ANEMIA OF INFLAMMATION ASSOCIATED WITH PERIODONTITIS: ANALYSIS OF TWO CLINICAL STUDIES

Running title: Erythrocytes and periodontitis

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One sentence summary: Patients with periodontitis may have a tendency to anemia of chronic inflammation compared with periodontally healthy patients.

KEYWORDS: periodontitis, periodontal medicine, periodontal-systemic disease interactions, inflammation and innate immunity.

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ABSTRACT

Background: While leukocytosis is a common feature of severe periodontitis, a smaller amount of evidence has been produced on erythrocytes counts in periodontitis, suggesting a possible tendency to anemia. The aim of this study was to investigate the associations between periodontitis and circulating leukocytes, erythrocytes and platelets.

Methods: The study included 471 patients with periodontitis (including aggressive periodontitis, AgP, and chronic periodontitis, CP) and periodontal health. A separate sample of 333 patients from a previous study (127 AgP and 206 periodontally healthy) was used as replication. Periodontal clinical data were collected and a blood sample was obtained from each participant for hematological analysis of leukocytes, erythrocytes and platelets.

Results: Adjusted linear regression analyses revealed associations between periodontitis and total leukocytes counts ($p<0.001$), neutrophil counts ($p<0.001$) and hematocrit levels ($p=0.045$). Sub-analysis revealed no statistically significant differences between AgP and CP. Disease severity was correlated with total leukocyte and neutrophil counts and hematocrit ($p<0.001$, $p<0.001$ and $p=0.004$ respectively). In the replication sample, adjusted linear regression analysis revealed associations between periodontitis and decreased hemoglobin ($p=0.009$), mean corpuscular hemoglobin ($p=0.023$) and mean corpuscular hemoglobin concentration ($p<0.001$). A subset of patients included in these studies (14%-15.7% of periodontitis vs. 8.4%-10.2% of healthy respectively in the two cohorts) were anemic based on the WHO criteria.

Conclusion: This paper, reporting results of periodontal examination and blood sampling in over 800 patients, suggest that not just leukocytosis but also tendency to ‘anemia of inflammation’ are typical features of periodontitis.

BACKGROUND

Several studies have now clarified that inflammatory diseases of specific organs or tissues, such as periodontitis, are often not just a local event, but may have systemic ramifications, including elevations in the numbers of circulating leukocytes, acute-phase proteins and oxidative stress markers [1,2]. It is now emerging that also erythrocytes are affected by chronic inflammatory diseases. This phenomenon, named 'anemia of inflammation' may be mediated by the effect of pro-inflammatory cytokines, such as Interleukin (IL)-6, which promote production of hepcidin, resulting in higher iron trapping within macrophages and liver cells; alternative mechanisms may be reduced erythropoiesis and mildly shortened erythrocyte survival [3,4]. Some studies have reported reductions in hemoglobin levels, mean corpuscular hemoglobin, circulating erythrocytes and hematocrit in both chronic periodontitis [5-7] and aggressive periodontitis [8]. A recent systematic review and meta-analysis including data from 342 cases and 359 controls revealed a significant decrease in erythrocyte counts, hematocrit and hemoglobin in patients with chronic periodontitis when compared to healthy individuals [9]. However, no specific comparisons between aggressive and chronic periodontitis have been published. Severe periodontitis has also been associated with increases in platelet count [10], possibly through a process of reactive thrombocytosis mediated by systemic inflammation [11].
The aim of this study, based on secondary analyses of two case-control studies (main study and replication sample), was to assess associations between periodontitis and leukocyte, erythrocyte and platelet counts. The null hypothesis was that no differences existed in leukocyte, erythrocyte, hemoglobin, hematocrit and platelet levels by periodontal diagnosis.

MATERIAL AND METHODS

Patient population - main study

This study had a case–control design, with a total of 471 participants cases selected among patients referred to the Eastman Dental Hospital, University College London by general dental practitioners. Healthy controls were recruited among patients referred to other Departments of the Hospital. All of the patients gave written informed consent and the study had been reviewed and approved by the Joint UCL/UCLH Committees on the Ethics of Human Research. The study was conducted in accordance with the Helsinki Declaration as revised in 2013. All participants in the study had no signs or symptoms of systemic infection or disease as assessed by the examining clinician and had not taken any systemic antibiotics in the last 3 months.

Inclusion criteria for periodontitis patients were the presence of at least one site with ≥ 5mm probing pocket depth (PPD) and clinical attachment level (CAL) (excluding third molars and distal surfaces of second molars). Inclusion criteria for control participants were absence of any site with ≥ 5mm PPD and CAL (excluding third molars or distal surfaces of second molars) or history of periodontitis and periodontal treatment.

Exclusion criteria for all study patients included (i) known systemic diseases (cardiovascular, respiratory, renal, malignancy, etc.), (ii) history and/or presence of any other infections, (iii) systemic antibiotic treatment in the preceding 3 months, (iv) long-term treatment with any medication suspected to affect the periodontium (e.g. non-steroidal anti-inflammatory drugs), (v) pregnant or lactating females and (vi) <20 teeth present.

Demographic data

Demographic data was taken as self-reported by all patients, including age, gender, ethnicity, smoking status and medical history. Patients' height and weight were measured to obtain their body mass index (BMI). Socio-economic factors were derived as quintiles of Index of Multiple Deprivation (IMD) obtained from the patients' post code [12]

Clinical examination and diagnosis

One clinician (author LN) assessed all participants and assigned a diagnosis. A comprehensive clinical examination was performed by three calibrated examiners (authors LN, TR and UD). Full-mouth measures of PPD, recessions (REC) and CAL were obtained at six points per tooth. Diagnosis of alveolar bone loss was confirmed by radiographic assessment from each patient. Patients were diagnosed as having aggressive periodontitis (AgP) or chronic periodontitis (CP), according to the 1999 Consensus Classification [13]. The differential diagnosis between AgP and CP was conducted as described before [14]. Generally, diagnosis of AgP was given to systemically healthy patients ≤ 45 years old who had at least 3 teeth with CAL ≥ 6 mm and BOP. Therefore, 'rapid' progression was based on young age, in the absence of previous radiographic records. The cases were also then subdivided according to CAL and PPD as no-mild vs. moderate-severe periodontitis according to Page and Eke criteria [15].

Replication sample

A previous study with case–control design was used as replication sample to re-test the associations observed in the main study. This population, including a total of 455 patients, has been described before [16]. A total of 333 of these patients had blood samples taken and sent for

hematology analysis. All patients were identified among the population referred for care to the Eastman Dental Hospital. All participants gave written informed consent and the study protocol had been reviewed and approved by the University College London Hospitals ethics committee [16]. The study was conducted in accordance with the Helsinki Declaration as revised in 2013. Inclusion criteria for cases were diagnosis of Aggressive Periodontitis (AgP) [17], taking into account systemic health, rapid disease progression and familial aggregation as described before [16]. The control population was enrolled based on (i) absence of sites with PPD and CAL ≥4mm or radiographic evidence of bone loss, (ii) at least 20 teeth present, (iii) no history of periodontal treatment, (iv) no history of systemic diseases (e.g. diabetes), (v) no pregnancy, (vi) minimum age of 25 years [16]. Results relative to leukocytes in this population have already been reported [18].

Blood sampling and analysis

For both studies, a blood sample was obtained from each patient via venipuncture of the right arm at the examination visit and processed in a blind fashion at the University College London Hospital laboratories for leukocyte and differential counts, erythrocyte count (RBC), red blood cell distribution width (RDW), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count and mean platelet volume (MPV).

Statistical analysis

Data from all patients who took part in the study were entered into a spreadsheet by independent staff not involved in the study, proofed for entry errors and analyzed by a statistical package[§]. Continuous variables are reported as means and standard deviations. The primary outcome was hemoglobin levels. Secondary outcomes were HCT, RBC, RDW, MCV, MCH, MCHC, platelet count, MPV and leukocyte counts. Associations were first sought independently between periodontal status (periodontitis vs. healthy using protocol criteria; periodontitis vs. healthy using Page and Eke's criteria) and laboratory parameters by ANOVA. A sub-analysis was performed for AgP vs. CP vs. healthy. Analyses adjusted for confounders (age, gender, ethnicity, IMD, smoking, BMI) were then carried out by linear regression (for continuous outcomes). The same analysis was repeated in the replication sample, for which linear regression was adjusted only for age, gender, ethnicity and smoking, since no data on BMI and IMD were available.

RESULTS

Table 1 reports characteristics of patients included in the main study, subdivided into AgP, CP and healthy. Patients in the three different groups differed for age, ethnicity, smoking, BMI and average PPD. Table 2 shows associations between leukocyte and erythrocytes parameters and platelets with periodontal diagnosis (divided into male and female patients for erythrocyte parameters). Unadjusted analyses suggested associations between periodontal phenotype and number of total leukocytes, neutrophils and lymphocytes. and for hematocrit (only in females). Adjusted linear regression analyses revealed statistically significant associations between periodontitis (AgP +CP) and total leukocytes counts ($p<0.001$), neutrophil counts ($p<0.001$) and hematocrit levels ($p=0.045$). Adjusted sub-analysis by periodontal diagnosis revealed associations for hematocrit for AgP vs. healthy ($p=0.015$). No statistically significant differences were detected for any laboratory parameters between AgP and CP.

When patients were divided based on Page & Eke's classification (supplemental table 1), only total leukocytes and neutrophil counts were different between periodontitis and healthy volunteers at adjusted analysis ($p=0.013$ and $p<0.001$ respectively).

The correlations between number of PPDs≥5mm and total leukocyte and neutrophil counts and hematocrit were investigated by adjusted linear regression and found to be statistically significant ($p<0.001$, $p<0.001$ and $p=0.004$ respectively). Figure 1 shows hemoglobin and hematocrit levels in patients with periodontitis (excluding controls) divided by tertile of disease extent (based on number of PPDs >4mm), showing trend for association by increase disease severity.

Table 3 reports characteristics of patients included in the replication sample. AgP patients were younger than healthy volunteers. Leukocytosis has already been reported in this subgroup, as part of a larger analysis[17]; in this subgroup analysis, leukocytes and neutrophils were increased in AgP ($p=0.003$ for both, data not presented in tables). Table 4 reports erythrocyte parameters in this population, including results of unadjusted analyses of comparison between AgP and healthy patients. Adjusted linear regression analysis revealed statistically significant associations between AgP and decreased hemoglobin ($p=0.011$), MCH ($p=0.014$) and MCHC ($p<0.001$). Results of adjusted regression analyses for both main study and replication sample are reported in table 5. Gender, ethnicity and smoking exhibited strong associations with erythrocyte parameters.

When both cohorts (n=804) were analyzed together by adjusted linear regression, periodontitis was associated with hemoglobin (p=0.005), hematocrit (p=0.008), MCV (p=0.049) and MCH (p=0.021) (data not presented in tables).

By using the World Health Organization definition for anemia (hemoglobin<12 g/dL in women and <13 g/dL in men) [19], a total of 14% of periodontitis and 8.4% of healthy patients respectively were anemic (p=0.202 at Chi-square) in the main study (data not presented in tables). In the replication cohort, 15.7% of AgP and 10.2% of healthy patients respectively were anemic (p=0.169).

DISCUSSION

'Anemia of inflammation' (AI), previously referred to as anemia of chronic disorders or anemia of chronic inflammation is a secondary anemic status associated with the presence of chronic inflammatory conditions [3]. Although the association between periodontitis and AI was already suggested several years ago [5], only a few studies have investigated it since [20]. A recent systematic review on erythrocyte parameters included 9 studies with a total sample size of 342 periodontitis and 359 healthy patients [9]. The authors found reduced erythrocyte counts, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit and packed cell volume in patients with periodontitis. In agreement with the above review, by investigating 471 patients ranging from AgP to CP to healthy, we found that patients with periodontitis had reduced hematocrit level. This tendency to an anemic state has been suggested for both chronic periodontitis [5-7] and aggressive periodontitis [8]. In the present study, patients with AgP had particularly low hematocrit (statistically significant difference for AgP vs. healthy), although the difference between AgP and CP was not statistically significant. An inverse linear association was observed between disease severity (measured as number of PPDs ≥5 mm) and hematocrit, strengthening the argument for a non-coincidental association. However, interestingly, when Page & Eke's classification[15] was used, the difference in hematocrit between no-mild periodontitis and moderate-severe periodontitis did not reach statistical significance at adjusted analysis. No differences between groups were detected for the other erythrocyte parameters. The observed findings are strengthened by analysis of a replication cohort consisting of 333 patients (127 AgP and 206 healthy patients), showing decreased hemoglobin, mean corpuscular hemoglobin

concentration (MCH) and mean corpuscular hemoglobin concentration (MCHC) in AgP cases, although the association with reduced hematocrit was not statistically significant. In the replication cohort, it appeared that the differences in erythrocyte parameters were particularly marked in male patients. If data from both cohorts were grouped together, increasing statistical power, periodontitis was significantly associated with hemoglobin, hematocrit, MCV and MCH.

It has been suggested that AI is mediated by the effect of pro-inflammatory cytokines, such as Interleukin (IL)-6, in turn stimulating production of hepcidin, resulting in a combination of decrease in erythrocyte survival, impaired production of erythrocytes and hypoferremia [3,4]. Based on the initial evidence gathered so far [5,9] and enhanced by the larger sample size of this study compared with previous studies, it is possible that the local and systemic inflammatory reactions elicited by presence of periodontitis act on the cascade leading to increased production of hepcidin and to reduced production and survival of erythrocytes. Initial evidence suggests that hemoglobin and hematocrit levels may further decrease short-term following intensive periodontal therapy [21], in line with traumatic anemia following surgery [22]. In support of a role of periodontitis in affecting anemia, some studies showed an increase in erythrocytes 3- to 6-months following periodontal treatment [7,23]. However, since most studies on this association are cross-sectional [5,6,8], no strong inference can yet be made on the direction of the association.

In AI, erythrocytes are usually of normal size with normal hemoglobin content but are reduced in number. However, in some long-term chronic conditions, they may become reduced in size and hemoglobin content [3]. Interestingly, although in the main study presented here, patients with periodontitis had reduced erythrocyte numbers, this was not the case for the replication sample. Despite slightly higher erythrocyte counts than controls, AgP patients in the replication sample had lower hemoglobin, MCH and MCHC, suggesting a tendency for erythrocytes to be smaller and with less uptake of iron (less 'hemoglobinised'). The relatively small magnitude of differences in erythrocyte parameters, poses the question of whether the differences between cases and controls are clinically relevant. In this context, it is important to highlight that diagnosis of AI is based on the presence of anemia and hypoferremia with concomitant normal serum ferritin; however, no data on iron absorption and ferritin levels were available in our cohorts [3]. Despite this, it is important that a small subset of patients included in these studies (14%-15.7% of periodontitis vs. 8.4%-10.2% of healthy respectively in the two cohorts) were

actually anemic based on the WHO criteria. This shows that for a small subset of patients, anemia might be of possible periodontal origin.

The proposed association between presence of periodontitis and increased number of platelets could not be confirmed in this study, although a slight increase was found in AgP patients compared with controls in both main study and replication study, of a similar magnitude to that previously reported in a larger sample [10].

This study further confirms that periodontitis is associated with leukocytosis, especially with increased numbers of neutrophils. This is in agreement with several studies, convincingly showing that elevations in neutrophils and often lymphocytes are a typical response to periodontitis [8,18,24,25]. This is part of an inflammatory cascade, initiated by the accumulation of periodontopathogenic bacteria subgingivally and continued by a local inflammatory reaction and subsequently by an overall higher systemic inflammation, linked with vascular dysfunction [26,27] and higher risk of cardiovascular events [28,29].

The main strengths of the present study are a larger sample size compared with previous studies, analysis of association with disease severity measured as PPD≥ 5mm and sub-analysis in AgP and CP. This showed no differences in leukocyte and erythrocyte parameters between these now obsolete disease entities, supporting the concepts that led to the elimination of AgP-CP differentiation in the new classification [30]. Important limitations are that no serum iron levels were studied and that this was a secondary analysis of a study not powered to test differences in erythrocyte and leukocyte parameters. Furthermore, no information on other systemic inflammatory markers such as IL-6 or C-reactive protein (CRP) are available.

CONCLUSION: Whilst confirming that leukocytosis is a typical finding in patients with periodontitis, this paper suggests that erythrocyte parameters (mainly hematocrit and hemoglobin) are reduced in periodontitis, likely as part of a process named anemia of inflammation. Clinically, periodontal diagnosis should be requested as a possible investigation in cases of anemia of unclear origin. Mechanisms of association between periodontitis and AI should be studied, as they might contribute to explaining the systemic burden of periodontal diseases.

Footnotes: ǁ IBM SPSS 25.0, Armonk, New York

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REFERENCES

1. Linden GJ, Lyons A, Scannapieco FA. Periodontal systemic associations: review of the evidence. *J Periodontol* 2013;84:S8-S19.
2. Tonetti MS, Van Dyke TE; working group 1 of the joint EFP/AAP workshop. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Periodontol* 2013;84:S24-9.
3. Nemeth E, Ganz T. Anemia of inflammation. *Hematol Oncol Clin North Am* 2014;28:671-681
4. Schop A, Stouten K, van Houten R, et al. Diagnostics in anemia of chronic disease in general practice: a real-world retrospective cohort study. *BJGP Open* 2018;25;2(3).
5. Hutter JW, van der Velden U, Varoufaki A, Huffels RA, Hoek FJ, Loos BG. Lower numbers of erythrocytes and lower levels of hemoglobin in periodontitis patients compared to control subjects. *J Clin Periodontol* 2001;28:930-936.
6. Gokhale SR, Sumanth S, Padhye AM. Evaluation of blood parameters in patients with chronic periodontitis for signs of anemia. *J Periodontol* 2010;81:1202-1206.
7. Malhotra R, Kapoor A, Grover V, Grover D, Kaur A. Effect of scaling and root planing on erythrocyte count, hemoglobin and hematocrit in patients with chronic periodontal disease. *J Dent Hyg* 2012;86:195-203.
8. Anand PS, Sagar DK, Ashok S, Kamath KP. Association of aggressive periodontitis with reduced erythrocyte counts and reduced hemoglobin levels. *J Periodont Res* 2014 ;49:719-728.
9. França LFC, da Silva FRP, di Lenardo D, et al. Comparative analysis of blood parameters of the erythrocyte lineage between patients with chronic periodontitis and healthy patients: Results obtained from a meta-analysis. *Arch Oral Biol* 2019;97:144-149.
10. Romandini M, Laforí A, Romandini P, Baima G, Cordaro M. Periodontitis and platelet count: A new potential link with cardiovascular and other systemic inflammatory diseases. *J Clin Periodontol* 2018;45:1299-1310.

11. Zareifar S, Farahmand Far MR, Golfeshan F, Cohan N. Changes in platelet count and mean platelet volume during infectious and inflammatory disease and their correlation with ESR and CRP. *J Clin Lab Anal* 2014;28:245-248.
12. http://imd-by-postcode.opendatacommunitites.org/, accessed December 10th, 2018).
13. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;4:1–6.
14. Onabolu O, Donos N, Tu YK, Darbar U, Nibali L. Periodontal progression based on radiographic records: an observational study in chronic and aggressive periodontitis. *J Dent* 2015;43:673–682.
15. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. *J Periodontol* 2007;78:1387-1399.
16. Nibali L, Parkar M, Brett P, Knight J, Tonetti MS, Griffiths GS. NADPH oxidase (CYBA) and FcgammaR polymorphisms as risk factors for aggressive periodontitis: a case-control association study. *J Clin Periodontol* 2006;33:529-539.
17. Lang NP, Bartold M, Cullinan M. Consensus report: aggressive periodontitis. *Ann Periodontol* 1999;4:53.
18. Nibali L, D'Aiuto F, Griffiths G, Patel K, Suvan J, Tonetti MS. Severe periodontitis is associated with systemic inflammation and a dysmetabolic status: a case-control study. *J Clin Periodontol* 2007;34:931-937.
19. World Health Organization. Worldwide prevalence of anemia 1993–2005 (PDF). Geneva: World Health Organization 2008. Accessed December 10th, 2018.
20. Loos BG. Systemic Markers of Inflammation in Periodontitis. *J Periodontol* 2005;76 Suppl 11S:2106-2115.
21. D'Aiuto F, Nibali L, Mohamed-Ali V, Vallance P, Tonetti MS. Periodontal therapy: a novel non-drug-induced experimental model to study human inflammation. *J Periodont Res* 2004;39:294-299.
22. van Iperen CE, Kraaijenhagen RJ, Biesma DH, Beguin Y, Marx JJ, van de Wiel A. Iron metabolism and erythropoiesis after surgery. *Brit J Surg* 1998;85:41-45.
23. Pradeep AR, Anuj S. Anemia of chronic disease and chronic periodontitis: does periodontal therapy have an effect on anemic status? *J Periodontol* 2011;82:388-394.
24. Kwon YJ, Jeon KJ, Chung TH, Lee YJ. Elevated leukocyte count is associated with periodontitis in Korean adults: the 2012-2014 KNHANES. *Oral Dis* 2017;23:241-246.
25. Loos BG, Craandijk J, Hoek FJ, Wertheim-van Dillen PM, van der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *J Periodontol* 2000:71:1528-1534.
26. Tonetti MS, D'Aiuto F, Nibali L, et al. Treatment of periodontitis and endothelial function. *New Eng J Med* 2007;356:911-920

27. 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.
28. Söder B, Jin LJ, Klinge B, Söder PO. Periodontitis and premature death: a 16-year longitudinal study in a Swedish urban population. *J Periodont Res* 2007;42:361-366.
29. Cowan LT, Lakshminarayan K, Lutsey PL, et al. Periodontal disease and incident venous thromboembolism: The Atherosclerosis Risk in Communities study. *J Clin Periodontol* 2019;46:12-19.
30. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol* 2018;89 Suppl 1:S159-S172.

FIGURE LEGENDS

Figure 1. Box plots showing associations between periodontitis extent (tertiles by number of PPDs≥5 mm) and both hemoglobin and hematocrit in the main study.

TABLES

Patient characteristics		**AgP (n=125)**	**CP (n=121)**	**Healthy (n=225)**	**Comparison perio vs. healthy P=**
Age		33.79 ± 6.18	45.12 ± 10.04	37.65 ± 11.52	0.086
Gender (male)		44 (35.2%)	50 (41.3%)	104 (46.2%)	0.048
Ethnicity	**Caucasian**	55 (44.0%)	71 (59.7%)	168 (75.0%)	
	African	23 (18.4%)	2 (1.7%)	11 (4.9%)	
	Black Caribbean	23 (18.4%)	16 (13.4%)	6 (2.7%)	<0.001
	Asian	19 (15.2%)	25 (21.0%)	35 (15.6%)	
	Other	5 (4.0%)	5 (4.2%)	4 (1.8%)	
Smoking	**Current**	25 (20.0%)	27 (22.3%)	32 (14.2%)	
	Former	34 (27.2%)	33 (27.3%)	41 (18.2%)	0.002
	Never	66 (52.8%)	61 (50.4%)	152 (67.6%)	
IMD quintile	**1**	27 (23.5%)	20 (17.1%)	30 (14.2%)	
	2	31 (27.0%)	43 (36.8%)	74 (34.9%)	
	3	29 (25.2%)	20 (17.1%)	57 (26.9%)	0.333
	4	21 (18.3%)	13 (11.1%)	29 (13.7%)	
	5	7 (6.1%)	21 (17.9%)	22 (10.4%)	
BMI		26.94 ± 6.80	26.49 ± 5.00	24.25 ± 4.17	<0.001
Average PPD (mm)		4.16 ± 1.17	3.40 ± 0.77	1.97 ± 0.28	<0.001

Table 1. Characteristics of patients included in the main study, subdivided into AgP, CP and healthy. Differences between groups were analysed by Chi-square (categorical variables) or ANOVA (continuous variables). IMD: Index of Multiple Deprivation, ranging from 1 (less deprived) to 5 (more deprived area) (please note IMD data were missing for 27 patients).

Blood analytes		AgP (n=125)	CP (n=121)	Healthy (n=225)	Perio (AgP + CP) vs. Healthy
Leukocytes (10^9/L)		6.83 ± 2.03	6.52 ± 1.42	5.87± 1.48	<0.001
Neutrophils (10^9/L)		4.11 ± 1.63	3.83 ± 1.26	3.28 ± 1.19	<0.001
Lymphocytes (10^9/L)		2.10 ± 0.56	2.03 ± 0.51	1.92 ± 0.55	0.007
Monocytes (10^9/L)		0.45 ± 0.15	0.47 ± 0.15	1.92 ± 0.55	0.144
Eosinophils (10^9/L)		0.15 ± 0.13	0.15 ± 0.10	0.17 ± 0.12	0.084
Basophils (10^9/L)		0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.764
RBC (10^9/mcL)	Males	5.12 ± 0.35	5.02 ± 0.40	5.01 ± 0.38	0.395
	Females	4.47 ± 0.44	4.45 ± 0.34	4.48 ± 0.34	0.780
HB (g/dL)	Males	14.77 ± 1.06	14.76 ± 1.15	14.79 ± 0.97	0.860
	Females	12.74 ± 1.19	12.82 ± 1.31	12.91 ± 0.95	0.328
HCT (%)	Males	0.44 ± 0.03	0.44 ± 0.03	0.44 ± 0.02	0.675
	Females	0.39 ± 0.03	0.39 ± 0.04	0.40 ± 0.03	0.035
MCV (fL)	Males	86.78 ± 4.77	88.14 ± 5.85	88.71 ± 4.552	0.111
	Females	87.05 ± 6.96	88.45 ± 7.21	89.16 ± 5.75	0.076
MCH (picograms/cell)	Males	28.91 ± 1.71	29.48 ± 2.12	29.37 ± 2.48	0.677
	Females	28.59 ± 2.53	28.81 ± 2.58	28.93 ± 2.17	0.409
MCHC (g/dL)	Males	33.33 ± 1.20	33.46 ± 1.34	33.33 ± 1.02	0.656
	Females	32.86 ± 1.14	32.57 ± 1.47	32.45 ± 1.05	0.066
RDW (%)	Males	13.29 ± 0.77	13.50 ± 1.02	13.40 ± 1.98	0.961
	Females	13.68 ± 1.51	13,76 ± 1.48	13.54 ± 0.95	0.268
MPV (fL)	Males	11.13 ± 0.90	11.22 ± 1.05	10.91 ± 0.87	0.053
	Females	10.85 ± 0.84	11.13 ± 0.94	10.85 ± 0.89	0.278
Platelets (number/µL)	Males	230.32 ± 56.33	227.04 ± 52.07	233.80 ± 43.98	0.467
	Females	263.40 ± 56.59	256.80 ± 60.99	262.84 ± 48.86	0.717

Table 2: Associations between leukocyte and erythrocytes parameters and platelets with periodontal diagnosis. Differences between groups were analysed by ANOVA. RBC: red blood cell count; HB: Hemoglobin; HCT: Hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width; MPV: mean platelet volume

Patient characteristics		AgP (n=127)	Healthy (n=206)	Comparisons p=
Age		29.72 ± 7.59	38.25 ± 11.51	<0.001
Gender	Male	48 (37.8%)	89 (43.2%)	0.360
	Female	79 (62.2%)	117 (56.8%)	
Ethnicity	Caucasian	69 (54.3%)	129 (62.6%)	0.576
	African	11 (8.7%)	12 (5.8%)	
	Caribbean	21 (16.5%)	28 (13.6%)	
	Asian	15 (11.8%)	24 (11.7%)	
	Other	11 (8.7%)	11 (8.7%)	
Smoking	Never	70 (55.1%)	120 (58.5%)	0.174
	Former	32 (25.2%)	35 (17.1%)	
	Current	25 (19.7%)	50 (24.4%)	
Average PPD (mm)		3.78 ± 1.17	-	-

Table 3. Characteristics of patients included in the replication sample. Differences between groups were analysed by Chi-square (categorical variables) or ANOVA (continuous variables).

		AgP (n=127)	Healthy (n=206)	Comparisons P=
RBC (10^9/mcL)	Males	**4.98** ± 0.47	4.90 ± 0.38	0.331
	Females	4.40 ± 0.44	4.37 ± 0.35	0.568
HB (g/dL)	Males	14.42 ± 1.15	14.93 ± 1.01	0.008
	Females	12.74 ± 1.19	12.95 ± 1.12	0.226
HCT (%)	Males	0.43 ± 0.29	0.44 ± 0.27	0.217
	Females	0.39 ± 0.03	0.39 ± 0.03	0.431
MCV (fL)	Males	87.62 ± 8.50	89.20 ± 5.18	0.181
	Females	88.46 ± 7.11	89.73 ± 5.96	0.177
MCH (picograms/cell)	Males	29.17 ± 3.08	30.48 ± 1.50	0.001
	Females	29.07 ± 2.76	29.68 ± 2.34	0.100
MCHC (g/dL)	Males	33.28 ± 1.17	34.05 ± 0.87	<0.001
	Females	32.83 ± 1.02	33.06 ± 0.98	0.108
RDW (%)	Males	13.42 ± 1.33	13.12 ± 0.60	0.071
	Females	13.69 ± 1.45	13.74 ± 1.48	0.846
MPV (fL)	Males	10.49 ± 1.08	11.03 ± 0.89	0.003
	Females	10.57 ± 1.15	10.87 ± 0.81	0.042
Platelets (number/µL)	Males	258.77 ± 54.52	235.75 ± 40.70	0.006
	Females	267.73 ± 68.44	281.01 ± 61.13	0.157

Table 4. Erythrocyte parameters and platelet counts in the replication sample. Differences between groups were analysed by ANOVA.

	HB (g/dL)		**HCT (%)**		**MCH (picograms/cell)**		**MCHC (g/dL)**	
	Main study	Replication	Main study	Replication	Main study	Replication	Main study	Replication
Periodontitis	0.347	0.009	0.045	0.140	0.865	0.023	0.085	<0.001
Age	0.506	0.414	0.985	0.484	0.014	0.151	0.244	0.563
Gender	<0.001	<0.001	<0.001	<0.001	0.079	0.038	<0.001	<0.001
Ethnicity	0.005	0.090	0.011	0.227	<0.001	<0.001	0.211	0.058
Smoking	0.003	0.227	0.040	0.347	0.001	0.007	0.003	0.310
BMI	0.444	-	0.420	-	0.091	-	0.955	-
IMD	0.584	-	0.888	-	0.820	-	0.443	-

Table 5. P values of adjusted linear regression analyses for both main study and replication sample..