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

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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.\textsuperscript{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).\textsuperscript{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.\textsuperscript{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)\textsuperscript{14}, revised Cochrane tool (RoB 2)\textsuperscript{15}, and ROBINS-I tool\textsuperscript{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 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-enhanced⁵²-⁵⁴, ⁵⁷, ⁵⁸, ⁶⁰, ⁶⁴ or lucigenin-enhanced¹⁵¹, 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.\textsuperscript{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. Natural killer cells (CD16^+^CD56^+^), B cells (CD19^+^), and CD25^+^ cells were all reduced after periodontal surgical treatment. A declined percentage of monocytes (CD14^+^) expressing CD36, CD80, TLR2 or TLR4 were also reported after non-surgical periodontal treatments. 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-17^+^Foxp3^+/^-). Interestingly, periodontal treatment with SRP only was able to decrease both IL-17^+^ and IL-17^+^IFN-γ^+^ cells.

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. Intensive periodontal therapy reduced activated (CD8^+^CD38^+^), immunosenescent (CD8^+^CD28^null^), and CD57^+^CD8^+^ T cells, while control periodontal therapy did not. 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. Periodontal treatment reduced HLADR-, CD44-, CD49d-, CD62-expressing CD4^+^ T cells, and effector memory T helper (Th) cells.
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.\textsuperscript{46}

The alteration on the proportion of antigen (Ag)-specific T cells after periodontal treatment were reported in one study.\textsuperscript{129} PBMCs were stimulated with \textit{Fusobacterium nucleatum} or \textit{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.\textsuperscript{129} The final single study investigated the effect of the treatment on T cell receptor (TCR) V$\alpha$/V$\beta$ in CD3$^{+}$, but only TCR V$\beta$22 were decreased at 24 months post-intervention.\textsuperscript{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-1$\alpha$, MIP-1$\beta$, RANTES), and cytokines (IFN-$\gamma$, IL-10, IL-12, IL-17, IL-1$\beta$, IL-4, IL-6, IL-8, and TNF-$\alpha$) (Supplementary Table 10).

Proliferative response of T lymphocytes measured by autologous mixed lymphocyte reaction was increased after successful periodontal treatment (45, 82, 127). The treatment also improved the phagocytic activity of both peripheral blood neutrophils and monocytes. Circulating neutrophils and monocytes demonstrated increased chemotactic activity following the intervention. 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. Periodontal treatment decreased NET production, neutrophil speed in response to chemoattractants (fMLP and CXCL8), neutrophil velocity, and accuracy after fMLP stimulation, while neutrophil velocity and accuracy were normalized for CXCL8-stimulated neutrophils. The ROS production of peripheral blood neutrophil was lower in post-treatment patients than that in pre-treatment. In addition, periodontal therapy did not affect PGE$_2$ production in whole blood cell culture. Transcriptomic analysis on peripheral monocytes revealed that the periodontal therapy altered the expression of genes relevant to innate immunity, apoptosis, and cell signaling. 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.
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.\textsuperscript{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).\textsuperscript{124, 136} Periodontal treatment reduced IFN-γ production of tetanus toxoid- or Porphyromonas gingivalis (\textit{P.gingivalis}) or Concanavalin A (ConA)-stimulated PBMCs or stimulated PBMCs, IL-4 level of \textit{P.gingivalis} or ConA-stimulated PBMCs, and lactoferrin supplementation decreased IL-1β, IL-6, and TNF-α in PBMCs.\textsuperscript{47, 129, 136} Lastly, monocyte IL-12p70 level increased in patients following SRP and systemic antibiotic administration when compared to baseline values.\textsuperscript{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. Meanwhile, for adaptive inflammatory cells, increased CD5+ and memory B cells as reported in patients with SLE as well as higher IFN-γ-expressing Th CD4+ and cytotoxic T CD8+ lymphocyte 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.

**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. 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.\textsuperscript{147} This is supported by recent evidence suggesting that IL-6, induced
by \textit{P.gingivalis} infection triggers osteoclast progenitor (OCP) expansion in the bone
marrow which ultimately activate osteoclastogenesis.\textsuperscript{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.\textsuperscript{149} Systemic inflammation caused by periodontitis could also
stimulate trained granulopoiesis, resulting in hyper-responsive neutrophils increasing
their ROS and TNF-α productions.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\(^{\text{null}}\)), 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. 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. 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. Endothelial dysfunction which is partly induced by vascular inflammation is evident in patients with periodontitis. 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. A reduced proportion of Treg cells which are usually involved in controlling excessive pro-inflammatory responses has been reported in patients with diabetes. Similarly, B cells play an important role in metabolic diseases and in experimental models, B2 B cells promote pro-inflammatory responses and insulin resistance, whereas B1a and B1b 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. Experimental animal models suggested that non-classical monocytes recruited to synovial joint, differentiate into inflammatory macrophages inducing arthritis in mice. 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). Lastly, memory B cells contribute to osteoclastogenesis via expression of RANKL (181), whereas the generation of IgM autoantibody by CD5+ B cells 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. 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
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Scholarship).
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