finally, in endothelial cells [35]. It may be hypothesized that endothelial damage in pSS may be associated, at least in part, with enhanced production of EMPs from endothelial cells and that the increased EPC release may represent an attempt to counteract endothelial damage and preserve endothelium homeostasis. However, in patients with longer disease duration, this reparative potentiality seems to be lost, as demonstrated by the significant reduction of circulating EPCs during the course of the disease in our population. Of note, this is in line with results from other studies involving RA and SSC patients and suggests that chronic exposure of endothelial cells to multiple risk factors may lead to progressive exhaustion of EPCs, thus contributing to progressive endothelial damage and dysfunction [31, 32, 34].

In addition, the quantification, in our cohort, of more mature CD133− EPCs as a separate population allows for some intriguing considerations. Mature EPCs represent a late cell population with a high proliferative rate favoring vessel formation in vitro and in vivo [36]. In patients with systemic autoimmune disorders, their direct contribution to vasculogenesis underlying synovitis in RA or vasculitic manifestations in SSc has been hypothesized [30, 32]. Interestingly, the low EPC:mature EPC ratio detected in our population compared with controls suggests that many early EPCs are committed to differentiate in mature EPCs in patients with autoimmune disorders, further supporting a contribution of mature CD34+CD309+CD133− EPCs to abnormal vasculogenesis.

On the other hand, it should be born in mind that in patients with systemic autoimmune disorders, both early and mature EPCs seem to exhibit defective functionality, characterized by impaired ability to differentiate to more mature cells or by defective migration and adhesive properties [16, 37]. In this setting, reduced migratory capacity and/or impaired functional phenotype characterizing our mature EPC population cannot be ruled out, as recently demonstrated in SLE and SSc [14, 32]. Nevertheless, other mechanisms of impaired endothelial repair can be hypothesized in pSS. Prolonged and repeated endothelial exposure to oxidative stress, which has been demonstrated to be increased in pSS, may exhaust vascular layer antioxidant protective mechanisms and may induce endothelial cell apoptosis and detachment [38]. Moreover, endothelial cell apoptosis may be induced by anti-endothelial cell antibodies (AECAs) through antibody-dependent cell-mediated cytoxicity, as demonstrated in SSc [37]. Interestingly, increased levels of both AECAs, correlated with RP, and von Willebrand factor antigen, a specific product of endothelial cells and an index of endothelial damage, have been identified in pSS patients, thus postulating their contribution to vessel injury [39, 40]. Finally, an impaired balance between angiogenic and angiostatic factors and altered expression of endothelial adhesion molecules induced by chemokines and pro-inflammatory cytokines may be an additional cause of reduced endothelial cell layer repair in pSS [41].

The lack of instrumental measurements of endothelial dysfunction may represent a limit of the present study, since it does not allow validation of EMPs and EPCs as indirect biomarkers of subclinical atherosclerosis and speculation of their role in endothelium damage in SS. A strength of this study is the quantification of EPCs and mature EPCs as separate populations—rarely performed in other studies—and their characterization with an accurate combination of validated markers. The CD133 molecule is a marker characterizing CD34+CD309+ EPCs in
their immature phase and is no longer expressed on mature EPCs in vitro, leading to the conclusion that CD34+CD309+CD133+ cells represent early EPCs [24]. The use of different surface markers to identify and define EPCs and more mature EPCs in other rheumatic diseases and the lack of standardized procedures make the comparison of literature data very difficult and probably represent the main source of variability between studies [42].

In addition, it should be noted that EPC and EMP evaluation has been performed in the present study in a homogeneous population without interference of potent immunosuppressive therapies commonly employed in other systemic autoimmune diseases. It is known that high-dose CS and immunosuppressive drugs, such as CYC and ciclosporin A, have been demonstrated to induce EPC mobilization, whereas MTX induces EPC apoptosis in vitro [16].

In our cohort, only HCQ and low-dose CS were permitted. Antimalarials have been demonstrated to induce endothelial cell apoptosis, but they also seem to stimulate nitric oxide synthesis in human endothelial cells, contributing to improved endothelial function [43, 44]. Moreover, experimental data have shown that HCQ is able to induce EPC differentiation in vitro, but the effect of antimalarial in vivo is more controversial, as HCQ exposure has been associated with both reduced and increased EPC levels in SLE patients [16]. Importantly, no difference in EMP concentration was found in our study between patients receiving and those not receiving HCQ (data not shown).

It is also important to note that our results can be interpreted without the effect of other pharmacological agents known to influence EMP and EPC levels. Among these, statins have been demonstrated to decrease EMP levels and increase EPC proliferative capacity [45]. Indeed, in our cohort, no patient was on lipid-lowering therapy with statins and other drugs known to affect EPC number, such as ACE inhibitors and A2RBs, as these were withdrawn before inclusion. Moreover, our patients were relatively young and, of more importance, free from some traditional CV risk factors, including DM and dyslipidaemia, known to potentially affect EMP and EPC concentration. Finally, we assume that close interplay between concomitant factors, including inflammation, vascular damage and immune dysregulation, may influence EPC count, thus precluding the identification of a correlation with a single clinical or immunological feature.

We are aware that the cross-sectional nature of this study does not allow clarification of whether EMPs and EPCs are causally involved in CV pathophysiology and outcomes or in specific clinical manifestations or whether they are solely indirect markers of endothelial damage and subsequent increased CV risk. In fact, prospective studies are required to evaluate the effect of EMP and EPC levels on endothelial homeostasis and whether they contribute to the excess of CV risk in SS. However, our data highlight for the first time a condition of chronic persistent endothelial fragmentation characterizing pSS patients, independently of disease clinical activity, inflammatory background or disease severity. Endothelial layer reparative potentiality appears to be preserved in the earliest stages of disease. However, during the course of the disease a progressive exhaustion of the precursor endothelial pool may be hypothesized, leading to defective vascular layer restoration and consequently to endothelial dysfunction. Certainly the identification of a mature EPC subset with altered functional and/or proliferative and migratory ability requires further investigation in order to define its role in disease pathogenesis and endothelial damage. Furthermore, studies are needed to further clarify the function and role of these molecules as markers of subclinical endothelial damage and to understand the pathogenesis of endothelial repair mechanisms in order to prevent endothelial dysfunction in patients with systemic autoimmune diseases.

**Rheumatology key messages**

- Primary SS is characterized by chronic endothelial layer damage.
- Increased endothelial microparticle release and progressive endothelial progenitor cell exhaustion may contribute to endothelial damage in primary SS.
- Disease duration seems to influence altered balance between endothelial fragmentation and repair in primary SS.

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**References**

Clinical vignette

Granulomatosis with polyangiitis granulomata show increased uptake of FDG

We present a case of granulomatosis with polyangiitis (GPA) in which [18F]fluorodeoxyglucose ([18F-FDG] PET/CT gave a false-positive result for tumour-like activity before the diagnosis of GPA was made histologically. Our patient, a 73-year-old male, was referred with chest pain. Initial investigations (chest X-ray, CT thorax) revealed two ill-defined opacities in the right upper zone, suspicious for lung cancer. Subsequent FDG-PET/CT findings were consistent with metastases (Fig. 1). Lung biopsy showed a granulomatous vasculitis consistent with GPA. He was PR3-ANCA positive. Although FDG-PET is widely used in oncology, it is less well recognized that FDG accumulates not only in malignant tissues, but also at sites of infection and inflammation as well as in patients with autoimmune disease [1]. The use of [18F-FDG PET/CT has been well established in the assessment of large vessel vasculitis, but its potential use in the assessment of granulomatous vasculitis is less well recognized [2]. Two recent case series have highlighted the potential use of [18F-FDG PET/CT in determining the extent of granulomata in GPA and in selecting a site for biopsy [1, 2]. Our case highlights the importance of remembering that uptake of FDG is not specific for malignancy and occurs in other metabolically active lesions.

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References


This corresponds to a 62 mm bilobar speculated lung nodule. There are further smaller foci of tracer accumulation within the right upper lobe (maximum SUV 1.4) and two in the right middle lobe (maximum SUV 2.9). These correspond to nodules measuring up to 12 mm.

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