Decreased circulating endothelial progenitor cells as an early risk factor of subclinical atherosclerosis in systemic lupus erythematosus

Raquel Castejon1, Carlos Jimenez-Ortiz2, Sara Valero-Gonzalez1, Silvia Rosado1, Susana Mellor1 and Miguel Yebra-Bango1

Abstract

Objective. Endothelial progenitor cells (EPCs) play an important role in vascular damage repair and it has been suggested that a decreased number of these cells is associated with increased subclinical atherosclerosis. Our study aim was to evaluate whether the number of circulating EPCs in patients with SLE is associated with subclinical atherosclerosis, the presence of cardiovascular (CV) risk factors and SLE-specific factors.

Methods. Forty-six female SLE patients were included. At the time of each patient’s appointment, CV risk factors, SLE-specific factors and EPCs were assessed in peripheral blood by flow cytometry. Simultaneously, atherosclerosis was assessed by measuring the carotid-femoral pulse wave velocity (PWV) by Doppler velocimetry, intima media thickness (IMT) and carotid plaque by B-mode US scanning.

Results. Patients were classified according to PWV following the reference values adjusted by age and blood pressure published by the European Society of Cardiology. Patients with pathological values of PWV showed a significant decrease of circulating EPC percentage compared with normal PWV patients. Decreased EPC counts were also associated with certain risk factors, including hypertension, tobacco use, impaired glucose metabolism, and metabolic syndrome, and correlate with high levels of high-sensitivity CRP (hsCRP) or fibrinogen. The presence of carotid plaque and IMT measurement were unrelated with EPC quantification.

Conclusion. Patients with a reduced percentage of EPCs showed pathological arterial stiffness and association with certain CV risk factors, suggesting that the measurement of circulating EPCs can be used as a biological marker to determine subclinical atherosclerosis in SLE.

Key words: systemic lupus erythematosus, endothelial progenitor cells, arterial stiffness, subclinical atherosclerosis.

Introduction

Evidence from epidemiological studies demonstrates that SLE is associated with a striking increase in the risk of premature cardiovascular (CV) complications due to accelerated atherosclerosis, which significantly contributes to morbidity and mortality in these patients. While preliminary studies suggest that traditional risk factors may play a role, they do not seem to fully explain the increased prevalence of atherosclerosis [1–4]. Therefore, additional so-called non-traditional factors, such as systemic chronic inflammation, the presence of autoantibodies and the use of immunosuppressive drugs, have been suggested to contribute.

It has also been previously reported that SLE is characterized by accelerated endothelial cell (EC) apoptosis, which correlates with reduced endothelial function, and that circulating EC can be used as a marker of vessel damage [5]. A crucial aspect to preserve endothelial
integrity is maintaining the balance between endothelial damage and repair. Vascular repair appears to be mediated by the recruitment of bone marrow-derived endothelial progenitor cells (EPCs) to the site of endothelial injury [6]. The EPCs are a heterogeneous population that contribute to the restoration of endothelial function because of their ability to differentiate into mature ECs once incorporated into circulating blood [7].

A variety of biomarkers, such as soluble inflammatory molecules, endothelial microparticles and circulating ECs, have been used as indicators of endothelial dysfunction. Recently it has been shown that circulating EPCs could be a surrogate marker for vascular dysfunction and that a reduced number of these cells are associated with an increased risk of CV disease (CVD) [8] and even with preclinical atherosclerosis [9].

In SLE patients, several studies have independently reported significantly reduced numbers and functional abnormalities of peripheral blood EPCs [10–16] or just impaired EPC function [17, 18], suggesting that this cell population could potentially be involved in the development of premature atherosclerosis in this disease. However, only one of these studies has suggested an association with preclinical vascular disease in SLE patients [16].

Usually determination of the common carotid arteries intima media thickness (IMT) and the presence of plaque in the carotid system have been considered reliable indicators of atherosclerosis. Recently several longitudinal studies directly demonstrated that aortic stiffness, measured by carotid-femoral pulse wave velocity (PWV), is an independent predictor of all-cause and CV mortality, coronary events and stroke [19–21], emerging as the gold standard method to assess subclinical atherosclerosis because it is a non-invasive, easy to assess and reproducible marker of early atherosclerosis. Our purpose is to evaluate if the number of circulating EPCs is associated with arterial stiffness measured by PWV, CV factors and SLE-specific factors.

**Patients and methods**

**Study population, protocol and design**

This cross-sectional study included 46 consecutive female SLE patients (four or more ACR criteria) with a median age of 48 years (range 19–64) attending a scheduled appointment in the outpatient autoimmune diseases unit at our hospital. Patients were enrolled over a 6-month period between February and July 2010. The study was approved by the Research Ethics Committee of the Hospital Universitario Puerta de Hierro and written informed consent was obtained from all participants.

At the time of the study, patients were assessed for co-morbidity, traditional and non-traditional CV risk factors and SLE-related factors. At the patient’s visit for blood extraction, subclinical atherosclerosis was assessed. The median SLE duration and demographic and clinical data were obtained from the medical records.

CV factor definitions are summarized below. The following SLE-related factors were considered positive: ANA (immunofluorescence) >1:40, anti-dsDNA antibodies (ELISA) >15 U/ml, anti-ENA antibodies (ELISA) >10 U/ml, lupus anticoagulant (Russell viper venom; confirmatory ratio 0–1.12), anti-cardiolipin and anti-phosphatidylglyceroprotein 1 antibodies (ELISA) >18 U/ml. The activity of SLE was assessed by the SLEDAI, with inactive disease defined as SLEDAI ≤4, and organ damage by the SLICC/ACR, with no organ damage defined as SLICC/ACR = 0. Other lupus-related parameters were determined.

Information concerning patients’ treatment at the time of recruitment was recorded. The kind of therapy and doses were HCQ (200 mg/day), corticosteroids (2.5–10 mg/day), MMF (1–2 g/day) and AZA (75 mg/day).

Information about concomitant medication was also recorded.

**Quantification of circulating EPCs and apoptotic ECs**

Peripheral blood mononuclear cells (PBMCs) were isolated from 15 ml of heparinized venous blood samples. Quantification of circulating EPCs was carried out following the recommendations published by the expert European League Against Rheumatism (EULAR) working group [22] and using the human EPC Enrichment and Enumeration Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) by staining with fluorescent-conjugated monoclonal antibodies to CD34-FITC, CD133-PE, VEGFR-2-APC and CD14-PECy5. Propidium iodide was used for real-time viability staining before flow cytometry analysis (BD Bioscience, San Jose, CA, USA).

The EPCs in peripheral blood have been defined as mononuclear cells positive for CD34, VEGFR-2 and CD133, but negative for monocyte antigen CD14. Similarly, a mature EPC subpopulation was CD34+VEGFR-2+ and CD133+ and the population including cells in both states of maturation was reported as CD34+ and VEGFR-2+.

Apoptotic circulating EC detection was carried out in the isolated PBMCs stained with CD45/CD3-APC antibodies (BD Bioscience), CD146-PE antibody (clone P1H12; Pharmingen, San Diego, CA, USA), Annexin V-FITC (Pharmingen) and 7-amino-actinomycin D (7-AAD; Immunostep, Salamanca, Spain) to exclude necrotic cells. Circulating apoptotic ECs were identified as a CD146+/Annexin V− subpopulation in the CD45+/CD3−/CD14+ gate. Results from both subpopulations were expressed as the percentage of cells in the lymphocytes or PBMC pool and as absolute numbers of cells per millilitre of blood.

**Subclinical atherosclerosis assessment**

**PWV measurement**

Arterial stiffness was assessed by measuring the carotid–femoral PWV by Doppler velocimetry and simultaneous ECG. Simultaneous recordings of the arterial flow waves from the right common carotid artery and the right femoral artery were made using a bidirectional transcutaneous Doppler velocimeter using an 8-MHz probe. After waveform collection, distance measurements between the carotid and femoral sampling sites were...
Determination in SLE patients was 7.8 (S.D. 2.2) m/s. Aortic stiffness was measured by PWV. The mean of PWV and arterial stiffness

Association between circulating EPCs, apoptotic ECs and carotid atherosclerosis

Carotid atherosclerosis was determined by assessing the IMT and carotid plaque using B-mode US scanning (Phillips IU22) equipped with eco-Doppler and IMT automatic quantification. All measurements of IMT were made in the longitudinal plane at the point of the maximum thickness on the far wall of the common carotid artery along a 1 cm section of the artery proximal to the carotid bulb. Values from each location were then averaged to provide an overall measure of IMT. The presence of carotid plaque was defined as a distinct area protruding into the vessel lumen.

Statistical analysis

For quantitative data with a non-Gaussian distribution, statistical analysis was performed with the nonparametric Mann-Whitney U-test, and when a normal distribution was followed, Student’s t-test was carried out. The \( \chi^2 \) test (with the two-sided Fisher’s exact test) was used to compare categorical variables. For all analyses, significance was defined as a P-value <0.05. Statistical analysis was performed using SPSS software (version 15.0).

Results

Subject characteristics

The clinical characteristics of patients included in the study and data related to classic CV risk factors are shown in supplementary Tables S1 and S2, available at Rheumatology Online. Mean disease duration was 14 (S.D. 9) years and 25 patients (54.3%) had an evolution of >10 years. Four patients had established CVD not due to APS.

Regarding the features of the SLE patients at the time of blood extraction for the study, nine patients (19.5%) had serological activity, based on the presence of positive anti-DNA antibodies and/or low complement levels. Although 20 patients (43.4%) met the laboratory criteria for APS, only 3 patients fulfilled clinical criteria for APS.

Eight patients (17.4%) were on no medication and 36 patients (78.3%) were taking antimalarials regularly either as the only treatment or in combination with some immunosuppressive drug (22 patients).

Association between circulating EPCs, apoptotic ECs and arterial stiffness

Aortic stiffness was measured by PWV. The mean of PWV determinations in SLE patients was 7.8 (S.D. 2.2) m/s. Significant correlation was found between PWV values, patient age \( (r=0.7, \ P=0.0001) \) and blood pressure (BP) \( (r=0.41, \ P=0.006) \). SLE patients were divided in two different groups—PWV normal range and PWV pathological range—according to the results of PWV adjusted for age and BP, based on normal standards published by the European Society of Cardiology [23]. Subjects were categorized by age decade and further subdivided according to BP category. The distribution of PWV with age and BP category is described and reference values for PWV were established. Following these values, 23 of the patients included in our study had a normal PWV and 23 patients had a pathological PWV. The association between EPCs, apoptotic ECs and arterial stiffness was examined in the two groups defined.

There were no differences in the haematopoietic progenitor cells CD34+ subpopulation, but we did find that patients with normal PWV had a significantly higher percentage of EPCs that were CD34+CD133+VEGFR-2+ [0.90 (S.D. 0.43) vs 0.67 (S.D. 0.59), \( P=0.01 \)], mature EPCs that were CD34+VEGFR-2-CD133- [0.43 (S.D. 0.24) vs 0.33 (S.D. 0.24), \( P=0.04 \)] and the population that includes stem cells in both states of maturation, CD34+VEGFR-2+ [1.33 (S.D. 0.56) vs 1.00 (S.D. 0.78), \( P=0.01 \)] (Table 1). Both groups of patients showed no differences in the quantification of apoptotic circulating ECs.

In these data analyses, patients with established CVD were not excluded. The same significant association \( (P<0.03) \) was found between increased arterial stiffness and a decreased percentage of EPCs in a second analysis performed excluding the four patients with symptomatic CVD not due to APS (Table 2).

Association between circulating EPCs, apoptotic ECs and carotid atherosclerosis

The mean of our patients IMT measurements was 0.54 (S.D. 0.09) mm. IMT was categorized using the median obtained from our patient group. Twenty patients had an increased IMT \( (>0.55 \text{ mm}) \) and 26 patients were in the normal IMT group \( (<0.55 \text{ mm}) \). Our data showed that IMT measurement was unrelated with EPC quantification. However, the patients with an increased IMT showed a statistically significant higher percentage of apoptotic circulating ECs \( (P=0.03) \) (Table 1).

We did not find in our patients significant differences between the percentages of cell subpopulations determined in the presence or absence of carotid plaque. However, as shown in Table 1, it was observed that patients without carotid plaque tend to have more circulating EPCs than patients with established plaque.

Differences between circulating EPCs and apoptotic EC quantification according to CV risk factors and SLE-related factors

We investigate the concept that the EPC count and apoptotic ECs were associated with the presence of CV risk factors. In our study, patients with a family history of CVD had lower amounts of haematopoietic CD34+ progenitor cells than those without [11.8 (S.D. 0.8) vs 18.6 (S.D. 9.7), \( P=0.05 \)].

Hypertensive patients, smokers, those with impaired fasting glucose or those with metabolic syndrome showed a decreased percentage of EPCs when compared with non-hypertensive patients \( (P=0.03) \), those who did not smoke \( (P=0.008) \), those with normal blood


**Table 1: Circulating EPC and EC quantification**

<table>
<thead>
<tr>
<th>IMT</th>
<th>No plaque</th>
<th>Plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT &lt; 0.55</td>
<td>(n = 20)</td>
<td>(n = 26)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>PWV (n = 23)</td>
<td>1.37 (1.39)</td>
<td>1.48 (1.32)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.42</td>
<td>0.92</td>
</tr>
<tr>
<td>C34+VSGFR-2-CD133-</td>
<td>0.96 (0.98)</td>
<td>0.49 (0.30)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.99</td>
<td>0.06</td>
</tr>
<tr>
<td>C34+VSGFR-2+CD133</td>
<td>0.78 (0.81)</td>
<td>0.68 (0.60)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.99</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Discussion**

The goal of the current study was to evaluate the association between circulating EPC counts and subclinical atherosclerosis determined as arterial stiffness in SLE patients. We found that SLE patients with pathological arterial stiffness have decreased circulating EPCs when compared with SLE patients without evidence of subclinical atherosclerosis determined as arterial stiffness in SLE patients. We found that SLE patients with pathological arterial stiffness have decreased circulating EPCs when compared with SLE patients without evidence of subclinical atherosclerosis determined by PWV.

Recently, it has been reported that EPCs play an important role in endothelial repair and regeneration; they have been identified as regulators of CV integrity and a decrease in the number and altered function of EPCs are involved in the pathogenesis of atherosclerosis [8].

Some studies have described reduced numbers of EPCs in SLE patients compared with healthy controls, even in periods of inactive disease [10-13]. Supporting this hypothesis, some studies have concluded that SLE patients circulating CD34+ progenitor cells present an increased susceptibility to undergo apoptosis [11, 12], induced by the large amounts of IFN-α present in these patients.

When we investigated the influence of concomitant CV drugs on EPC levels, we found no difference in EPC levels among those patients with or without statin, antihypertensive or aspirin therapy. It was also found that patients who had impaired fasting glucose present a significant increase in the apoptotic EC subpopulation (P = 0.03).

Regarding SLE-related factors, no significant correlation was found between any of the cell subpopulations quantified and the levels of autoantibodies, complement C3 or C4 or the other lupus-related parameters recorded. Looking specifically at disease activity, patients were classified into two groups by taking into account the SLEDAI: inactive disease patients (SLEDAI ≤ 4) and patients with active disease (SLEDAI > 4). No differences were found in the quantification of EPCs and apoptotic ECs between both groups of patients. With respect to the organ damage score, the concentration of EPCs and apoptotic ECs was not significantly different between patients with some organ damage (SLICC/ACR ≥ 1) and those with no organ damage (SLICC/ACR = 0).

**Peripheral blood quantification of EPCs and ECs assessed in all SLE patients included in the study (n = 46) by flow cytometry. Results are expressed as the mean (%).**

<table>
<thead>
<tr>
<th>Cell subpopulation</th>
<th>Normal (n = 23)</th>
<th>Pathological (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C34+Annexin V+</td>
<td>0.05 (0.99)</td>
<td>0.05 (0.99)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>PWV (n = 23)</td>
<td>13.33 (3.03)</td>
<td>12.31 (8.33)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.49</td>
<td>0.06</td>
</tr>
<tr>
<td>C34+VEGFR-2-CD133-</td>
<td>0.57 (0.48)</td>
<td>0.67 (0.59)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.97</td>
<td>0.53</td>
</tr>
<tr>
<td>C34+VEGFR-2+CD133</td>
<td>0.67 (0.49)</td>
<td>0.67 (0.49)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.97</td>
<td>0.53</td>
</tr>
<tr>
<td>C34+VEGFR-2+</td>
<td>0.34 (1.06)</td>
<td>0.34 (1.06)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.97</td>
<td>0.53</td>
</tr>
</tbody>
</table>

**Discussion**

The goal of the current study was to evaluate the association between circulating EPC counts and subclinical atherosclerosis determined as arterial stiffness in SLE patients. We found that SLE patients with pathological arterial stiffness have decreased circulating EPCs when compared with SLE patients without evidence of subclinical atherosclerosis determined by PWV.

Recently it has been reported that EPCs play an important role in endothelial repair and regeneration; they have been identified as regulators of CV integrity and a decrease in the number and altered function of EPCs are involved in the pathogenesis of atherosclerosis [8].

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Analysing the possible association between the cell subpopulations quantified and the patients’ therapeutic option at the time of the study, the data showed no differences in the EPC count or apoptotic ECs between treated and untreated patients or when the analysis was performed considering the use of HCQ and/or immunosuppressive intake.
However, a recent study showed an increased absolute number of circulating EPCs in patients with SLE associated with enhanced CD34+ bone marrow progenitor cell release [24]. Other authors have not found these differences in EPC quantification, suggesting impaired function, adhesion and migratory capacity of EPCs when comparing SLE patients with healthy controls [12, 14, 17, 18].

Discrepancies in EPC quantification could be explained by the use of non-homogeneous methods, as the EPC sub-population is not determined by the expression of the same surface marker combination. In addition, patients included present different prevalences of CV risk factors, in disease activity, in mean disease duration or in medication. The mean age of the patients included in our study is high and therefore it is related with long mean disease duration.

<table>
<thead>
<tr>
<th>Cell subpopulation</th>
<th>Normal PWV (n = 22)</th>
<th>Pathological PWV (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD146+Annexin V+%, %</td>
<td>0.08 (0.06)</td>
<td>0.06 (0.08)</td>
<td>0.39</td>
</tr>
<tr>
<td>cells/µl</td>
<td>0.88 (1.39)</td>
<td>1.11 (1.27)</td>
<td>0.69</td>
</tr>
<tr>
<td>CD34+, %</td>
<td>12.75 (8.76)</td>
<td>11.76 (7.16)</td>
<td>0.03*</td>
</tr>
<tr>
<td>cells/µl</td>
<td>227.52 (243.80)</td>
<td>180.99 (113.37)</td>
<td>0.04*</td>
</tr>
<tr>
<td>CD34+VEGFR-2+CD133+, %</td>
<td>0.90 (0.44)</td>
<td>0.69 (0.62)</td>
<td>0.03*</td>
</tr>
<tr>
<td>cells/µl</td>
<td>1.84 (1.92)</td>
<td>1.29 (1.93)</td>
<td>0.04*</td>
</tr>
<tr>
<td>CD34+VEGFR-2+CD133−, %</td>
<td>0.44 (0.24)</td>
<td>0.34 (0.26)</td>
<td>0.02*</td>
</tr>
<tr>
<td>cells/µl</td>
<td>0.80 (0.71)</td>
<td>0.55 (0.45)</td>
<td>0.05</td>
</tr>
<tr>
<td>CD34+VEGFR-2−, %</td>
<td>1.34 (0.57)</td>
<td>1.03 (0.82)</td>
<td>0.03*</td>
</tr>
<tr>
<td>cells/µl</td>
<td>2.64 (2.54)</td>
<td>1.84 (2.31)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Circulating EPC values are expressed as the mean (s.d.) of the percentage determined by flow cytometry in peripheral blood. Hypertension is defined as systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg or the patient has antihypertensive treatment. Diabetes mellitus was considered when the patient was treated with oral agents or insulin or if fasting glucose was ≥ 126 mg/dl. Hyperlipidaemia is defined as total cholesterol ≥ 190 mg/dl or low-density lipoprotein cholesterol ≥ 115 mg/dl or triglycerides ≥ 150 mg/dl or lipid-lowering therapy. The presence of metabolic syndrome was calculated according to the definitions used by the Adult Treatment Panel III. Family history of CVD was considered if the patient had a first-degree relative who had suffered a heart attack or stroke before age 65 years. High BMI was considered > 25 kg/m². *Statistically significant differences in the percentage of EPCs associated with CV risk factors.

**Table 3** Association between circulating EPCs and the presence of CV or SLE-related risk factors

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Presence</th>
<th>Absence</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial hypertension</td>
<td>0.82 (0.40)</td>
<td>1.28 (0.74)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Tobacco habit</td>
<td>0.77 (0.34)</td>
<td>1.30 (0.73)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
<td>0.63 (0.29)</td>
<td>1.24 (0.70)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>1.10 (0.68)</td>
<td>1.23 (0.73)</td>
<td>0.37</td>
</tr>
<tr>
<td>Hypertriglyceridaemia</td>
<td>1.09 (0.76)</td>
<td>1.18 (0.68)</td>
<td>0.50</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>0.79 (0.33)</td>
<td>1.31 (0.74)</td>
<td>0.02*</td>
</tr>
<tr>
<td>BMI</td>
<td>1.26 (0.77)</td>
<td>1.09 (0.64)</td>
<td>0.44</td>
</tr>
<tr>
<td>At least one CV risk factor</td>
<td>1.09 (0.70)</td>
<td>1.37 (0.65)</td>
<td>0.23</td>
</tr>
<tr>
<td>Family history of CVD</td>
<td>1.25 (0.86)</td>
<td>1.14 (0.66)</td>
<td>0.70</td>
</tr>
<tr>
<td>ANA</td>
<td>1.21 (0.72)</td>
<td>0.80 (0.21)</td>
<td>0.16</td>
</tr>
<tr>
<td>Anti-dsDNA antibodies</td>
<td>1.48 (0.82)</td>
<td>0.94 (0.47)</td>
<td>0.05</td>
</tr>
<tr>
<td>aPL</td>
<td>1.53 (0.71)</td>
<td>1.15 (0.69)</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypocomplementaemia</td>
<td>1.28 (0.65)</td>
<td>1.10 (0.72)</td>
<td>0.44</td>
</tr>
<tr>
<td>Disease activity (SLEDAI)</td>
<td>1.22 (0.55)</td>
<td>1.15 (0.72)</td>
<td>0.66</td>
</tr>
<tr>
<td>Organ damage (SLICC/ACR)</td>
<td>1.14 (0.67)</td>
<td>1.21 (0.78)</td>
<td>0.87</td>
</tr>
</tbody>
</table>
In our study the EPCs were quantified following the recommendations published by the expert EULAR working group for quantification of EPCs in the blood, using CD133, VEGFR-2 and CD34 in combination with a viability marker [22]. The use of these three markers allows us to analyse the EPC subpopulation in any state of maturation.

Finally, there are some studies that have observed an increased number of circulating apoptotic ECs in SLE patients compared with healthy subjects [25–27] and patients with coronary artery disease [5, 11]. In our study, although we observed no significant differences, we found that patients with pathological PWV tended to show higher counts of circulating apoptotic ECs.

In the current study, premature atherosclerosis was assessed by PWV, the gold standard for arterial stiffness measurement. The association found between decreased EPC counts in patients with pathological PWV was also described by Westerweel et al. [11]. Previous studies have not taken into account that arterial stiffness is intimately linked to age and arterial pressure, providing a potential confounding effect in the PWV assessment. Thus in the current study, PWV determinations were adjusted for age and arterial pressure applying the reference values published by the European Society of Cardiology [23].

The association between EPCs and PWV found in our study supports the hypothesis suggested by other authors, that decreased levels of EPCs could be predictors of CV events in SLE patients, as it is in patients with coronary artery disease [8]. In view of our results, we suggest that patients with SLE, because of the high demand for endothelial regeneration, are unable to repair vascular damage due to the decreased number of circulating EPCs.

In addition, we found no association between IMT measurement and EPC numbers in the SLE patients included. Similarly, Baker et al. [16] observed that a reduced number of EPCs in SLE patients did not correlate with carotid IMT measurements. However, we found that patients with higher IMT presented a significant increase in apoptotic EC levels compared with patients with lower IMT. In agreement with the results obtained from PWV assessment, it was expected that patients with an increased IMT should have lower EPC counts. This could be explained because the mean IMT in our patients was not a pathological value. Other authors have also showed that the carotid IMT of SLE patients is only mildly increased compared with that of healthy controls [28], suggesting that other parameters that more accurately reflect endothelial damage in SLE patients should be used.

Regarding the presence of carotid plaque, no differences were found in the EPC levels and the circulating apoptotic ECs of our patients. According to the results obtained from the PWV assessment, patients with plaque tended to have fewer EPCs than patients without plaque. No relationship was found probably because only nine of our patients had carotid plaque, which was insufficient to find statistical significance.

The association between the presence of traditional CV risk factors and low levels of EPCs, even with the loss of EPC migration capacity, has been extensively studied in the general population and especially in patients with CVD [29–32]. However, few studies have reported the relationship between EPCs and CV risk factors in patients with SLE. Denny et al. [13] found no significant statistical correlation between low levels of EPCs and specific Framingham risk factors, explaining these results by the low prevalence of risk factors in their sample. Grisar et al. [14] also found no differences in circulating EPC levels in SLE patients with CV risk factors.

In our study we observed that certain known CV risk factors such as hypertension, smoking, impaired fasting glucose or the presence of metabolic syndrome were associated with a decrease in EPC levels, which would agree with the mentioned investigation on the general population. Regarding the non-classical CV risk factors, as in the work of Lee et al. [10], we found that a reduction of EPCs correlated with elevated serum levels of hsCRP, probably due to the role of this protein in the inflammation process of the vascular endothelium.

There are several publications investigating the potential association of circulating EPCs with SLE-related factors such as the duration of disease, disease activity, complement levels or the presence of different autoantibodies. The disparity in the results makes it difficult to come to any conclusion; while one study demonstrated that decreased EPC numbers correlated with the SLEDAI score [13], other authors have not found any association [11, 12, 17, 18]. Similar different results are shown for complement levels and the presence of autoantibodies [12, 17, 26]. More homogeneous are the results of studies examining the relationship between circulating ECs and disease activity, concluding that in SLE patients there is a strong correlation between circulating EC counts and SLEDAI score [5, 25, 27, 33]. In our study, no differences were found between EPC and EC counts related to SLEDAI score, perhaps because of the small number of patients with active disease. We also found no association with the SLICC/ACR score, which could similarly be explained by the small number of patients with high organ damage scores.

Regarding whether any of the different treatments of SLE disease is related with circulating EPC or EC counts, in patients with SLE the use of steroids has been associated with decreased numbers of circulating ECs, but with no change in EPC counts [27]. Inconclusive findings appear if we review studies examining the effect of antimalarials on circulating EPCs [11, 14]. Recently it was suggested that statins may increase EPC numbers and enhance their function [34]. In our results we observed no EPC difference between treated and untreated patients or in considering any of the therapeutic options. Taken together, circulating EPC levels of SLE patients were not explained by the use of common medications, including steroids, antimalarials, cytotoxic agents and statins.

Our study has several limitations. First, the number of patients included was limited. Second, our study did not include healthy controls, and several publications have
demonstrated different EPC levels between SLE patients and controls. Finally, four patients with established CVD were included in this study, although the results obtained excluding these patients did not show any difference. In summary, the findings of this study suggest that a decreased number of circulating EPCs could be considered as an early risk factor for subclinical atherosclerosis in SLE patients, providing additional information to traditional risk factors and allowing us to use it as a biomarker to identify patients at increased risk of atherosclerosis.

**Rheumatology key messages**

- Reduced endothelial progenitor cell (EPC) counts are associated with arterial stiffness and cardiovascular risk factors in SLE patients.
- Circulating EPC quantification could be useful to identify SLE patients at increased risk of atherosclerosis.

**Supplementary data**

Supplementary data are available at *Rheumatology* Online.

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