Title:

Circulating inflammatory cell profiling and periodontitis: a systematic review and meta-analysis

Authors:

Rizky A. Irwandi[1], Sandra O. Kuswandani[1,2], Simon Harden[3], Debora Marletta[4], Francesco D'Aiuto[1]

Institutional affiliations:

1. Periodontology Unit, Eastman Dental Institute, University College London, London, United Kingdom
2. Department of Periodontology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
3. Department of Statistical Science, University College London, London, United Kingdom
4. Cruciform Hub, University College London, London, United Kingdom

Corresponding author:

Francesco D'Aiuto

Periodontology Unit

UCL Eastman Dental Institute

21 University Street

London WC1E 6DE

United Kingdom

f.daiuto@ucl.ac.uk

Abstract

Inflammation is a key driver of common non-communicable diseases. Among common triggers of inflammation, chronic gingival inflammation (periodontitis) triggers a consistent humoral host inflammatory response, but little is known on its impact on circulating inflammatory cell profiles. We aimed to systematically appraise all the evidence linking periodontitis and its treatment to circulating inflammatory cell profiles. From six databases, 157 studies were eligible for qualitative synthesis and 29 studies for meta-analysis. Our meta-analysis showed that participants with periodontitis exhibited a significant mean increase in circulating $CD4^+$, $CD4^+CD45RO^+$, IFNγ-expressing $CD4^+$ and $CD8^+$ T cells, $CD19^+CD27^+$ and $CD5^+$ B cells, $CD14^+CD16^+$ monocytes, and $CD16^+$ neutrophils but decrease in $CD8^+$ T and $CD14^{++}CD16^-$ monocytes. Our qualitative synthesis revealed that peripheral blood neutrophils of patients with periodontitis consistently showed elevated production of reactive oxygen species (ROS) when compared to those of healthy controls. Some evidence suggested that the treatment of periodontitis reversed the exaggerated ROS production, but limited and inconclusive data was found on several circulating inflammatory cell profiling. We conclude that periodontitis and its treatment are associated with minor but consistent alterations in circulating inflammatory cell profiles. These changes could represent key mechanisms explaining the association of periodontitis with other co-morbidities such as cardiovascular disease, diabetes, and rheumatoid arthritis.

Introduction

Inflammation is a body response to infection or injury aimed at promoting tissue and overall homeostasis. Evidence accumulated in the past three decades confirmed that inflammation not only forces a transient impair in tissue function, but in turn could contribute to the pathogenesis of other systemic diseases and altered homeostasis.[1] Inflammatory cells, such as neutrophils, monocytes, macrophages, and dendritic cells initiate inflammation as part of an innate response.[2] The host mounts an adaptive inflammatory response, which is mediated by dendritic cells and NK cells to promote T and B lymphocytes functions. The expected outcome of these changes is to achieve a complete resolution of the inflammatory response and alleviate the damage in tissues where the response takes place.[3] Inflammation however plays a key role in the onset and progression of several chronic non-communicable diseases such as Cardiovascular Disease (CVD), Type 2 Diabetes (T2D), and Rheumatoid Arthritis (RA).[4] Sources of inflammation in non-communicable diseases are still not completely understood.

Periodontitis is a common chronic inflammatory disease caused by a specific oral dysbiosis and characterized by a progressive loss of soft and hard tissues keeping the teeth.[5] The disease onset and progression could span over decades and when left untreated it leads inevitably not only to tooth loss but also to masticatory impairment and negative influences on the patient's quality of life.[5, 6] Periodontitis is a major public health concern as it affects over the half of world's population and increases costs of oral healthcare.[7, 8]

There is convincing evidence to suggest that periodontitis triggers a systemic inflammatory response, and this could explain its association with an increased incidence of systemic health outcomes including cardiovascular events, T2D

complications, and onset and progression of RA.[9-11] One of the plausible mechanisms that could explain these relationships is a change in key circulating inflammatory cell profiles (both innate and adaptive immune systems).[12] No collective evaluation of the whole available evidence, however, has been attempted to date. Our aim was to perform a critical and systematic appraisal of the existing evidence linking periodontitis and its treatment to circulating inflammatory cell profiles including cell populations and their functions. The inflammatory cells profiled were neutrophils, monocytes, and lymphocytes, whereas the cell functions evaluated were inflammatory mediator releases, cellular functional activities and gene expression or transcripts.

Material and Methods

The review process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.[13] This study was registered with PROSPERO (Registration Number CRD42020199995), an international prospective register of systematic reviews (https://www.crd.york.ac.uk/prospero/).

Eligibility Criteria

Adults aged no less than 18 years old suffering from all forms of periodontitis and undergoing any type of treatment of periodontitis (including whole mouth subgingival scaling, surgical periodontal therapy, supra-gingival scaling and polishing, the adjunctive use of locally delivered antimicrobial therapy, and/or systemic antibiotic therapy) were the main inclusion criteria. With regards to study design, case control studies, nonrandomized, and randomized controlled trials reporting the impact of

periodontitis and its treatment on circulating inflammatory cell populations and functions were included.

Case reports and series, reviews, animal studies and studies including participants under 18 years old, pregnant, or suffering from other systemic diseases were excluded.

The inflammatory cells that were profiled were neutrophils, monocytes/macrophages, lymphocytes which are T, B, and NK cells. The circulating inflammatory cell populations were the proportion of the cells indicated by cell surface and/or intracellular markers expressed by peripheral blood mononuclear cells (PBMCs) using flow cytometry. Meanwhile, the function of the cells was the activity of the peripheral blood-derived cells including the investigation on their intracellular cytokine expressions, cytokine release, cellular functional activities, and gene expression or transcripts.

Search Strategy

Six different electronic databases including the Cochrane CENTRAL, MEDLINE, Embase, Web of science, Scopus and CINAHL up to March 2021 with no year restriction but limited to English language were accessed using medical subject headings and free text terms **(Supplementary Appendix)**. Further, manual searches from original manuscripts references list and review articles were conducted.

Study Selection

Two reviewers (RAI and SOK) independently searched titles and abstracts (when available) and any disagreement was resolved by discussion or moved to full text

screening. Full reports were retrieved and examined independently in duplicates and any disagreement was resolved by discussion and if necessary, a third reviewer (FD) was consulted. If manuscripts were lacking all information necessary for the appraisal, authors were contacted at least twice to retrieve missing data. Detailed reasons for exclusion of studies were recorded in particular when randomized clinical trials were identified **(Supplementary Table 1)**.

Data Extraction

Data were grouped according to study design and reported in evidence tables consisted of study characteristics data, population (age, sex, ethnicity, smoking habit, and systemic health), exposure (case definition for periodontitis), intervention (periodontal treatment modalities), outcome (inflammatory cell populations and functional analysis to determine cell functions), and publication results/conclusions.

Quality Assessment

Quality assessment and risk of bias in observational studies, randomized controlled trials, and non-randomized studies of interventions were assessed by Newcastle-Ottawa Scale (NOS)[14], revised Cochrane tool (RoB 2)[15], and ROBINS-I tool[16], respectively.

Data Analysis

Descriptive (summary of evidence retrieved) and quantitative (meta-analysis) methods were used to appraise the included evidence. For meta-analysis, Weighted Mean difference (WMD) and 95% confidence intervals (CI) of the percentage of cell populations between cases (patients with periodontitis) and healthy controls were

calculated using random-effect models when at least two studies with data were available whilst fixed effect models were used for the remaining studies. Heterogeneity was assessed using The Cochrane Q heterogeneity statistic and quantified with the I^2 statistic. The overall effect was considered statistically significant if $p < 0.05$. Publication bias was ascertained using Egger's test[17] and visually assessed using funnel plots. Sensitivity analyses were performed in the subgroup of studies with medium-low of risk of bias to confirm/confute the results of the meta-analysis. Statistical analyses were performed using R package metafor (version 2.0).[18]

Results

Study Characteristics

The electronic and manual searches identified 10875 articles of potential relevance after removal of duplicates (**Figure 1**). Following title-abstracts screening, 320 articles were eligible for a full-text assessment. A total of 157 articles were included, consisting of 129 case-control studies, 3 randomized controlled trials (RCTs) and 25 non-RCT interventional studies (**Supplementary Table 2 and 3**). Almost perfect agreement between the two reviewers was observed (Kappa score of 0.94)

The majority of studies identified (144 studies) included participants with periodontitis and healthy controls, whereas a small minority recruited patients with periodontitis and reported the effect of periodontal treatment (without control). After screening for available data 29 studies were included in quantitative analysis (**Table 1**) whilst the remaining (157 studies) in qualitative analyses including intervention studies on inflammatory cell populations (11 studies) and cell functions (21 studies).

Risk of bias varied across observational studies (**Supplementary Table 4**) because of differences in a) the selection and definition of controls, b) hospital rather than community settings where controls were recruited and c) ambiguous definitions and descriptions of history of periodontitis. Meanwhile, the risk of bias for non-randomized trials were of low (six studies), moderate (four studies), and serious (three studies) (**Supplementary Table 5**). One RCT study of low risk of bias and two of some concerns mainly due to the randomization, blinding of participants, and research personnel descriptions completed our assessments (**Supplementary Table 6**).

Periodontitis on Circulating Inflammatory Cell Populations

Total proportion of 21 cell populations originating from 29 studies were identified and eligible for meta-analyses (**Table 2**). A statistically significant overall effect was observed in twelve cell populations: two cell populations analyses revealed reduced cell number proportions whilst other ten comparison of cell populations exhibited elevated cell number proportions in patients with periodontitis when compared to healthy controls. Heterogeneity varied enormously between meta-analyses (ranging from 0%-94.24%).

Figure 2A shows a significant increased proportion of peripheral $CD4^+$ (WMD of 4%, 95% CI 1% to 7%, p = 0.0144), whilst **Figure 2B** shows a reduced proportion of peripheral $CD8^+$ cells (WMD of 2%, 95% CI 1% to 4%, p = 0.0075) in patients with periodontitis compared to healthy controls were observed in ten studies (**Table 2**). No evidence of publication bias was observed (**Supplementary Figure 1**). Three studies reported that the proportion of non-classical monocytes, $CD14^+CD16^+$ (WMD of 4%, 95% CI 2% to 5%, p < 0.0001) and $CD5^+$ B cells (WMD of 6%, 95% CI 3% to

10%, p = 0.0001) were increased peripheral blood of patients with periodontitis compared to healthy controls (**Table 2**; **Figure 3A-B**). Lastly, fewer studies confirmed that the peripheral blood of patients with periodontitis on average exhibited higher proportions of $CD16^+$ neutrophils **(Table 2; Figure 4A)**, memory B ($CD19^+CD27^+$), $CD5^+CD19^+$, $CD20^+CD23^+$, $CD20^+CD69^+$, $CD25^+$ cells (**Table 2**; **Supplementary Figure 2 – 3**), and lower proportions of classical monocytes ($CD14^{++}CD16^-$) **(Table 2; Figure 4B)**. These findings were confirmed in studies with low-moderate risk of bias.

Periodontitis on Circulating Inflammatory Cell Functions

We identified 26 functional analyses on circulating inflammatory cells between patients with periodontitis and healthy controls in 97 included studies (**Table 3**). These analyses, which were at least reported in two studies, consisted of reactive oxygen species (ROS) production, proliferation, chemotaxis, phagocytosis, adhesion, diacylglycerol kinase (DAGK) activity, tartrate-resistant acid phosphatase formation and elastase activity, actin polymerization, 4 different intracellular cytokines, and 13 different soluble inflammatory mediators.

Most of the included studies (24 studies) reported on ROS production in neutrophils. Of these, 14 studies indicated that peripheral neutrophils from patients with periodontitis exhibited higher production of ROS when compared to healthy controls (**Table 3A**). ROS production detected using either luminol-enhanced[52-54, 57, 58, 60, 64] or lucigenin-enhanced[51, 62, 71] chemiluminescence were consistently elevated in stimulated neutrophils derived from peripheral blood of periodontitis patients. Stimulations used in these experiments was heterogeneous including fMLP, PMA, periodontal pathogens, opsonized *S.aureus* and *E.coli.* Unstimulated PBMCs and

neutrophils in patients with periodontitis exhibited increased ROS production using flow cytometry and lucigenin-enhanced chemiluminescence respectively.[49, 62, 71] Similarly, the majority of studies reported an increased level of TNF-α in cells (5 out of 9 studies) (**Table 3B**), a higher proliferative response of peripheral blood mononuclear cells (PBMCs) (7 out of 15 studies) (**Table 3C**) and neutrophil elastase activity (2 out of 2 studies) (**Table 3D**) were observed in patients with periodontitis.

In comparison to healthy controls, a significant mean increase of intracellular IFN-γ expression was observed in both $CD4^+$ cells (WMD of 1%, 95% CI 0% to 2%, p = 0.0242) and $CD8^+$ cells (WMD of 2%, 95% CI 1% to 4%, p = 0.002) from periodontitis patients (**Table 4; Figure 5**), whereas the expression of intracellular IL-4 and IL-17 in $CD4^+$ cells as well as IL-12 in $CD14^+$ cells were not significantly different between groups (**Table 4, Supplementary Figure 4**). Levels of IL-2, IL-4, IL-12p70, DAGK, chemotactic response, and phagocytic activity were, however, lower in the peripheral blood-derived cells from patients with periodontitis when compared to that from healthy controls (**Table 3E – 3J**). The majority of studies confirmed no difference in IL-6, IL-10, IL-1β, IFN-γ, IL-8, PGE2, TGF-β, MCP-1, IL-13 levels, and adhesion of cells isolated from peripheral blood in patients versus controls (**Table 3K – 3T**), whereas the remaining analyses were inconclusive (**Table 3U – 3V**).

Periodontitis Treatment on Circulating Inflammatory Cell Populations

After a comprehensive search, 12 interventional studies reporting the effect of periodontal treatment on peripheral inflammatory cell populations were identified (**Supplementary Table 7**). Three studies were RCTs, and eight studies were non-RCT interventional studies (**Supplementary Table 3**). These studies referred to

various periodontal treatment modalities including follow-up of various lengths. In summary after periodontal treatment, reduced proportion of 32 circulating inflammatory cell populations and increased percentage of 11 circulating inflammatory cell populations were reported (**Supplementary Table 8**).

Within the neutrophil subset, suppressive neutrophils ($CD16^{dim}CD62L^{bright}$) and $CD62L^{-}$ neutrophils were reported to decrease up to 6 and 12 months after the treatment, respectively, while an elevated proportion of normal neutrophils ($CD16^{bright}CD62L^{bright}$) was reported at 3- and 6-month intervals.[121] Natural killer cells ($CD16^{+}CD56^{+}$), B cells ($CD19^{+}$), and $CD25^{+}$ cells were all reduced after periodontal surgical treatment.[122] A declined percentage of monocytes ($CD14^{+}$) expressing CD36, CD80, TLR2 or TLR4 were also reported after non-surgical periodontal treatments.[123] The periodontal treatment including scaling and root planning (SRP) followed by a systemic antibiotic therapy decreased circulating myeloid dendritic cells ($CD1C^{+}CCR6^{+}$) and Th17 cells ($CD4^{+}IL\text{-}17^{+}Foxp3^{+/-}$).[124] Interestingly, periodontal treatment with SRP only was able to decrease both $IL\text{-}17^{+}$ and $IL\text{-}17^{+}IFN\text{-}\gamma^{+}$ cells.[125]

Following periodontal treatment, the proportion of $CD3^{+}$ and $CD3^{+}CD25^{+}$ cells were higher, while $CD3^{+}CD45RA^{+}$ was lower than that in baseline of patients with periodontitis.[122, 126] Intensive periodontal therapy reduced activated ($CD8^{+}CD38^{+}$), immunosenescent ($CD8^{+}CD28^{null}$), and $CD57^{+}CD8^{+}$ T cells, while control periodontal therapy did not.[127] Further, the percentage of $CD8^{+}$ T cells and their effector memory ($CCR7^{-}CD45RA^{-}$) were lower after periodontal treatment compared to baseline. The naive cytotoxic T (Tc) cells ($CD8^{+}CCR7^{+}CD45RA^{+}$) were higher following treatment compared to baseline.[46] Periodontal treatment reduced HLADR-, CD44-, CD49d-, CD62-expressing $CD4^{+}$ T cells[123], and effector memory T helper (Th) cells

(CD4$^+$CCR7$^-$CD45RA$^-$)[46], whereas CD4+, naive Th cells (CD4$^+$CCR7$^+$CD45RA$^+$), and CD4$^+$CD45RA$^+$ cells were increased after the treatment (46, 127).[46, 128]

Supra- and sub-gingival tooth cleaning modified the proportion of both double positive (CD4$^+$CD8$^+$) or negative (CD4$^-$CD8$^-$) cells. Effector memory (CD4+CD8+CCR7-CD45RA-) and central memory (CD4$^+$CD8$^+$CCR7$^+$CD45RA$^-$) double positive cells were reduced, but their naive (CD4$^+$CD8$^+$CCR7$^+$CD45RA$^+$) and effector memory expressing CD45RA (CD4$^+$CD8$^+$CCR7$^-$CD45RA$^+$) cells were elevated following the intervention. Likewise, a reduction in the percentage of effector memory double negative (CD4$^-$CD8$^-$CCR7$^-$CD45RA$^-$) and an increase in the proportion of naive double negative cells (CD4$^-$CD8$^-$CCR7$^+$CD45RA$^+$) were also observed after the treatment.[46]

The alteration on the proportion of antigen (Ag)-specific T cells after periodontal treatment were reported in one study.[129] PBMCs were stimulated with *Fusobacterium nucleatum* or *Treponema denticola* Ag, labelled as FadA and Td92, respectively then the observation of Ag-specific CD4$^+$ and regulatory T cells (CD4$^+$Foxp3$^+$) were accomplished. Periodontal treatment increased the FadA- and Td92-specific CD4$^+$ cells, whereas only Td92-specific regulatory T cells were reduced in response to the intervention.[129] The final single study investigated the effect of the treatment on T cell receptor (TCR) Vα/Vβ in CD3$^+$, but only TCR Vβ22 were decreased at 24 months post-intervention.[126]

Periodontitis Treatment on Circulating Inflammatory Cell Functions

In 21 non-RCT interventional studies, we identified 34 functional analyses to assess the peripheral inflammatory cell functions following periodontal treatment (**Supplementary Table 9**). The included analyses were proliferation, phagocytosis,

chemotactic activity, migration inhibition, neutrophil extracellular trap (NET), speed, velocity, resultant vector length, ROS production, PGE_2, transcriptomic analysis, inflammasomes (ASC and NLRP), enzymes (ATPase and caspase-1), transcription factors (GATA-3, RORC, and T-bet), toll-like receptors (TLR-2 and TLR-4), chemokines (MCP-1, MDC, MIP-1α, MIP-1β, RANTES), and cytokines (IFN-γ, IL-10, IL-12, IL-17, IL-1β, IL-4, IL-6, IL-8, and TNF-α) (**Supplementary Table 10**).

Proliferative response of T lymphocytes measured by autologous mixed lymphocyte reaction was increased after successful periodontal treatment (45, 82, 127).[45, 128, 130] The treatment also improved the phagocytic activity of both peripheral blood neutrophils and monocytes.[66, 131] Circulating neutrophils and monocytes demonstrated increased chemotactic activity following the intervention.[96] Meanwhile, a high variation of leukocyte migration inhibition was observed after the treatment, depending on the group of samples, type of cell stimulation, and day of observation, while the treatment consistently reduced leukocyte ATPase activity.[132] Periodontal treatment decreased NET production[133], neutrophil speed in response to chemo-attractants (fMLP and CXCL8), neutrophil velocity, and accuracy after fMLP stimulation, while neutrophil velocity and accuracy were normalized for CXCL8-stimulated neutrophils.[97] The ROS production of peripheral blood neutrophil was lower in post-treatment patients than that in pre-treatment.[61, 62] In addition, periodontal therapy did not affect PGE_2 production in whole blood cell culture.[118]

Transcriptomic analysis on peripheral monocytes revealed that the periodontal therapy altered the expression of genes relevant to innate immunity, apoptosis, and cell signaling.[134] Further, the alteration at transcriptional level in PBMCs involving ASC, an inflammasome was decreased after therapy, whilst NLPR3 and its downstream enzyme, Caspase-1 as well as TLR2 and TLR4 were not affected.[135, 136]

In CD4+ cells, SRP modified the expression of genes encoding transcription factors, RORC was reduced, while GATA-3 was increased after the treatment. No changes in T-bet gene were reported in patients after treatment.[125]

The production of chemokines and cytokines by peripheral blood-derived cells in most of the collected evidence were not affected by periodontal treatment. The only exception was for MCP-1, as either 4-week supplementation of liposomal bovine lactoferrin or SRP and systemic antibiotic therapy reduced MCP-1 production by PMBCs (when compared to pre-treatment values).[124, 136] Periodontal treatment reduced IFN-γ production of tetanus toxoid- or *Porphyromonas gingivalis* (*P.gingivalis*) or Concanavalin A (ConA)-stimulated PBMCs or stimulated PBMCs, IL-4 level of *P.gingivalis* or ConA-stimulated PBMCs, and lactoferrin supplementation decreased IL-1β, IL-6, and TNF-α in PBMCs.[47, 129, 136] Lastly, monocyte IL-12p70 level increased in patients following SRP and systemic antibiotic administration when compared to baseline values.[107, 118]

Discussion

This is the systematic review confirming that periodontitis is not only a local inflammatory disease, but it is accompanied by changes in proportion and function of circulating inflammatory cells. Patients with periodontitis exhibited increased numbers of circulating neutrophils (CD16+), T helper (CD4+), Tc1 ($CD8^+$), memory ($CD19^+CD27^+$), $CD5^+$ B cells, and non-classical ($CD14^+CD16^+$) monocytes, whilst reduced cytotoxic T cells ($CD8^+$) and classical ($CD14^{++}CD16^-$) monocytes when compared to healthy controls. Patients with periodontitis also presented altered functions of neutrophils and PBMCs (higher production and release of ROS and TNF-α) when compared to controls. Reduced levels of IL-2, IL-4, IL-12p70, DAGK,

chemotactic responses, and phagocytic activity in peripheral inflammatory cells were also found in patients suffering from periodontitis when compared to control. Collectively a distinct systemic inflammatory cell-profiling is triggered by periodontitis, and this could contribute to the aggravation or even initiation of other systemic diseases.

Our quantitative analysis on innate inflammatory cells, including classical and non-classical monocytes demonstrates that the host response profile present in patients with periodontitis is similar to that was observed in other systemic inflammatory diseases such as systemic lupus erythematosus (SLE), RA, and psoriasis. Common features include lower proportion of classical monocytes and higher numbers of non-classical monocytes.[137-139] Meanwhile, for adaptive inflammatory cells, increased $CD5^+$ and memory B cells as reported in patients with SLE[140, 141] as well as higher IFN-γ-expressing Th $CD4^+$[142] and cytotoxic T $CD8^+$ lymphocyte[143] as observed in patients with psoriasis, have been reported in patients with periodontitis when compared to healthy controls. Even hyperactive peripheral neutrophils features reported in patients with periodontitis have been observed in patients with inflammatory bowel diseases, including Crohn's disease and Ulcerative colitis.[144, 145]

Periodontitis to Altered Inflammatory Cell Profiles: Direct and Indirect Mechanisms

A number of direct and indirect mechanisms could be responsible for the systemic alteration of circulating inflammatory cell proportions in patients with periodontitis. There is sufficient preclinical evidence confirming that the dental plaque biofilm in periodontitis stimulates antigen-presenting cells in the gingival tissues which in turn trigger Th cells differentiation into Th1 cells.[146] This direct mechanism could explain

the increased proportion of $CD4^+$ Th and IFN-γ-expressing $CD4^+$ Th1 cells found in the systemic circulation of patients with periodontitis. Further, increased cytotoxic activity to eliminate damaged periodontal-derived cells could also explain the lower proportions of peripheral $CD8^+$ cytotoxic T cells but elevated IFN-γ-expressing $CD8^+$ Tc1 cells. Meanwhile, antigen activation of periodontal bacteria on naive T cell may contribute to an elevated proportion of $CD4^+CD45RO^+$ memory T cell population in peripheral blood presented in our meta-analysis.

Alternatively, the increased local and systemic production of inflammatory biomarkers could be an indirect mechanism altering myelopoiesis and granulopoiesis of the bone marrow. IL-6 and IL-1β influence hematopoietic stem and progenitor cell (HPSC) differentiation towards the myeloid lineage, also known as trained myelopoiesis.[147] This is supported by recent evidence suggesting that IL-6, induced by *P.gingivalis* infection triggers osteoclast progenitor (OCP) expansion in the bone marrow which ultimately activate osteoclastogenesis.[148] An alternative mechanism could involve neural circuits, particularly those of the sympathetic nervous system which innervates the bone marrow hematopoietic compartment, resulting in altered hematopoiesis.[149] Systemic inflammation caused by periodontitis could also stimulate trained granulopoiesis, resulting in hyper-responsive neutrophils increasing their ROS and TNF-α productions.[147, 150, 151] This is consistent with our qualitative analysis of available evidence suggesting an elevated ROS and TNFα production in neutrophils derived from peripheral blood of patients with periodontitis when compared to healthy controls. In this context, TNFα is also a regulator of ROS generation.[152] Besides indirect stimulation by cytokines, HSPCs could directly respond to commensal oral bacteria via their toll-like receptors (i.e. TLR4), resulting in increased proliferation and differentiation towards myeloid lineage, and

preferential differentiation of lymphoid lineage into dendritic cells.[153] Our review confirmed that alteration of peripheral lymphocytes could be a direct effect of skewed myelopoiesis and/or due to the migration of activated lymphocytes previously primed in inflamed periodontal tissues.[154]

Treatment of Periodontitis Modifies Circulating Inflammatory Cell Profiles

This review also provides some initial evidence that periodontal treatment alters the proportion and function of circulating inflammatory cells. Suppressive neutrophils, TLR-expressing monocyte, immunosenescent cytotoxic T cells, naive, central, and effector memory T cells were affected by periodontal treatment.[46, 121, 123, 127] Further improvement in phagocytic activity, chemotactic response, and ROS production of peripheral blood-derived cells, including neutrophils and monocytes were noted.[61, 62, 66, 96, 131] Collectively, this suggests a possible causal association between periodontitis and proportion and function of circulating inflammatory cells. In turn, this could also explain the association between periodontitis and other common chronic co-morbidities, such as CVD and T2D. Further research, however, to address this hypothesis is required.

Evidence suggested that the treatment of periodontitis could alleviate the symptoms of patients with systemic inflammation such as, RA, SLE, and psoriasis. The current systematic review and meta-analysis revealed evidence for a favorable effect of periodontal treatment on RA activity.[155] Patients suffering from RA and concomitant periodontitis had lower disease activity score with 28 joint counts (DAS28), erythrocyte sedimentation rate (ESR), tender joint counts (TJC), swollen joint counts (SJC), visual analogical scale (VAS), and the level of serum CRP following periodontitis treatment compared to without the treatment.[156] Further in a prospective

study, SLE disease activity index (SLEDAI) of patients with SLE and chronic gingivitis was reduced six months after the management of the gingival inflammation.[157] Lastly evidence from a single RCT suggested that treatment of periodontitis improved clinical outcomes in patients with concomitant psoriasis. Indeed 8-weeks after management of periodontitis patients with psoriasis exhibited reduced disease area and severity index (PASI) score when compared to patients who had delayed periodontal therapy .[158]

Implication of Periodontitis-induced Circulating Inflammatory Cell Alteration to Non-communicable Diseases

Recent evidence confirms the role of inflammatory cells in a number of chronic diseases such as CVD, T2D, and RA. This includes cells involved in either innate immune response, such as monocyte subsets, or adaptive immune response which are T and B lymphocyte subsets. The contribution of these cells is evident in the onset and/or progression of the non-communicable diseases mentioned above.

T lymphocyte subsets play a prominent role in the pathogenesis of atherosclerosis, and the modification of these cells are linked to hypertension and the increased risk of cardiovascular events. Antigen presenting cells recognize oxidized LDL (OxLDL) antigenic peptide and ApoB activate $CD4^+$ T cells and their differentiation into Th1 cells. These cells (marked as IFN-γ-expressing $CD4^+$) have shown to promote atherogenesis.[159] An experimental mouse model confirmed a crucial role of $CD4^+$ T cell-priming with antigen presenting cells and traffic between circulation and vessel wall during early stages of atherosclerosis.[160] $CD4^+$ cell trafficking markers, CD4492, CD49d, CD62L, and CD11a were all implicated in the onset of atheroma.[161, 162] Interestingly, periodontal treatment reduced the proportion of CD44-, CD49d- and CD62-expressing $CD4^+$ T cells and activated ($CD8^+CD38^+$), immunosenescent ($CD8^+CD28^{null}$), and $CD8^+CD57^+$ T cells.[123, 127] Increased peripheral effector memory T helper cells have been linked to faster subclinical atherosclerosis and cardiovascular events.[163] In this review, we identified evidence suggesting that periodontal treatment reduced these T cell subsets.[46] Further research should address the hypothesis that T lymphocyte subsets could be influenced by periodontal treatment and link them to vascular phenotypes including endothelial dysfunction and hypertension.

Monocyte subsets are also involved in atherosclerosis. Classical monocytes are short-lived cells that can differentiate into monocyte-derived macrophages and monocyte-derived dendritic cells.[164, 165] These cells are recruited to the site of inflammation, recognize and phagocytose pathogens, secrete various inflammatory cytokines, and recruit other immune cells for regulation of the inflammatory response.[166, 167] A reduced number of peripheral classical monocytes in periodontitis could be an indicator of enhanced inflammation within the periodontal tissue. On the other hand, non-classical monocytes are considered patrolling cells that exhibit a distinct motility and crawling pattern along the vasculature, at the luminal side of vascular endothelium. Besides that, these cells recognize and clear dying endothelial cells to maintain vascular homeostasis.[168] Endothelial dysfunction which is partly induced by vascular inflammation is evident in patients with periodontitis.[169] It is easy to speculate that alterations in peripheral non-classical monocytes in periodontitis could be responsible of the vascular dysfunction (**Figure 6**).

Th1 and Th17 are both pro-inflammatory T cell subsets that are increased in T2D and they are also linked to impaired insulin signaling and glucose tolerance.[170, 171] A reduced proportion of Treg cells which are usually involved in controlling excessive pro-inflammatory responses has been reported in patients with diabetes.[172, 173] Similarly, B cells play an important role in metabolic diseases and in experimental models, B2 B cells promote pro inflammatory responses and insulin resistance[174], whereas B1a[175] and B1b[176] B cells ameliorated insulin resistance and glucose intolerance. The results of our review confirmed increased proportions of IFN-γ-expressing $CD4^+$ Th1, $CD20^+CD23^+$ B (B2), and $CD5^+$ B (B1a) cells, indicating that alteration of these subsets in periodontitis may contribute to diabetes complications

(**Figure 6**). Further research investigating the role of Th1-Th17 subsets in patients with periodontitis and the impact of its treatment to T2D is recommended.

Several inflammatory cells, including monocytes, T and B cells orchestrate the pathogenesis of RA. Conflicting evidence exists on the role of peripheral classical monocytes and non-classical monocytes in patients with RA.[138] Experimental animal models suggested that non-classical monocytes recruited to synovial joint, differentiate into inflammatory macrophages inducing arthritis in mice.[177] Further, a reduced proportion of circulating Treg cells is associated with early signs of the disease (172) as these cells are responsible for immunosuppression (163), particularly suppressing the pro-inflammatory function of Th17 (173) and Th1 (174).[169, 178-180] Lastly, memory B cells contribute to osteoclastogenesis via expression of RANKL[181], whereas the generation of IgM autoantibody by $CD5^+$ B cells[182, 183] in RA forms immune complex and drives synovial inflammation.[11, 184, 185]

Our meta-analyses confirmed an increased percentage of non-classical monocytes ($CD14^+CD16^+$), circulating memory ($CD19^+CD27^+$) and $CD5^+$ B cells in patients with periodontitis suggesting these cell subsets could explain a two-way relationship between periodontitis and RA (**Figure 6**).

Study Limitation

Some limitations should be highlighted in this systematic review starting with high level of heterogeneity observed in the published evidence (mainly due the case definition of periodontitis). Despite our sensitivity analyses in studies with medium-low Risk of Bias, we urge caution in interpreting the results of the review especially when inferring a causal association between periodontitis and inflammatory cell subpopulation and their functions.[186] A wide variety of cell-functions assays/analyses

reported in small and often uncontrolled studies, undermine the potential impact of periodontitis on cell proportions and their function. Nevertheless, this was the first collective attempt of comprehensively appraise the evidence linking periodontitis to circulating cell proportions and functional differences.

Conclusion

In conclusion, periodontitis is associated with alterations in peripheral inflammatory cell profiles, and this could mediate the association of the disease with other common systemic co-morbidities such as CVD, T2D and RA. Further research should focus on models of inflammation to unravel the exact mechanisms of these association as well as to demonstrate a potential benefit in treating periodontitis over systemic complications in patients with other common co-morbidities by large randomized clinical trials.

Data Availability Statement

Data supporting the finding of the study are available in this article and its supplementary file, or from the corresponding author upon request.

Conflict of Interest Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authorship

Rizky Aditya Irwandi (RAI), Sandra Olivia Kuswandani (SOK), Simon Harden (SH), Debora Marletta (DM), Francesco D'Aiuto (FD).

RAI was involved in the formulation of study design, literature search, data curation, data interpretation and writing the original draft. DM was involved in the search strategies. SOK was involved in literature search and data curation. SH performed the statistical analysis. FD was involved in the formulation of study design, writing the original draft, data interpretation, and supervision of the study. All authors approved the final version of the manuscript.

Acknowledgement

This study was completed at UCL Biomedical Research Centre which receives funding from the NIHR. RAI and SOK received funding from the Indonesia Endowment Fund for Education S-1692/LPDP.4/2019 (LPDP – Indonesia Scholarship).

References

1. Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008;454:428-435.
2. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer* 2004;4:11-22.
3. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science* 2010;327:291-295.
4. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med* 2019;25:1822-1832.
5. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Primers* 2017;3:1-14.
6. Graziani F, Music L, Bozic D, Tsakos G. Is periodontitis and its treatment capable of changing the quality of life of a patient? *Br Dent J* 2019;227:621-625.
7. Kassebaum N, Bernabé E, Dahiya M, Bhandari B, Murray C, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. *J Dent Res* 2014;93:1045-1053.
8. Baehni P, Tonetti M, Periodontology GotEWo. Conclusions and consensus statements on periodontal health, policy and education in Europe: a call for action–consensus view 1: Consensus report of the 1st European Workshop on Periodontal Education. *Eur J Dent Educ* 2010;14:2-3.
9. Sanz M, Marco del Castillo A, Jepsen S, et al. Periodontitis and cardiovascular diseases: Consensus report. *J Clin Periodontol* 2020;47:268-288.
10. Preshaw PM, Bissett SM. Periodontitis and diabetes. *Br Dent J* 2019;227:577-584.
11. Potempa J, Mydel P, Koziel J. The case for periodontitis in the pathogenesis of rheumatoid arthritis. *Nat Rev Rheumatol* 2017;13:606-620.

12. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nat Rev Immunol* 2021:1-15.
13. Page MJ, McKenzie JE, Bossuyt PM, et al. Updating guidance for reporting systematic reviews: development of the PRISMA 2020 statement. *J Clin Epidemiol* 2021;134:103-112.
14. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. In: Oxford, 2000.
15. Sterne JA, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 2019;366.
16. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016;355.
17. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-634.
18. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw* 2010;36:1-48.
19. Afar B, Engel D, Clark E. Activated lymphocyte subsets in adult periodontitis. *J Periodontal Res* 1992;27:126-133.
20. Aoyagi T, Sugawara-Aoyagi M, Yamazaki K, Hara K. Interleukin 4 (IL-4) and IL-6-producing memory T-cells in peripheral blood and gingival tissues in periodontitis patients with high serum antibody titers to Porphyromonas gingivalis. *Oral Microbiol Immunol* 1995;10:304-310.
21. Berglundh T, Liljenberg B, Tarkowski A, Lindhe J. Local and systemic TCR V gene expression in advanced periodontal disease. *J Clin Periodontol* 1998;25:125-133.

22. Berglundh T, Liljenberg B, Tarkowski A, Lindhe J. The presence of local and circulating autoreactive B cells in patients with advanced periodontitis. *J Clin Periodontol* 2002;29:281-286.

23. Buduneli N, Bıçakçı N, Keskinogˇlu A. Flow-cytometric analysis of lymphocyte subsets and mCD14 expression in patients with various periodontitis categories. *J Clin Periodontol* 2001;28:419-424.

24. Cheng WC, van Asten SD, Burns LA, et al. Periodontitis-associated pathogens P. gingivalis and A. actinomycetemcomitans activate human CD14+ monocytes leading to enhanced Th17/IL-17 responses. *European journal of immunology* 2016;46:2211-2221.

25. Cheng WC, Saleh F, Abuaisha Karim B, Hughes FJ, Taams LS. Comparative analysis of immune cell subsets in peripheral blood from patients with periodontal disease and healthy controls. *Clin Exp Immunol* 2018;194:380-390.

26. Demoersman J, Pochard P, Framery C, et al. B cell subset distribution is altered in patients with severe periodontitis. *PloS One* 2018;13:e0192986.

27. Gemmell E, Seymour G. Phenotypic analysis of B-cells extracted from human periodontal disease tissue. *Oral Microbiol Immunol* 1991;6:356-362.

28. Gemmell E, Seymour G. Cytokine profiles of cells extracted from humans with periodontal diseases. *J Dent Res* 1998;77:16-26.

29. Gómez-Moreno G, Cutando-Soriano A, Arana C, et al. Melatonin expression in periodontal disease. *J Periodontal Res* 2007;42:536-540.

30. Hetta HF, Mwafey IM, Batiha GE-S, et al. Cd19+ cd24hi cd38hi regulatory b cells and memory b cells in periodontitis: Association with pro-inflammatory and anti-inflammatory cytokines. *Vaccines* 2020;8:340.

31. Jagannathan R, Lavu V, Rao SR. Comparison of the proportion of non-classic (CD14+ CD16+) monocytes/macrophages in peripheral blood and gingiva of healthy individuals and patients with chronic periodontitis. *J Periodontol* 2014;85:852-858.
32. Kundu D, Bandyopadhyay P, Nair V, Chowdhury M, Mukherjee S, Nayek M. Aggressive periodontitis: A clinico-hematological appraisal. *Journal of Indian society of Periodontology* 2014;18:166.
33. Lima PMA, Souza PEA, Costa JE, Gomez RS, Gollob KJ, Dutra WO. Aggressive and chronic periodontitis correlate with distinct cellular sources of key immunoregulatory cytokines. *J Periodontol* 2011;82:86-95.
34. Mouynet P, Picot C, Nicolas P, et al. Ex vivo studies of polymorphonuclear neutrophils from patients with early-onset periodontitis: (III). CR3 and LFA-1 expression by peripheral blood and gingival crevicular polymorphonuclear neutrophils. *J Clin Periodontol* 1995;22:110-117.
35. Nagai A, Takahashi K, Sato N, et al. Abnormal proportion of γδ T cells in peripheral blood is frequently detected in patients with periodontal disease. *J Periodontol* 1993;64:963-967.
36. Nagasawa T, Nitta H, Watanabe H, Ishikawa I. Reduced CD8+ peripheral blood T lymphocytes in rapidly progressive periodontitis. *Arch Oral Biol* 1995;40:605-608.
37. Nagasawa T, Kobayashi H, Aramaki M, Kiji M, Oda S, Izumi Y. Expression of CD14, CD16 and CD45RA on monocytes from periodontitis patients. *J Periodontal Res* 2004;39:72-78.
38. Nemoto E, Nakamura M, Shoji S, Horiuchi H. Circulating promyelocytes and low levels of CD16 expression on polymorphonuclear leukocytes accompany early-onset periodontitis. *Infect Immun* 1997;65:3906-3912.

635 39. Okada K, Fujimura T, Kikuchi T, et al. Effect of interleukin (IL)-35 on IL-17
636 expression and production by human CD4+ T cells. *PeerJ* 2017;5:e2999.
637 40. Schmidt J, Jentsch H, Stingu C-S, Sack U. General immune status and oral
638 microbiology in patients with different forms of periodontitis and healthy control
639 subjects. *PLoS One* 2014;9:e109187.
640 41. Seymour G, Taubman M, Eastcott J, Gemmell E, Smith D. CD29 expression on
641 CD4+ gingival lymphocytes supports migration of activated memory T lymphocytes
642 to diseased periodontal tissue. *Oral Microbiol Immunol* 1997;12:129-134.
643 42. Sugawara M, Yamashita K, Yoshie H, Hara K. Detection of, and anti-collagen
644 antibody produced by, CD5-positive B cells in inflamed gingival tissues. *J*
645 *Periodontal Res* 1992;27:489-498.
646 43. Tjoa ST, De Vries TJ, Schoenmaker T, Kelder A, Loos BG, Everts V. Formation of
647 osteoclast-like cells from peripheral blood of periodontitis patients occurs without
648 supplementation of macrophage colony-stimulating factor. *J Clin Periodontol*
649 2008;35:568-575.
650 44. Watanabe K, Blew B, Scherer M, et al. CD11b mRNA expression in neutrophils
651 isolated from peripheral blood and gingival crevicular fluid. *J Clin Periodontol*
652 1997;24:814-822.
653 45. Kimura S, Fujimoto N, Okada H. Impaired autologous mixed-lymphocyte reaction of
654 peripheral blood lymphocytes in adult periodontitis. *Infect Immun* 1991;59:4418-
655 4424.
656 46. Medara N, Lenzo JC, Walsh KA, et al. Peripheral memory T-cell profile is modified
657 in patients undergoing periodontal management. *J Clin Periodontol* 2021;48:249-262.

47. Medara N, Lenzo JC, Walsh KA, O'Brien-Simpson NM, Reynolds EC, Darby IB. Peripheral T helper cell profiles during management of periodontitis. *J Clin Periodontol* 2021;48:77-91.

48. Biasi D, Bambara LM, Carletto A, et al. Neutrophil migration, oxidative metabolism and adhesion in early onset periodontitis. *J Clin Periodontol* 1999;26:563-568.

49. Bullon P, Cordero MD, Quiles JL, Morillo JM, del Carmen Ramirez-Tortosa M, Battino M. Mitochondrial dysfunction promoted by Porphyromonas gingivalis lipopolysaccharide as a possible link between cardiovascular disease and periodontitis. *Free Radic Biol Med* 2011;50:1336-1343.

50. Carvalho RP, Mesquita JS, Bonomo A, Elsas PX, Colombo AP. Relationship of neutrophil phagocytosis and oxidative burst with the subgingival microbiota of generalized aggressive periodontitis. *Oral Microbiol Immunol* 2009;24:124-132.

51. Dias IH, Chapple IL, Milward M, et al. Sulforaphane restores cellular glutathione levels and reduces chronic periodontitis neutrophil hyperactivity in vitro. *PLoS One* 2013;8:e66407.

52. Fredriksson M, Gustafsson A, Asman B, Bergström K. Hyper-reactive peripheral neutrophils in adult periodontitis: generation of chemiluminescence and intracellular hydrogen peroxide after in vitro priming and FcgammaR-stimulation. *J Clin Periodontol* 1998;25:394-398.

53. Fredriksson MI, Figueredo CM, Gustafsson A, Bergström KG, Asman BE. Effect of periodontitis and smoking on blood leukocytes and acute-phase proteins. *J Periodontol* 1999;70:1355-1360.

54. Fredriksson MI, Gustafsson AK, Bergström KG, Asman BE. Constitutionally hyperreactive neutrophils in periodontitis. *J Periodontol* 2003;74:219-224.

682 55. Fredriksson MI. Effect of priming in subpopulations of peripheral neutrophils from
683 patients with chronic periodontitis. *J Periodontol* 2012;83:1192-1199.
684 56. Gronert K, Kantarci A, Levy BD, et al. A molecular defect in intracellular lipid
685 signaling in human neutrophils in localized aggressive periodontal tissue damage. *J*
686 *Immunol* 2004;172:1856-1861.
687 57. Guentsch A, Puklo M, Preshaw PM, et al. Neutrophils in chronic and aggressive
688 periodontitis in interaction with Porphyromonas gingivalis and Aggregatibacter
689 actinomycetemcomitans. *J Periodontal Res* 2009;44:368-377.
690 58. Gustafsson A, Ito H, Asman B, Bergström K. Hyper-reactive mononuclear cells and
691 neutrophils in chronic periodontitis. *J Clin Periodontol* 2006;33:126-129.
692 59. Johnstone AM, Koh A, Goldberg MB, Glogauer M. A hyperactive neutrophil
693 phenotype in patients with refractory periodontitis. *J Periodontol* 2007;78:1788-1794.
694 60. Kim KY, Kim M-K, Choi YS, et al. A Hyperactive Neutrophil Phenotype in
695 Aggressive Periodontitis. *Int J Oral Biol* 2012;37:69-75.
696 61. Kimura S, Yonemura T, Kaya H. Increased oxidative product formation by peripheral
697 blood polymorphonuclear leukocytes in human periodontal diseases. *J Periodontal*
698 *Res* 1993;28:197-203.
699 62. Ling MR, Chapple IL, Matthews JB. Neutrophil superoxide release and plasma C-
700 reactive protein levels pre-and post-periodontal therapy. *J Clin Periodontol*
701 2016;43:652-658.
702 63. Loesche WJ, Robinson JP, Flynn M, Hudson JL, Duque RE. Reduced oxidative
703 function in gingival crevicular neutrophils in periodontal disease. *Infect Immun*
704 1988;56:156-160.

64. Matthews JB, Wright HJ, Roberts A, Cooper PR, Chapple IL. Hyperactivity and reactivity of peripheral blood neutrophils in chronic periodontitis. *Clin Exp Immunol* 2007;147:255-264.

65. Mouynet P, Delamaire M, Legoff MC, Genetet B, Yardin M, Michel JF. Ex vivo studies of polymorphonuclear neutrophils from patients with early-onset-periodontitis (II). Chemiluminescence response analysis. *J Clin Periodontol* 1994;21:533-539.

66. Naiff PF, Carneiro V, Guimarães MdCM, et al. Mechanical Periodontal Therapy Recovered the Phagocytic Function of Monocytes in Periodontitis. *Int J Dent* 2020;2020.

67. Nicu EA, Rijkschroeff P, Wartewig E, Nazmi K, Loos BG. Characterization of oral polymorphonuclear neutrophils in periodontitis patients: a case-control study. *BMC Oral Health* 2018;18:149.

68. Paknejad M, Rokn AR, Touhid Amoli H. An Investigation on Chemotaxis Activity, Respiratory Explosion and the Phagocytosis of Peripheral Blood Neutrophils in Patients Affected With EOP. *J Dent* 2004;1:49-55.

69. Takahashi K, Ohyama H, Kitanaka M, et al. Heterogeneity of host immunological risk factors in patients with aggressive periodontitis. *J Periodontol* 2001;72:425-437.

70. Tufano MA, Ianniello R, Sanges MR, Rossano F. Neutrophil function in rapidly progressive and adult periodontitis. *Eur J Epidemiol* 1992;8:67-73.

71. Žilinskas J, Žekonis J, Žekonis G, et al. Inhibition of peripheral blood neutrophil oxidative burst in periodontitis patients with a homeopathic medication Traumeel S. *Med Sci Monit* 2011;17:284-291.

72. Akramas L, Akramienė D, Sakalauskienė J, Kubilius R, Gleiznys A. Effect of (1→3),(1→6)-β-glucan on in vitro production of cytokines by leukocytes of patients with periodontitis. *Medicina* 2012;48:186-191.

730 73. Fokkema SJ, Loos BG, Slegte C, van der Velden U. A type 2 response in
731 lipopolysaccharide (LPS)-stimulated whole blood cell cultures from periodontitis
732 patients. *Clin Exp Immunol* 2002;127:374-378.
733 74. Gonçalves TO, Costa D, Brodskyn CI, Duarte PM, César Neto JB, Nogueira-Filho G.
734 Release of cytokines by stimulated peripheral blood mononuclear cells in chronic
735 periodontitis. *Arch Oral Biol* 2010;55:975-980.
736 75. Grenier D, Cazalis J, Gagnon G. Response of periodontitis and healthy patients in a
737 Porphyromonas gingivalis-stimulated whole-blood model. *J Investig Clin Dent*
738 2011;2:38-42.
739 76. Ling MR, Chapple IL, Matthews JB. Peripheral blood neutrophil cytokine hyper-
740 reactivity in chronic periodontitis. *Innate Immun* 2015;21:714-725.
741 77. Sakalauskiene J, Giedrimiene D, Kubilius R, et al. Cytokine production by leukocytes
742 in patients with periodontitis. *Cent Eur J Med* 2014;9:821-829.
743 78. Shapira L, Soskolne WA, Sela MN, Offenbacher S, Barak V. The secretion of PGE2,
744 IL-1 beta, IL-6, and TNF alpha by adherent mononuclear cells from early onset
745 periodontitis patients. *J Periodontol* 1994;65:139-146.
746 79. Aoyagi T, Yamazaki K, Kabasawa-Katoh Y, et al. Elevated CTLA-4 expression on
747 CD4 T cells from periodontitis patients stimulated with Porphyromonas gingivalis
748 outer membrane antigen. *Clin Exp Immunol* 2000;119:280-286.
749 80. Boyatzis S, Seymour GJ. Effect of age and periodontal disease status in man on the
750 spontaneous proliferation of peripheral blood lymphocytes. *Arch Oral Biol*
751 1986;31:749-755.
752 81. Engel D, Monzingo S, Rabinovitch P, Clagett J, Stone R. Mitogen-induced
753 hyperproliferation response of peripheral blood mononuclear cells from patients with

severe generalized periodontitis: lack of correlation with proportions of T cells and T-cell subsets. *Clin Immunol Immunopathol* 1984;30:374-386.

82. Evans RI, Mikulecky M, Seymour GJ. Effect of initial treatment of chronic inflammatory periodontal disease in adults on spontaneous peripheral blood lymphocyte proliferation. *J Clin Periodontol* 1989;16:271-277.

83. Figueira EA, de Rezende ML, Torres SA, et al. Inhibitory signals mediated by programmed death-1 are involved with T-cell function in chronic periodontitis. *J Periodontol* 2009;80:1833-1844.

84. Getka TP, Alexander DC, Parker WB, Miller GA. Immunomodulatory and superantigen activities of bacteria associated with adult periodontitis. *J Periodontol* 1996;67:909-917.

85. Kopp W, Marggraf E. Blastogenetic responsiveness of peripheral blood lymphocytes from patients with adult periodontitis, evaluated by a reverse hemolytic plaque assay. *J Clin Periodontol* 1986;13:805-809.

86. Mangan DF, Laughon BE, Bower B, Lopatin DE. In vitro lymphocyte blastogenic responses and titers of humoral antibodies from periodontitis patients to oral spirochete isolates. *Infect Immun* 1982;37:445-451.

87. Page RC, Clagett JA, Engel LD, Wilde G, Sims T. Effects of prostaglandin on the antigen- and mitogen-driven responses of peripheral blood lymphocytes from patients with adult and juvenile periodontitis. *Clin Immunol Immunopathol* 1978;11:77-87.

88. Patters MR, Genco RJ, Reed MJ, Mashimo PA. Blastogenic response of human lymphocytes to oral bacterial antigens: comparison of individuals with periodontal disease to normal and edentulous subjects. *Infect Immun* 1976;14:1213-1220.

89. Patters MR, Chen P, McKenna J, Genco RJ. Lymphoproliferative responses to oral bacteria in humans with varying severities of periodontal disease. *Infect Immun* 1980;28:777-784.

90. Petit MD, Wassenaar A, van der Velden U, van Eden W, Loos BG. Depressed responsiveness of peripheral blood mononuclear cells to heat-shock proteins in periodontitis patients. *J Dent Res* 1999;78:1393-1400.

91. Tew JG, Miller GA, Greene EJ, et al. Immunological studies of young adults with severe periodontitis. II. Cellular factors. *J Periodontal Res* 1981;16:403-416.

92. Trindade SC, Olczak T, Gomes-Filho IS, et al. Porphyromonas gingivalis antigens differently participate in the proliferation and cell death of human PBMC. *Arch Oral Biol* 2012;57:314-320.

93. Yamazaki K, Ohsawa Y, Tabeta K, et al. Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. *Infect Immun* 2002;70:2492-2501.

94. Figueredo CM, Gustafsson A, Asman B, Bergström K. Expression of intracellular elastase activity in peripheral neutrophils from patients with adult periodontitis. *J Clin Periodontol* 2000;27:572-577.

95. Champagne CM, Vaikuntam J, Warbington ML, Rose L, Daniel MA, Van Dyke TE. Cytoskeletal actin reorganization in neutrophils from patients with localized juvenile periodontitis. *J Periodontol* 1998;69:209-218.

96. Kumar RS, Prakash S. Impaired neutrophil and monocyte chemotaxis in chronic and aggressive periodontitis and effects of periodontal therapy. *Indian J Dent Res* 2012;23:69.

97. Roberts HM, Ling MR, Insall R, et al. Impaired neutrophil directional chemotactic accuracy in chronic periodontitis patients. *J Clin Periodontol* 2015;42:1-11.

802 98. Srinivas M, Chethana KC, Padma R, et al. A study to assess and compare the
803 peripheral blood neutrophil chemotaxis in smokers and non smokers with healthy
804 periodontium, gingivitis, and chronic periodontitis. *J Indian Soc Periodontol*
805 2012;16:54-58.
806 99. Van Dyke TE, Levine MJ, Tabak LA, Genco RJ. Reduced chemotactic peptide
807 binding in juvenile periodontitis: a model for neutrophil function. *Biochem Biophys*
808 *Res Commun* 1981;100:1278-1284.
809 100. Van Dyke TE, Levine MJ, Tabak LA, Genco RJ. Juvenile periodontitis as a model for
810 neutrophil function: reduced binding of the complement chemotactic fragment, C5a. *J*
811 *Dent Res* 1983;62:870-872.
812 101. Zhang F, Yang XM, Jia SY. Characteristics of neutrophil extracellular traps in
813 patients with periodontitis and gingivitis. *Braz Oral Res* 2020;34:e015.
814 102. Asif K, Kothiwale SV. Phagocytic activity of peripheral blood and crevicular
815 phagocytes in health and periodontal disease. *J Indian Soc Periodontol* 2010;14:8-11.
816 103. Carneiro VM, Bezerra AC, Guimarães Mdo C, Muniz-Junqueira MI. Decreased
817 phagocytic function in neutrophils and monocytes from peripheral blood in
818 periodontal disease. *J Appl Oral Sci* 2012;20:503-509.
819 104. Gonzales JR, Gröger S, Boedeker RH, Meyle J. Expression and secretion levels of
820 Th1 and Th2 cytokines in patients with aggressive periodontitis. *Clin Oral Investig*
821 2012;16:1463-1473.
822 105. Berker E, Kantarci A, Hasturk H, Van Dyke TE. Blocking proinflammatory cytokine
823 release modulates peripheral blood mononuclear cell response to Porphyromonas
824 gingivalis. *J Periodontol* 2013;84:1337-1345.
825 106. Hurttia HM, Pelto LM, Leino L. Evidence of an association between functional
826 abnormalities and defective diacylglycerol kinase activity in peripheral blood

neutrophils from patients with localized juvenile periodontitis. *J Periodontal Res* 1997;32:401-407.

107. Fokkema S, Loos B, De Slegte C, et al. Increased release of IL-12p70 by monocytes after periodontal therapy. *J Clin Periodontol* 2003;30:1091-1096.

108. Bajestan MN, Radvar M, Afshari JT, Naseh MR, Arab HR. Interleukin-6 production by cultured peripheral blood monocytes before and after stimulation by E. coli lipopolysaccharide in Iranian patients with aggressive periodontitis. *Med Sci Monit* 2006;12:393-396.

109. Gonzales JR, Groeger S, Johansson A, Meyle J. T helper cells from aggressive periodontitis patients produce higher levels of interleukin-1 beta and interleukin-6 in interaction with Porphyromonas gingivalis. *Clin Oral Investig* 2014;18:1835-1843.

110. Radvar M, Tavakkol-Afshari J, Bajestan MN, Naseh MR, Arab HR. The effect of periodontal treatment on IL-6 production of peripheral blood monocytes in aggressive periodontitis and chronic periodontitis patients. *Iran J Immunol* 2008;5:100-106.

111. Borilova Linhartova P, Danek Z, Deissova T, et al. Interleukin Gene Variability and Periodontal Bacteria in Patients with Generalized Aggressive Form of Periodontitis. *Int J Mol Sci* 2020;21.

112. Nogueira-Filho G, Rosa BT, Santos PF, et al. Whole-blood cultures from patients with chronic periodontitis respond differently to Porphyromonas gingivalis but not Escherichia coli lipopolysaccharide. *J Periodontol* 2014;85:e18-23.

113. Galbraith GM, Hagan C, Steed RB, Sanders JJ, Javed T. Cytokine production by oral and peripheral blood neutrophils in adult periodontitis. *J Periodontol* 1997;68:832-838.

114. Mahanonda R, Sa-Ard-Iam N, Charatkulangkun O, et al. Monocyte activation by Porphyromonas gingivalis LPS in aggressive periodontitis with the use of whole-blood cultures. *J Dent Res* 2004;83:540-545.

115. de Heens GL, van der Velden U, Loos BG. Cigarette smoking enhances T cell activation and a Th2 immune response; an aspect of the pathophysiology in periodontal disease. *Cytokine* 2009;47:157-161.

116. Restaíno CG, Chaparro A, Valenzuela MA, et al. Stimulatory response of neutrophils from periodontitis patients with periodontal pathogens. *Oral Dis* 2007;13:474-481.

117. Shapira L, Soskolne WA, Van Dyke TE. Prostaglandin E2 secretion, cell maturation, and CD14 expression by monocyte-derived macrophages from localized juvenile periodontitis patients. *J Periodontol* 1996;67:224-228.

118. Fokkema S, Loos B, Van Der Velden U. Monocyte-derived RANTES is intrinsically elevated in periodontal disease while MCP-1 levels are related to inflammation and are inversely correlated with IL-12 levels. *Clin Exp Immunol* 2003;131:477-483.

119. MacFarlane GD, Herzberg MC, Wolff LF, Hardie NA. Refractory periodontitis associated with abnormal polymorphonuclear leukocyte phagocytosis and cigarette smoking. *J Periodontol* 1992;63:908-913.

120. Brunetti G, Colucci S, Pignataro P, et al. T cells support osteoclastogenesis in an in vitro model derived from human periodontitis patients. *J Periodontol* 2005;76:1675-1680.

121. Medara N, Lenzo JC, Walsh KA, Reynolds EC, O'Brien-Simpson NM, Darby IB. Peripheral neutrophil phenotypes during management of periodontitis. *J Periodontal Res* 2021;56:58-68.

122. Górska R, Laskus-Perendyk A, Gregorek H, Kowalski J. The influence of surgical treatment of periodontal disease on selected lymphocyte subpopulations important for cellular and humoral immune responses. *J Periodontol* 2005;76:1304-1310.
123. Piconi S, Trabattoni D, Luraghi C, et al. Treatment of periodontal disease results in improvements in endothelial dysfunction and reduction of the carotid intima-media thickness. *FASEB J* 2009;23:1196-1204.
124. Rajendran M, Looney S, Singh N, et al. Systemic antibiotic therapy reduces circulating inflammatory dendritic cells and Treg–Th17 plasticity in periodontitis. *J Immunol* 2019;202:2690-2699.
125. Zhao L, Zhou Y, Xu Y, Sun Y, Li L, Chen W. Effect of non-surgical periodontal therapy on the levels of Th17/Th1/Th2 cytokines and their transcription factors in Chinese chronic periodontitis patients. *J Clin Periodontol* 2011;38:509-516.
126. Berglundh T, Liljenberg B, Lindhe J. Some effects of periodontal therapy on local and systemic immunological parameters. *J Clin Periodontol* 1999;26:91-98.
127. Czesnikiewicz-Guzik M, Osmenda G, Siedlinski M, et al. Causal association between periodontitis and hypertension: evidence from Mendelian randomization and a randomized controlled trial of non-surgical periodontal therapy. *Eur Heart J* 2019;40:3459-3470.
128. Okada H. Phenotypic and functional characterization of peripheral blood T cells in adult periodontitis. *J Periodontal Research* 1991;26:289-292.
129. Shin J, Kho S-A, Choi YS, Kim YC, Rhyu I-C, Choi Y. Antibody and T cell responses to Fusobacterium nucleatum and Treponema denticola in health and chronic periodontitis. *PLoS One* 2013;8:e53703.

130. Evans R, Mikulecky M, Seymour G. Effect of initial treatment of chronic inflammatory periodontal disease in adults on spontaneous peripheral blood lymphocyte proliferation. *J Clin Periodontol* 1989;16:271-277.
131. Carneiro V, Bezerra A, Guimarães M, Muniz-Junqueira MI. Effects of periodontal therapy on phagocytic activity of peripheral blood neutrophils–evidence for an extrinsic cellular defect. *Oral Health Prev Dent* 2012;10:195-203.
132. Budtz-Jörgensen E, Ellegaard B, Ellegaard J, Jörgensen F, Kelstrup J. The effect of levamisole on experimental gingivitis in juvenile periodontitis: Sequential clinical, immunological, and haematological changes. *J Periodontal Res* 1978;13:460-473.
133. White P, Sakellari D, Roberts H, et al. Peripheral blood neutrophil extracellular trap production and degradation in chronic periodontitis. *J Clin Periodontol* 2016;43:1041-1049.
134. Papapanou PN, Sedaghatfar MH, Demmer RT, et al. Periodontal therapy alters gene expression of peripheral blood monocytes. *J Clin Periodontol* 2007;34:736-747.
135. Higuchi K, Ziauddin S, Yamashita Y, Ozaki Y, Yoshimura A. Initial periodontal treatment affects nucleotide-binding domain leucine-rich repeat-containing protein 3 inflammasome priming in peripheral blood mononuclear cells. *Arch Oral Biol* 2020;110:104625.
136. Ishikado A, Uesaki S, Suido H, et al. Human trial of liposomal lactoferrin supplementation for periodontal disease. *Biol Pharm Bull* 2010;33:1758-1762.
137. Zhu H, Hu F, Sun X, et al. CD16+ monocyte subset was enriched and functionally exacerbated in driving T-cell activation and B-cell response in systemic lupus erythematosus. *Front Immunol* 2016;7:512.
138. Lacerte P, Brunet A, Egarnes B, Duchêne B, Brown JP, Gosselin J. Overexpression of TLR2 and TLR9 on monocyte subsets of active rheumatoid arthritis patients

contributes to enhance responsiveness to TLR agonists. *Arthritis Res Ther* 2016;18:1-14.

139. Chiu YG, Shao T, Feng C, et al. CD16 (FcRγIII) as a potential marker of osteoclast precursors in psoriatic arthritis. *Arthritis Res Ther* 2010;12:1-14.

140. Odendahl M, Jacobi A, Hansen A, et al. Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J Immunol* 2000;165:5970-5979.

141. Böhm I. Increased peripheral blood B-cells expressing the CD5 molecules in association to autoantibodies in patients with lupus erythematosus and evidence to selectively down-modulate them. *Biomed Pharmacother* 2004;58:338-343.

142. Szegedi A, Aleksza M, Gonda A, et al. Elevated rate of Thelper1 (TH1) lymphocytes and serum IFN-γ levels in psoriatic patients. *Immunol Lett* 2003;86:277-280.

143. Guo R, Zhang T, Meng X, et al. Lymphocyte mass cytometry identifies a CD3–CD4+ cell subset with a potential role in psoriasis. *JCI Insight* 2019;4.

144. Nikolaus S, Bauditz J, Gionchetti P, Witt C, Lochs H, Schreiber S. Increased secretion of pro-inflammatory cytokines by circulating polymorphonuclear neutrophils and regulation by interleukin 10 during intestinal inflammation. *Gut* 1998;42:470-476.

145. Somasundaram R, Nuij VJ, van der Woude CJ, Kuipers EJ, Peppelenbosch MP, Fuhler GM. Peripheral neutrophil functions and cell signalling in Crohns disease. *PLoS One* 2013;8:e84521.

146. Gemmell E, Carter C, Grieco D, Sugerman P, Seymour G. P. gingivalis-specific T-cell lines produce Th1 and Th2 cytokines. *J Dent Res* 2002;81:303-307.

147. Chavakis T, Mitroulis I, Hajishengallis G. Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation. *Nat Immunol* 2019;20:802-811.

148. Zhao Y, Li Z, Su L, et al. Frontline science: Characterization and regulation of osteoclast precursors following chronic Porphyromonas gingivalis infection. *J Leukoc Biol* 2020;108:1037-1050.
149. Konkel JE, O'Boyle C, Krishnan S. Distal consequences of oral inflammation. *Front Immunol* 2019;10:1403.
150. Dias IH, Matthews JB, Chapple IL, Wright HJ, Dunston CR, Griffiths HR. Activation of the neutrophil respiratory burst by plasma from periodontitis patients is mediated by pro-inflammatory cytokines. *J Clin Periodontol* 2011;38:1-7.
151. Morgan MJ, Kim Y-S, Liu Z-g. TNFα and reactive oxygen species in necrotic cell death. *Cell Res* 2008;18:343-349.
152. Blaser H, Dostert C, Mak TW, Brenner D. TNF and ROS Crosstalk in Inflammation. *Trends Cell Biol* 2016;26:249-261.
153. Nagai Y, Garrett KP, Ohta S, et al. Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. *Immunity* 2006;24:801-812.
154. Kitamoto S, Nagao-Kitamoto H, Jiao Y, et al. The intermucosal connection between the mouth and gut in commensal pathobiont-driven colitis. *Cell* 2020;182:447-462. e414.
155. Silva DS, Costa F, Baptista IP, et al. Evidence-based research on effectiveness of periodontal treatment in rheumatoid arthritis patients: a systematic review and meta-analysis. *Arthritis Care & Research* 2021.
156. Sun J, Zheng Y, Bian X, Ge H, Wang J, Zhang Z. Non-surgical periodontal treatment improves rheumatoid arthritis disease activity: a meta-analysis. *Clinical Oral Investigations* 2021:1-11.

157. Sete MRC, Carlos JC, Mello-Neto JM, et al. Impact of chronic gingivitis management on the cytokine and anti-PPAD expressions in juvenile systemic lupus erythematosus: A six-month follow-up. *Journal of Periodontal Research* 2021;56:1132-1140.
158. Ucan Yarkac F, Ogrum A, Gokturk O. Effects of non-surgical periodontal therapy on inflammatory markers of psoriasis: A randomized controlled trial. *Journal of clinical periodontology* 2020;47:193-201.
159. Saigusa R, Winkels H, Ley K. T cell subsets and functions in atherosclerosis. *Nat Rev Cardiol* 2020;17:387-401.
160. MacRitchie N, Grassia G, Noonan J, et al. The aorta can act as a site of naive CD4+ T-cell priming. *Cardiovasc Res* 2020;116:306-316.
161. Cuff CA, Kothapalli D, Azonobi I, et al. The adhesion receptor CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. *J Clin Invest* 2001;108:1031-1040.
162. Krieglstein CF, Granger DN. Adhesion molecules and their role in vascular disease. *Am J Hypertens* 2001;14:44S-54S.
163. Baragetti A, Garlaschelli K, Bonacina F, et al. Effector memory T cells predict atherosclerosis progression and cardiovascular events over 4 years follow-up. *Nutr Metab Cardiovasc Dis* 2017;27:e7.
164. Patel AA, Zhang Y, Fullerton JN, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. *J Exp Med* 2017;214:1913-1923.
165. Sander J, Schmidt SV, Cirovic B, et al. Cellular differentiation of human monocytes is regulated by time-dependent interleukin-4 signaling and the transcriptional regulator NCOR2. *Immunity* 2017;47:1051-1066. e1012.
166. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol* 2017;17:349-362.

994 167. Kapellos TS, Bonaguro L, Gemünd I, et al. Human monocyte subsets and phenotypes
995 in major chronic inflammatory diseases. *Front Immunol* 2019;10:2035.
996 168. Narasimhan PB, Marcovecchio P, Hamers AA, Hedrick CC. Nonclassical monocytes
997 in health and disease. *Ann Rev Immunol* 2019;37:439-456.
998 169. Higashi Y, Goto C, Jitsuiki D, et al. Periodontal infection is associated with
999 endothelial dysfunction in healthy subjects and hypertensive patients. *Hypertension*
1000 2008;51:446-453.
1001 170. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and
1002 inflammatory diseases. *Autoimmun Rev* 2014;13:668-677.
1003 171. Zeng C, Shi X, Zhang B, et al. The imbalance of Th17/Th1/Tregs in patients with
1004 type 2 diabetes: relationship with metabolic factors and complications. *J Mol Med*
1005 2012;90:175-186.
1006 172. Feuerer M, Herrero L, Cipolletta D, et al. Lean, but not obese, fat is enriched for a
1007 unique population of regulatory T cells that affect metabolic parameters. *Nat Med*
1008 2009;15:930-939.
1009 173. Qiao Y-c, Shen J, He L, et al. Changes of regulatory T cells and of proinflammatory
1010 and immunosuppressive cytokines in patients with type 2 diabetes mellitus: a
1011 systematic review and meta-analysis. *J Diabetes Res* 2016;2016.
1012 174. Winer DA, Winer S, Shen L, et al. B cells promote insulin resistance through
1013 modulation of T cells and production of pathogenic IgG antibodies. *Nat Med*
1014 2011;17:610-617.
1015 175. Shen L, Chng MHY, Alonso MN, Yuan R, Winer DA, Engleman EG. B-1a
1016 lymphocytes attenuate insulin resistance. *Diabetes* 2015;64:593-603.

176. Harmon DB, Srikakulapu P, Kaplan JL, et al. Protective role for B-1b B cells and IgM in obesity-associated inflammation, glucose intolerance, and insulin resistance. *Arterioscler Thromb Vasc Biol* 2016;36:682-691.
177. Misharin AV, Cuda CM, Saber R, et al. Nonclassical Ly6C− monocytes drive the development of inflammatory arthritis in mice. *Cell Rep* 2014;9:591-604.
178. Lawson CA, Brown AK, Bejarano V, et al. Early rheumatoid arthritis is associated with a deficit in the CD4+ CD25 high regulatory T cell population in peripheral blood. *Rheumatology* 2006;45:1210-1217.
179. van den Berg WB, Miossec P. IL-17 as a future therapeutic target for rheumatoid arthritis. *Nat Rev Rheumatol* 2009;5:549-553.
180. Loetscher P, Uguccioni M, Bordoli L, et al. CCR5 is characteristic of Th1 lymphocytes. *Nature* 1998;391:344-345.
181. Meednu N, Zhang H, Owen T, et al. Production of RANKL by Memory B Cells: A Link Between B Cells and Bone Erosion in Rheumatoid Arthritis. *Arthritis Rheumatol* 2016;68:805-816.
182. Mantovani L, Wilder RL, Casali P. Human rheumatoid B-1a (CD5+ B) cells make somatically hypermutated high affinity IgM rheumatoid factors. *J Immunol* 1993;151:473-488.
183. Elkon K, Casali P. Nature and functions of autoantibodies. *Nat Clin Pract Rheumatol* 2008;4:491-498.
184. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. *Nat Rev Immunol* 2017;17:60-75.
185. Bugatti S, Manzo A, Montecucco C, Caporali R. The Clinical Value of Autoantibodies in Rheumatoid Arthritis. *Front Med* 2018;5:339.

186. Grimes DA, Schulz KF. An overview of clinical research: the lay of the land. *Lancet* 2002;359:57-61.