


# Periodontal inflammation is associated with increased circulating levels of endothelial progenitor cells: a retrospective cohort study in a high vascular risk population

María Vázquez-Reza, Antía Custodia, Iria López-Dequidt, Marta Aramburu-Núñez, Daniel Romaus-Sanjurjo, Alberto Ouro, João Botelho, Vanessa Machado, Ramón Iglesias-Rey, Juan Manuel Pías-Peleiteiro, Rogelio Leira, Juan Blanco, José Castillo, Tomás Sobrino\* and Yago Leira\* 

## Abstract

**Background:** One of the main biological mechanisms behind the link between periodontitis and atherosclerotic vascular diseases is vascular endothelial dysfunction. Particularly, circulating endothelial progenitor cells (EPCs) have been considered a biomarker of altered vascular endothelial function.

**Objectives:** The aim of this study was to investigate relationship between periodontal inflammation and increased number of circulating EPCs.

**Design:** This is retrospective cohort study.

**Methods:** In this study, 85 elderly patients with a previous history of hypertension were followed up to 12 months. A baseline full-mouth periodontal assessment was carried out, and the amount of periodontal tissue inflamed per subject was calculated as a proxy of periodontal inflammation [periodontal inflamed surface area (PISA)]. The number of circulating EPCs (CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup>) was determined by flow cytometry from peripheral blood samples collected at baseline and 12 months.

**Results:** Mean concentrations of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> progenitor cells were higher in periodontitis patients than in those without periodontitis at baseline [55.4, 95% confidence interval (CI) = 20.8 to 90.0 *versus* 27.2, 95% CI = 13.6 to 40.8,  $p=0.008$ ] and 12 months (114.6, 95% CI = 53.5 to 175.7 *versus* 19.1, 95% CI = 10.8 to 27.4,  $p=0.003$ ). A significant increase over the follow-up was noticed in the group of subjects with periodontitis ( $p=0.049$ ) but not in the nonperiodontitis group ( $p=0.819$ ). PISA was independently associated with CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs at baseline ( $B$  coefficient = 0.031, 95% CI = 0.005 to 0.058;  $p=0.021$ ). The relationship between PISA and CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs at 12 months was confounded by increased baseline body mass index ( $B$  coefficient = 0.064, 95% CI = -0.005 to 0.132;  $p=0.066$ ).

**Conclusion:** Periodontal inflammation is associated with high number of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs, thus supporting a potential link between periodontitis and endothelial dysfunction.

**Keywords:** biomarker, endothelial function, endothelial progenitor cells, inflammation, periodontitis

Received: 21 December 2022; revised manuscript accepted: 9 May 2023.

## Introduction

Dysfunction of the vascular endothelium is considered an early step of atherosclerosis, preceding

atherosclerotic plaque formation.<sup>1</sup> Endothelial progenitor cells (EPCs) are a subset of stem cells involved in maintaining appropriate endothelial

*Ther Adv Chronic Dis*

2023, Vol. 14: 1–10

DOI: 10.1177/  
20406223231178276

© The Author(s), 2023.  
Article reuse guidelines:  
sagepub.com/journals-  
permissions

Correspondence to:

**Tomás Sobrino**  
NeuroAging Laboratory  
(NEURAL) Group, Clinical  
Neurosciences Research  
Laboratory (LINC), Health  
Research Institute of  
Santiago de Compostela  
(IDIS), University Clinical  
Hospital, Travesía da  
Choupana S/N, 15706  
Santiago de Compostela,  
Spain.

**Tomas.Sobrino.Moreiras@  
sergas.es**

**Yago Leira**  
NeuroAging Laboratory  
(NEURAL) Group, Clinical  
Neurosciences Research  
Laboratory (LINC), Health  
Research Institute of  
Santiago de Compostela  
(IDIS), University Clinical  
Hospital, Travesía da  
Choupana S/N, 15706  
Santiago de Compostela,  
Spain.

Periodontology Unit,  
Faculty of Medicine and  
Odontology, University of  
Santiago de Compostela,  
Santiago de Compostela,  
Spain

**yagoleira@gmail.com**

**María Vázquez-Reza**

**Juan Blanco**

Periodontology Unit,  
Faculty of Medicine and  
Odontology, University of  
Santiago de Compostela,  
Santiago de Compostela,  
Spain

**Antía Custodia**  
**Marta Aramburu-Núñez**  
**Daniel Romaus-Sanjurjo**  
**Alberto Ouro**  
**Juan Manuel Pías-  
Peleiteiro**

NeuroAging Laboratory  
(NEURAL) Group, Clinical  
Neurosciences Research  
Laboratory (LINC), Health  
Research Institute of  
Santiago de Compostela  
(IDIS), University Clinical  
Hospital, Santiago de  
Compostela, Spain

Iria López-Dequidt

Rogelio Leira  
Neurology Department,  
University Clinical  
Hospital, Santiago de  
Compostela, Spain

João Botelho

Vanessa Machado  
Periodontology  
Department and  
Evidence-Based Hub,  
Clinical Research Unit,  
Centro de Investigação  
Interdisciplinar Egas  
Moniz, Instituto  
Universitário Egas Moniz –  
Cooperativa de Ensino  
Superior, Caparica,  
Portugal

Ramón Iglesias-Rey

José Castillo  
Neuroimaging and  
Biotechnology Laboratory  
(NOBEL) Group, Clinical  
Neurosciences Research  
Laboratory (LINC), Health  
Research Institute of  
Santiago de Compostela  
(IDIS), University Clinical  
Hospital, Santiago de  
Compostela, Spain

\*Tomás Sobrino and Yago  
Leira contributed equally  
as joint senior authors.

function. Also, they have the ability to proliferate, differentiate and mature into endothelial cells.<sup>2</sup> EPCs are present in the bloodstream and can also be mobilised from the bone marrow, after vascular injury, to home into the site of damage and repair the vascular endothelium.<sup>2</sup> The most widely accepted characterization is the co-expression of the CD34 and CD133 antigens as well as kinase insert domain-containing receptor (KDR) cell surface antigens.<sup>3</sup> In the last decades, it has been suggested that levels of circulating EPCs could be used as a surrogate biomarker for endothelial function associated with subclinical atherosclerosis and clinical vascular events.<sup>4,5</sup>

In the last consensus report from the European Federation of Periodontology (EFP) that was based on the most updated scientific epidemiological evidence, it was concluded that periodontitis might predispose to increased risk for atherosclerotic vascular disease, in particular cardiovascular disease, ischemic stroke and peripheral artery disease.<sup>6,7</sup> Furthermore, periodontal treatment could have a positive effect mainly on surrogate markers of vascular disease.<sup>8</sup> Biological mechanistic pathways described in the literature so far include, on one hand, bacterial invasion of endothelial and phagocytic cells within atheroma leading to the progression of the same and, on the other hand, inflammatory mediators, haemostatic and pro-coagulant factors together with matrix metalloproteinases can be disseminated into the bloodstream through the ulcerated periodontal pocket could further accelerate atheroma formation and progression through oxidative stress and inflammatory and endothelial dysfunction.<sup>9</sup> Kebschull and co-workers demonstrated that recurrent transient bacteremias induced by repeated intravenous challenge with live *Porphyromonas gingivalis* (a bacteria causally related to periodontitis) in mice mobilised bone marrow-derived progenitor cells and led to increased peripheral concentrations of EPCs.<sup>10</sup> These experimental data are supported by human studies in which higher levels of circulating EPCs were found in patients with periodontitis compared with subjects without periodontitis.<sup>11,12</sup> In line with these findings, a randomised clinical trial demonstrated that periodontal treatment might reduce the number of peripheral CD34<sup>+</sup> EPCs.<sup>13</sup> Periodontitis, however, may have an effect on changes of EPCs levels over time is still unknown. Therefore, the aim of this study was to

evaluate if periodontal inflammation is associated with an increase in the number of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs over a period of 12 months.

## Materials and methods

### Study sample

This was a retrospective analysis using data from a longitudinal study carried out between 2016 and 2019 and performed following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.<sup>14</sup> In brief, dentate elderly (aged  $\geq 60$  years) subjects of both sexes with a previous history of hypertension and diabetes (at least 5 years of disease evolution) were included in the original prospective study.<sup>15</sup> All participants were recruited from two primary care centres in A Estrada and Porto do Son (Galicia, Spain) and referred for a detailed examination to the University Clinical Hospital of Santiago de Compostela (Spain). Exclusion criteria were as follows: (1) <10 teeth present (periodontal examination unreliable); (2) previous history of cerebrovascular disease, cardiovascular disease, dementia, malignancy, or other severe medical condition; (3) periodontal treatment in the last year; (4) active infectious/inflammatory diseases (e.g. HIV, hepatitis, chronic bronchitis, inflammatory bowel disease, rheumatoid arthritis, allergies or asthma); (5) treatment with systemic antibiotics, corticosteroids and immunosuppressive agents within 3 months prior to periodontal examination; (6) not able to consent.

### Socio-demographic data, clinical examination and vascular risk factors

Socio-demographic data recorded used in the present analysis included age and sex and educational level (low was defined as those participants who dropped out of school before age 14 years). Body weight was measured to the nearest 1 kg, and height was recorded to the nearest centimetre. Body mass index (BMI) was calculated with the formula weight (kg)/height (m<sup>2</sup>). Classical vascular risk factors were recorded: previous history of smoking (current smoker or former smoker with less than 1 year), alcohol consumption (>300 g of alcohol/week), history of diabetes [glycated haemoglobin (HbA1c)  $\geq 6.5\%$ , glycaemia  $\geq 200$  mg/dl in symptomatic patients, baseline

glycaemia  $\geq 126$  mg/dl in two determinations or glycaemia after oral glucose tolerance test  $\geq 200$  mg/dl or under antidiabetic medication], hypertension (blood pressure  $\geq 140/90$  mmHg in two determinations or under antihypertensive medication) and hypercholesterolemia [total cholesterol  $> 250$  mg/dl or low-density lipoprotein (LDL) cholesterol  $> 130$  mg/dl or under lipid-lowering medication].

### *Periodontal assessment*

The periodontal examination was described in detail elsewhere.<sup>16</sup> The periodontal calibration was performed by a single calibrated periodontist (Y.L.). The calibration was completed before the start of the study in the Periodontology Unit of the Faculty of Odontology (University of Santiago de Compostela) using 10 nonstudy patients suffering from moderate or severe periodontitis. Intraexaminer reliability was assessed by the intraclass correlation coefficients [for pocket depth (PD) and clinical attachment level (CAL)], which were  $> 0.75$  for both parameters, hence, demonstrating a good degree of reliability in the measurements.<sup>16</sup> In this study, the following periodontal parameters were evaluated in all teeth (except third molars): (1) PD, measured from the free gingival margin to the bottom of the sulcus or pocket; (2) CAL, measured from the cemento-enamel junction to the bottom of the sulcus or pocket; (3) full-mouth plaque score (FMPS), defined as the number of sites with detectable supragingival dental plaque divided by the total number of sites per mouth, multiplied by 100;<sup>17</sup> (4) full-mouth bleeding score (FMBS), defined as the number of sites with gingival bleeding on probing (BoP) divided by the total number of sites per mouth, multiplied by 100<sup>18</sup> and (5) the number of teeth present (excluding third molars and retained roots). All measurements were recorded at six sites per tooth (mesio-buccal, disto-buccal, mid-buccal, mesio-lingual, disto-lingual and mid-lingual), except for FMPS (four sites/tooth) using a sterile mouth mirror and with a calibrated University of North Carolina periodontal probe (UNC 15; Hu-Friedy, Chicago, IL, USA).

The presence of periodontitis was defined according to the Centers for Disease Control and Prevention (CDC)–American Academy of Periodontology (AAP) consensus for epidemiologic

studies.<sup>19,20</sup> Accordingly, a periodontitis case was defined as those subjects showing at least two interproximal sites with CAL  $\geq 3$  mm and at least two interproximal sites with PD  $\geq 4$  mm (not on the same tooth) or one site with PD  $\geq 5$  mm.<sup>19</sup>

In addition, a measure of periodontal inflammation, the PISA was calculated.<sup>21</sup> PISA reflects the surface area of bleeding pocket epithelium in mm<sup>2</sup>. PISA was calculated with a Microsoft Excel spreadsheet in the following steps: (1) mean CAL and gingival recession for each particular tooth is calculated and (2) linear mean CAL and gingival recession is translated into the periodontal epithelial surface area (PESA) for each specific tooth.<sup>22</sup> The PESA for a particular tooth consists of the root surface area of that tooth measured in mm<sup>2</sup>, which is covered with pocket epithelium. (3) The PESA for a specific tooth is then multiplied by the proportion of sites around the tooth that was affected by BoP, resulting in the PISA for that particular tooth. (4) The sum of all individual PISAs around the individual tooth is calculated, rendering the full mouth PISA value in mm<sup>2</sup> of each participant.

### *Laboratory analysis*

*Standard biochemical analysis.* Fasting blood samples were obtained in the morning at the same time as the periodontal assessment and interview. Briefly, 2 ml of venous blood was collected from the antecubital fossa by venepuncture using a 20-gauge needle with a 2-mL syringe. Blood samples were allowed to clot at room temperature and, after 1 h, serum was separated by centrifugation (15 min at 3000g at 4°C) and then 0.5 ml of extracted serum was immediately transferred to 1.5-mL aliquots. Each aliquot was stored at  $-80^{\circ}\text{C}$  until required for analysis. Biochemical parameters analysed in this study included (1) inflammatory biomarkers: fibrinogen (mg/dl), erythrocyte sedimentation rate (ESR) (mm/h) and leukocytes ( $\times 10^3/\text{mL}$ ); (2) lipid fractions (all expressed in mg/dl): total cholesterol, high-density lipoprotein (HDL) cholesterol and LDL cholesterol and (3) metabolic control: HbA1c (%).

*EPCs.* Circulating EPC levels were measured by flow cytometry according to methods described elsewhere<sup>23,24</sup> in blood samples obtained at baseline and at 12 months. Blood samples were processed within 1–2 h after collection by a single

researcher. In brief, circulating EPCs were analysed for the expression of specific surface antigens using flow cytometry (BD FACSAria IIu, BD, Franklin Lakes, NJ, USA). Cells were labelled with FITC-conjugated anti-CD34 (BD), APC-conjugated anti-CD133 (clone AC133 from Miltenyi Biotec, Bergisch Gladbach, Germany) and PE-conjugated anti-KDR (R&D Systems, Minneapolis, MN, USA) monoclonal antibodies. We considered EPCs as triple CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> staining cells in the mononuclear cell fraction. In all analyses,  $5 \times 10^5$  events were acquired, scored using an FACSAria IIu analyzer (BD) and processed using the FlowJo™ 7.6 software programme (Ashland, OR, USA). Cell count was always expressed per  $10^6$  events. The intraobserver correlation, which was assessed by the unique investigator who analysed two blood samples obtained from a single subject, was 0.96.

All determinations were performed in an independent laboratory blinded to clinical data (standard biochemistry at Central Laboratory of the Clinical University Hospital of Santiago de Compostela and EPCs at Clinical Neurosciences Research Laboratory of the same hospital). Clinical investigators were unaware of the laboratory results until the end of the study.

#### Statistical analysis

Mean values and standard deviation ( $\pm$ SD) or 95% confidence intervals (CIs) were calculated for continuous variables and compared using independent *t* test after normality was confirmed by Kolmogorov–Smirnov test. Non-normally distributed continuous variables were expressed as median [ $P_{25}$ ,  $P_{75}$ ] and compared with Mann–Whitney *U* test. Categorical data were reported as percentages (%) and compared by Chi-square test. Parametric correlation analyses between clinical periodontal parameters and circulating levels of EPCs were performed using Pearson's correlation coefficient. Analysis of variance (ANOVA) for repeated measures test was used to compare the changes of EPCs between study groups at baseline and at 12 months. Linear regression models were performed to test potential associations between periodontitis and EPCs. All tests were performed at a significance level of  $\alpha=0.05$ . All data analyses were performed with SPSS Statistics (version 24.0 for Mac; IBM Corporation, Armonk, NY, USA).

#### Results

Among all participants, 62.4% were diagnosed with periodontitis. Table 1 shows the main characteristics of patients according to periodontal status. Subjects with periodontitis had significantly higher BMI compared with those without periodontitis ( $p=0.007$ ). A tendency was observed towards increased levels of systemic inflammation in the periodontitis group in comparison with the nonperiodontitis group as measured by concentrations of ESR ( $p=0.055$ ). No statistically significant differences were noted for any socio-demographic variables and biochemical parameters as well as well-known vascular risk factors.

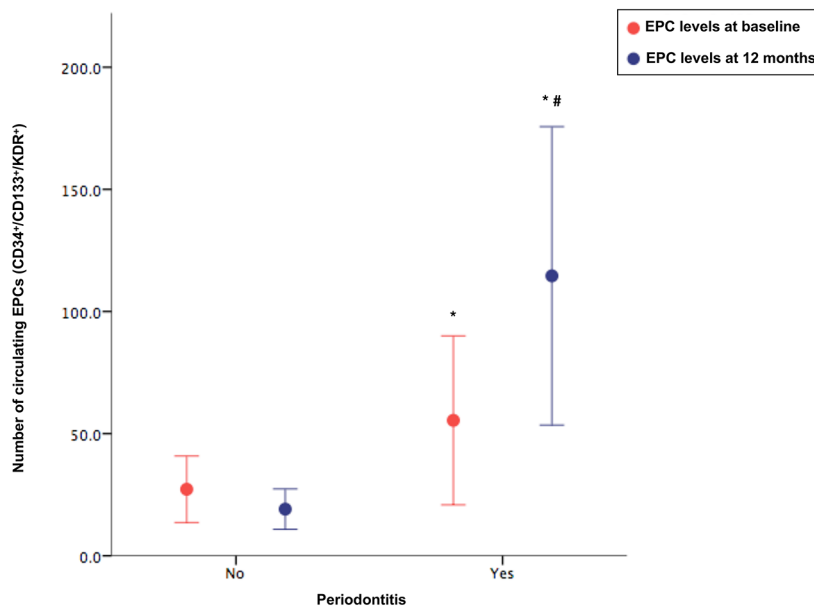
As expected, periodontitis patients exhibited worse periodontal conditions than in those without periodontitis in terms of plaque accumulation and gingival bleeding together with increased PDs and CALs as well as higher levels of periodontal inflammation ( $p<0.001$ ). Less number of teeth was also a feature observed in periodontitis patients when compared with subjects without periodontitis ( $p=0.009$ ).

The mean number with its 95% CIs of circulating EPCs according to periodontal status at baseline and 12 months are shown in Figure 1 (Perio-BL=55.4, 95% CI=20.8 to 90.0; NonPerio-BL=27.2, 95% CI=13.6 to 40.8; Perio-12M=114.6, 95% CI=53.5 to 175.7; and NonPerio-12M=19.1, 95% CI=10.8 to 27.4). At baseline, the number of EPCs (CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup>) was statistically significant higher in periodontitis subjects than those without periodontitis (mean difference in EPCs counts=35.1, 95% CI=9.6 to 60.6;  $p=0.008$ ). A substantial increase in EPC concentrations was noticed at 12 months in the periodontitis group ( $\Delta$ BL-12M=59.2, 95% CI=0.4 to 117.9;  $p=0.049$ ) while no major changes were seen in the nonperiodontitis group ( $\Delta$ BL-12M=-8.1, 95% CI=-79.0 to 62.7;  $p=0.819$ ). Intergroup comparisons at 12 months revealed that patients with periodontitis had higher levels of EPCs when compared with subjects without periodontitis (mean difference in EPCs counts=95.5, 95% CI=33.9 to 157.0;  $p=0.003$ ).

A positive moderate correlation was found between periodontal inflammation (PISA) and the number of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> progenitor cells at

**Table 1.** Main characteristics of study participants according to periodontal status.

	Periodontitis (n=53)	No periodontitis (n=32)	p value
Socio-demographic variables			
Age (years)	70.6 ± 5.1	70.9 ± 4.9	0.798
Males, n (%)	19 (35.8)	16 (50.0)	0.199
Low educational level, n (%)	16 (30.2)	16 (50.0)	0.155
BMI (kg/m <sup>2</sup> )	31.7 ± 5.0	29.1 ± 3.6	<b>0.007</b>
Vascular risk factors			
Smoking habit, n (%)	13 (24.5)	3 (9.4)	0.083
Alcohol consumption, n (%)	4 (7.5)	2 (6.2)	0.821
Hypertension, n (%)	53 (100)	32 (100)	1.000
Diabetes, n (%)	21 (39.6)	12 (37.5)	0.846
Dyslipidemia, n (%)	41 (77.4)	20 (62.5)	0.140
Periodontal parameters			
FMPS (%)	57.6	39.7	<b>&lt;0.001</b>
FMBS (%)	52.6	26.5	<b>&lt;0.001</b>
PD (mm)	3.7 ± 0.9	2.7 ± 0.7	<b>&lt;0.001</b>
PD6 (%)	15.2 ± 19.0	0.0 ± 0.0	<b>&lt;0.001</b>
CAL (mm)	4.0 ± 1.1	2.9 ± 0.9	<b>&lt;0.001</b>
CAL5 (%)	22.7 ± 29.1	0.0 ± 0.0	<b>&lt;0.001</b>
PISA (mm <sup>2</sup> )	606.1 ± 83.2	91.8 ± 16.2	<b>&lt;0.001</b>
Number of teeth	20.8 ± 4.8	23.4 ± 3.4	<b>0.009</b>
Biochemical parameters			
Fibrinogen (mg/dl)	428.0 [390.0, 483.0]	418.0 [392.75, 484.5]	0.681
ESR (mm/h)	16.5 ± 12.0	12.4 ± 6.3	0.055
Leukocytes (×10 <sup>3</sup> /mL)	7.8 ± 2.0	7.6 ± 2.2	0.667
HbA1c (%)	6.8 ± 1.4	6.8 ± 1.5	0.954
Total cholesterol (mg/dl)	195.0 [168.0, 220.7]	190.0 [163.2, 237.7]	0.782
HDL (mg/dl)	57.3 ± 15.6	59.7 ± 21.2	0.573
LDL (mg/dl)	112.9 ± 28.9	113.0 ± 32.0	0.987
BMI, body mass index; CAL, clinical attachment level; CAL5, percentage of sites with CAL ≥5mm; ESR, erythrocyte sedimentation rate; FMBS, full-mouth bleeding score; FMPS, full-mouth plaque score; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PD, pocket depth; PD6, percentage of sites with PD ≥6mm; PISA, periodontal inflamed surface area. Bold indicates P < 0.05.			



**Figure 1.** Mean and 95% CIs of number of EPCs (CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup>) according to periodontal status at baseline and after 12 months of follow-up.

\* $p < 0.01$  for intergroup comparisons; # $p < 0.05$  for intragroup comparisons.

baseline ( $r = 0.317$ ,  $p = 0.003$ ) and at 12 months ( $r = 0.283$ ,  $p = 0.038$ ). Linear regression analysis revealed that PISA was associated with baseline levels of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> progenitor cells in both unadjusted ( $B$  coefficient = 0.037, 95% CI = 0.013 to 0.061;  $p = 0.003$ ) and adjusted model for BMI ( $B$  coefficient = 0.031, 95% CI = 0.005 to 0.058;  $p = 0.021$ ). An association was also observed between PISA and CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> progenitor cells at 12 months in the unadjusted model ( $B$  coefficient = 0.063, 95% CI = 0.004 to 0.123;  $p = 0.038$ ); however, after adjustment for baseline BMI, a drop in the statistical significance was observed ( $B$  coefficient = 0.064, 95% CI = -0.005 to 0.132;  $p = 0.066$ ).

### Discussion

The main results of the study showed that in high vascular risk patients, PISA (a parameter reflecting periodontal inflammation) was associated with increase in levels of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs over 1 year of follow-up.

The link between periodontitis and endothelial dysfunction has been described years ago, when Amar and colleagues carried out a case-control

study in which they observed that in comparison with nonperiodontitis individuals; those with severe periodontitis had diminished brachial flow-mediated dilatation (FMD), a noninvasive ultrasonographic marker of endothelial function.<sup>25</sup> A further systematic review and meta-analysis including more studies has corroborated the initial results whereby almost a 5% mean-weighted difference in FMD values was observed between periodontitis and periodontally healthy patients.<sup>26</sup> In addition, intervention trials have shown that successful periodontal treatment carried out in advanced periodontitis cases is capable of improving endothelial function through an increase in FMD.<sup>27,28</sup>

In recent years, EPCs have become a widely used biomarker of endothelial function in several chronic inflammatory vascular conditions as well as a potential therapeutic target for the same.<sup>29</sup> In this regard, a few reports have demonstrated that EPCs are elevated in patients with periodontitis.<sup>11,12</sup> In a previous study, Li *et al.*<sup>11</sup> found a higher proportion of moderate-to-severe periodontitis patients among the top percentile ( $\geq 75$ th percentile) of CD34<sup>+</sup>/KDR<sup>+</sup> EPCs counts compared with those with mild form of periodontitis or without the disease. Authors also found that

advanced periodontitis was associated with both high counts of CD34<sup>+</sup>/KDR<sup>+</sup> and CD133<sup>+</sup>/KDR<sup>+</sup> EPCs.<sup>11</sup> Similarly, Jönsson and co-workers showed statistically significant different levels of haemangioblastic EPCs in a group of periodontitis patients compared with periodontally healthy controls.<sup>12</sup> Multivariable regression analyses revealed that periodontal parameters were associated with high levels of haemangioblastic EPCs.<sup>12</sup> Furthermore, in a recent case-control study, tooth loss (a common sequela in periodontitis patients) has also been associated with abnormal levels of CD133<sup>+</sup>/KDR<sup>+</sup> EPCs.<sup>30</sup> In line with all these findings, in the present longitudinal study, we observed that CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs increased progressively over 12 months in periodontitis subjects compared with those without periodontitis in which no statistically significant changes were observed over the study follow-up. There is also evidence coming from a clinical trial showing that periodontal treatment could reduce the number of CD34<sup>+</sup> progenitor cells after 3 months of therapy.<sup>13</sup> The biological rationale behind all of these observations could rely on the concept of mobilisation of bone marrow-derived EPCs in response to periodontal infection shown by Kebschull and colleagues.<sup>10</sup> These investigators carried out an experimental study in which they mimic acute recurrent bacteremias induced by *P. gingivalis* intravenous injections in mice, showing an increase in EPCs counts and decrease pools in the bone marrow, as well as that this mobilisation was toll-like 2 receptor dependent.<sup>10</sup> Based on our results might be interesting to design an intervention trial to investigate the potential impact of periodontal therapy on EPCs concentrations in the long-term and to evaluate whether this effect correlates with a risk reduction in clinical atherosclerotic vascular events (target population would be otherwise healthy subjects or at-risk individuals – primary prevention) or recurrent vascular events (patients diagnosed with atherosclerotic vascular disease – secondary prevention). Currently, there is moderate evidence showing a positive mid-term effect on surrogate markers such as C-reactive protein and FMD.<sup>8,31,32</sup> Whether this beneficial impact translates to a risk reduction in clinical events is still unknown, however.<sup>8,31</sup>

To our knowledge, this study is the first attempt to evaluate the potential fluctuations of EPCs

over time in periodontitis. It has to be highlighted that when multivariate regression analysis was performed, PISA (a marker of periodontal inflammation) was associated with baseline levels of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs but not with concentrations of EPCs at 12 months. This finding was in part confounded by the high BMI that periodontitis patients had at baseline, thus modifying the longitudinal association between periodontal inflammation and CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs. It has been suggested that BMI could negatively influence the number of circulating EPCs.<sup>33–36</sup> Weight loss, however, might have a beneficial impact on the changes in the number of circulating EPCs after undergoing a diet intervention<sup>33,35</sup> or bariatric surgery.<sup>36</sup> It is possible that the low-grade systemic inflammatory state that obese patients suffer might mask the effect of periodontal inflammation on EPCs levels in the long-term.

Some shortcomings are worth mentioning concerning this study. First, the study sample size was relatively small and no *a priori* calculation was done. Statistically significant differences, however, were observed for the primary outcome (number of EPCs) between periodontitis and nonperiodontitis patients. Second, the cohort of patients included in this study was comprised of individuals with several chronic comorbidities that could have an effect on circulating EPCs levels so we cannot rule out the possibility that increased EPCs are due to a synergetic effect of multiple inflammatory conditions rather than periodontitis itself. In fact, BMI appeared to be an important confounder in the perio-EPCs link. Future prospective studies should only include systemically healthy subjects with no other concomitant chronic conditions. Another important limitation is the use of the CDC-AAP classification system instead of the new one. We have to bear in mind that this study was design and performed before the new periodontal classification came out. Also, only clinical examination was done in the participants; thus, no X-rays were taken to analyse alveolar bone loss. This could have overestimated the prevalence of periodontitis. The classification used in the present study, however, is the one recommended for epidemiological studies like ours.<sup>19</sup> The next studies in this matter should apply the new classification system to avoid this issue.

## Conclusion

Periodontal inflammation was associated with increased numbers of CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup> EPCs, thus corroborating the link between periodontitis and endothelial dysfunction.

## Declarations

### *Ethics approval and consent to participate*

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Ethics Research Committee of Santiago de Compostela/Lugo approved this study (protocol ID: 2016/399). Written informed consent to participate in this study was obtained from all individual participants included in the study.

### *Consent for publication*

Not applicable.

### *Author contributions*

**María Vázquez-Reza:** Data curation; Formal analysis; Writing – original draft; Writing – review & editing.

**Antía Custodia:** Data curation; Formal analysis; Methodology; Software; Writing – review & editing.

**Iria López-Dequidt:** Data curation; Formal analysis; Investigation; Methodology; Resources; Writing – review & editing.

**Marta Aramburu-Núñez:** Data curation; Formal analysis; Writing – review & editing.

**Daniel Romaus-Sanjurjo:** Data curation; Formal analysis; Writing – review & editing.

**Alberto Ouro:** Data curation; Formal analysis; Writing – review & editing.

**João Botelho:** Data curation; Formal analysis; Writing – review & editing.

**Vanessa Machado:** Data curation; Formal analysis; Writing – review & editing.

**Ramón Iglesias-Rey:** Data curation; Formal analysis; Writing – review & editing.

**Juan Manuel Pías-Peleteiro:** Data curation; Formal analysis; Writing – review & editing.

**Rogelio Leira:** Conceptualisation; Data curation; Formal analysis; Project administration; Resources; Writing – review & editing.

**Juan Blanco:** Conceptualisation; Data curation; Funding acquisition; Methodology; Project administration; Supervision; Writing – review & editing.

**José Castillo:** Data curation; Formal analysis; Methodology; Writing – review & editing.

**Tomás Sobrino:** Conceptualisation; Data curation; Funding acquisition; Project administration; Resources; Supervision; Writing – review & editing.

**Yago Leira:** Conceptualisation; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualisation, Writing – original draft; Writing – review & editing.

### *Acknowledgements*

None.

### *Funding*

The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This study was partially supported by grants from the Xunta de Galicia (T.S. and J.C.: IN607A2018/3 and T.S.: IN607D 2020/09 and IN607A2022/07), the Institute of Health Carlos III (T.S.: PI22/00938 and CB22/05/00067) and the Spanish Ministry of Science (T.S.: RTI2018-102165-B-I00 and RTC2019-007373-1). Furthermore, this study was also supported by grants from the INTERREG Atlantic Area (T.S.: EAPA\_791/2018\_NEUROATLANTIC project), the INTERREG VA España Portugal (POCTEP) (T.S.: 0624\_2IQBIONEURO\_6\_E) and the European Regional Development Fund. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. M.A.-N. holds an iPFIS contract (IFI18/00008), D.R.-S. and Y.L. are the recipients of a Sara Borrell fellowship (CD21/00166 and CD22/00051, respectively) and T.S. and R.I.-R. hold a Miguel Servet contract (CPII17/00027 and CP22/00061, respectively), all of them supported by the Institute of Health Carlos III. Finally, A.C. is supported by a predoc contract of Xunta de Galicia (IN606A-2021/015).


### Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

### Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

### ORCID iD

Yago Leira  <https://orcid.org/0000-0001-5027-7276>

### References

1. Davignon J and Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation* 2004; 109: 27–32.
2. Asahara T, Kawamoto A and Masuda H. Concise review: circulating endothelial progenitor cells for vascular medicine. *Stem Cells* 2011; 29: 1650–1655.
3. Chong MSK, Ng WK and Chan JKY. Concise review: endothelial progenitor cells in regenerative medicine: applications and challenges. *Stem Cells Transl Med* 2016; 5: 530–538.
4. Hill JM, Zalos G, Halcox JPJ, *et al.* Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; 348: 593–600.
5. Fadini GP, Coracina A, Baesso I, *et al.* Peripheral blood CD34+KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population. *Stroke* 2006; 37: 2277–2282.
6. Sanz M, Del Castillo AM, Jepsen S, *et al.* Periodontitis and cardiovascular diseases: consensus report. *J Clin Periodontol* 2020; 47: 268–288.
7. Herrera D, Molina A, Buhlin K, *et al.* Periodontal diseases and association with atherosclerotic disease. *Periodontol* 2000; 83: 66–89.
8. Orlandi M, Graziani F and D’Aiuto F. Periodontal therapy and cardiovascular risk. *Periodontol* 2000; 83: 107–124.
9. Schenkein HA, Papapanou PN, Genco R, *et al.* Mechanisms underlying the association between periodontitis and atherosclerotic disease. *Periodontol* 2000; 83: 90–106.
10. Kebschull M, Haupt M, Jepsen S, *et al.* Mobilization of endothelial progenitors by recurrent bacteremias with a periodontal pathogen. *PLoS ONE* 2013; 8: e54860.
11. Li X, Tse HF, Yiu KH, *et al.* Increased levels of circulating endothelial progenitor cells in subjects with moderate to severe chronic periodontitis. *J Clin Periodontol* 2009; 36: 933–939.
12. Jönsson D, Spinell T, Vrettos A, *et al.* Circulating endothelial progenitor cells in periodontitis. *J Periodontol* 2014; 85: 1739–1747.
13. Li X, Tse HF, Yiu KH, *et al.* Effect of periodontal treatment on circulating CD34(+) cells and peripheral vascular endothelial function: a randomized controlled trial. *J Clin Periodontol* 2011; 38: 148–156.
14. Von Elm E, Altman DG, Egger M, *et al.* The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008; 61: 344–349.
15. López-Dequidt IA. *Clinical study of cerebral small vessel disease*. PhD Thesis, University of Santiago de Compostela, Santiago de Compostela, 2019.
16. Leira Y, Rodríguez-Yáñez M, Arias S, *et al.* Periodontitis as a risk indicator and predictor of poor outcome for lacunar infarct. *J Clin Periodontol* 2019; 46: 20–30.
17. O’Leary TJ, Drake RB and Naylor JE. The plaque control record. *J Periodontol* 1972; 43: 38.
18. Ainamo J and Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J* 1975; 25: 229–235.
19. Holtfreter B, Albandar JM, Dietrich T, *et al.* Joint EU/USA Periodontal Epidemiology Working Group. Standards for reporting chronic periodontitis prevalence and severity in epidemiologic studies: proposed standards from de Joint EU/ USA Periodontal Epidemiology Working Group. *J Clin Periodontol* 2015; 42: 407–412.
20. Eke PI, Page RC, Wei L, *et al.* Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol* 2012; 83: 1449–1454.
21. Nesse W, Abbas F, Van der Ploeg I, *et al.* Periodontal inflamed surface area: quantifying

- inflammatory burden. *J Clin Periodontol* 2008; 35: 668–673.
22. Hujoel PP, White BA, García RI, *et al.* The dentogingival epithelial surface area revisited. *J Periodontal Res* 2001; 36: 48–55.
  23. Fadini GP, De Kreutzenberg S, Agostini C, *et al.* Low CD34 cell count and metabolic syndrome synergistically increase the risk of adverse outcomes. *Atherosclerosis* 2009; 207: 213–219.
  24. Pías-Peleteiro J, Pérez-Mato M, López-Arias E, *et al.* Increased endothelial progenitor cells are associated with good outcome in intracerebral hemorrhage. *Sci Rep* 2016; 6: 28724.
  25. Amar S, Gokce N, Morgan S, *et al.* Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. *Arterioscler Thromb Vasc Biol* 2003; 23: 1245–1249.
  26. Orlandi M, Suvan J, Petrie A, *et al.* Association between periodontal disease and its treatment, flow-mediated dilatation and carotid intima-media thickness: a systematic review and meta-analysis. *Atherosclerosis* 2014; 236: 39–46.
  27. Seinost G, Wimmer G, Skerget M, *et al.* Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis. *Am Heart J* 2005; 149: 1050–1054.
  28. Tonetti MS, D’Aiuto F, Nibali L, *et al.* Treatment of periodontitis and endothelial function. *N Engl J Med* 2007; 356: 911–920.
  29. Edwards N, Langford-Smith AWW, Wilkinson FL, *et al.* Endothelial progenitor cells: new targets for therapeutics for inflammatory conditions with high cardiovascular risk. *Front Med* 2018; 5: 200.
  30. Isola G, Lo Giudice A, Polizzi A, *et al.* Periodontitis and tooth loss have negative systemic impact on circulating progenitor cell levels: a clinical study. *Genes* 2019; 10: 1022.
  31. Orlandi M, Muñoz Aguilera E, Marletta D, *et al.* Impact of treatment of periodontitis on systemic health and quality of life: a systematic review. *J Clin Periodontol* 2022; 49: 314–327.
  32. Luthra S, Orlandi M, Hussain SB, *et al.* Treatment of periodontitis and C-reactive protein: a systematic review and meta-analysis of randomized clinical trials. *J Clin Periodontol* 2023; 50: 45–60.
  33. Müller-Ehmsen J, Braun D, Schneider T, *et al.* Decreased number of circulating progenitor cells in obesity: beneficial effect of weight reduction. *Eur Heart J* 2008; 29: 1560–1568.
  34. MacEaney OJ, Kushner EJ, Van Guilder GP, *et al.* Endothelial progenitor cell number and colony-forming capacity in overweight and obese adults. *Int J Obes* 2009; 33: 219–225.
  35. Heida N-M, Müller J-P, Cheng I-F, *et al.* Effects of obesity and weight on the functional properties of early outgrowth endothelial progenitor cells. *J Am Coll Cardiol* 2010; 55: 357–567.
  36. De Ciuceis C, Rossini C, Porteri E, *et al.* Circulating endothelial progenitor cells, microvascular density and fibrosis in obesity before and after bariatric surgery. *Blood Press* 2013; 22: 165–172.