

INTERRELATIONSHIP OF PERIODONTAL AND ENDODONTIC INFECTIONS WITH SYSTEMIC INFLAMMATORY RESPONSES: A CLINICAL INVESTIGATION

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ABSTRACT

Periodontal and endodontic infections cause chronic inflammatory conditions of the oral cavity that can lead to systemic inflammatory activity via unique, but possibly overlapping, immune processes. This study evaluated the association between periodontal disease burden and systemic inflammatory markers and characterized inflammatory molecular signatures in chronic apical periodontitis. A secondary-data analytical design integrated independent clinical and transcriptomic analyses. Periodontal examination, C-reactive protein (CRP), and complete blood count data were used to assess systemic inflammatory profiles according to periodontal severity, probing pocket depth, and clinical attachment loss. Group comparisons, Spearman correlations, and robust linear regression were performed. Processed single-cell transcriptomic data from chronic apical periodontitis were independently analyzed to identify cell-specific inflammatory markers and mechanistic domains. CRP, white blood cell count, neutrophils, lymphocytes, monocytes, platelets, and platelet-to-lymphocyte ratio differed significantly across periodontal severity categories. Continuous periodontal measures showed weak but significant associations with CRP and selected leukocyte markers, while severe periodontitis was associated with higher log-transformed CRP. Chronic apical periodontitis showed high expression of inflammatory markers associated with myeloid/macrophage, epithelial, stromal and lymphoid cell populations, such as S100A8, S100A9, CXCL8, IL1B, CCL3, CCL4, and CCL5. Therefore, the systemic inflammatory associations of periodontal disease were modest, while those of chronic apical periodontitis had significant levels of local inflammatory transcriptional activity and did not indicate distinctive participant-level inflammatory links; however, inflammatory pathways converged.

KEYWORDS: Periodontitis, Apical periodontitis, C-reactive protein, Systemic inflammation, Transcriptomics

1. INTRODUCTION

Periodontal and endodontic infections are chronic oral inflammatory diseases that involve a host immune response to a microbial challenge that results in tissue damage. Periodontitis involves involvement of the tooth-supporting apparatus and is defined as dysregulated inflammatory responses that may move beyond the local periodontal tissues, via circulating cytokines and other mediators [1]. Endodontic inflammatory disease is a disease of the pulp–periapical continuum, and can also produce inflammatory signals that can have effects on the tooth beyond the endodontic disease itself [2]. The conditions can thus serve as complementary models to explore the relationship between localized oral infections and systemic inflammatory activity. This connection starts at the host/microbe interface. The pathogenic potential of oral microorganisms is not independent of immunity; immunity is influenced by the microorganisms, which in turn modulate the immune response, either to induce homeostasis or to continue to induce persistent inflammation [3]. The oral microbiota is comprised of dynamic, spatially structured communities, whose composition and function are ever-changing and are influenced by host factors and intermicrobial interactions [4]. This ecosystem balance can be disrupted, and inflammatory disease can be favored. In periodontitis, the disease is caused by microbial accumulation along with host susceptibility, and will express itself to a varying degree among individuals, but will result in progressive periodontal attachment and supporting bone destruction [5]. This complexity is typical of periodontal disease, as it is a multifactorial disease where the microbial, immunological, environmental and behavioural factors interact on the periodontal tissues [6].

An inflammatory process related to, but anatomically distinct from that of chronic apical periodontitis, is presented. Periapical immune response, characterized by cytokines, chemokines, recruitment of leukocytes and the activation and modulation of tissue and bone remodeling pathways, can occur in the root canal system and maintain this immune response [7]. The inflammatory reaction of the periodontium also has local ramifications. Microbial products, altered immune-cell responses and inflammatory mediators are suggested to contribute to oral dysbiosis and to lead to systemic immune imbalance via periodontal chronic immune activation [8]. Clinically and mechanistically, associations between periodontal inflammation and systemic diseases have since been established, albeit the type and the direction of the

association vary among the diseases and between the designs of specific studies [9]. There is also a wide range of support for microbial translocation of oral bacteria as well as by-products and altered inflammatory pathways into the systemic circulation as a mechanism by which the oral microbial community can influence systemic disease [10]. Circulating markers reflecting various components of this systemic inflammatory response can be used to assess systemic inflammatory status. The acute-phase response can be assessed by the level of C-reactive protein (CRP); cellular inflammatory responses by the total and differential leukocyte count. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII) are also easily accessible measures of low-grade systemic inflammation that have been explored [11]. These are specifically important for determining at the population level whether there is an increased periodontal burden that can be detected in circulating inflammatory activity. Inflammatory signaling in oral disease is a cooperative interplay between cells of the immune system, stromal cells, epithelium and bone. For periodontal and periapical disease, the involvement of cytokine networks, leukocytes and pathways controlling osteoclast activity are linked by osteoimmune mechanisms, and inflammation serves as a link to periodontal and periapical bone remodeling and destruction [12]. Transcriptomic techniques can be used to complement and add to traditional clinical evaluation by dissecting inflammatory signals by cell type. In chronic apical periodontitis, this sort of information could help determine the expression of cell-associated chemokines, cytokine-related genes, and immune activation markers, which cannot be determined by circulating biomarkers.

Although there is a lot of evidence of oral inflammation, endodontic and periodontal infections are usually studied as independent diseases. This separation creates a glaring lack of understanding of how periodontal burden clinically measured and the cell-specific inflammatory environment of endodontic infection can fit into a unified inflammatory framework in the oral-systemic system. Periodontal measurements (based on population) offer a means of quantifying relationships with CRP, leukocyte populations, and derived measures of inflammatory activity, and single-cell endodontic measurements offer complementary molecular resolution of endodontic inflammatory activity. While these independent data sources cannot directly link to the relationship of periodontal-endodontic status on the participant level, an independent parallel evaluation can help to identify different aspects of the inflammatory response, but not to assert causal or biological equivalence.

The interrelationship between periodontal and endodontic infections and systemic inflammatory response was therefore explored in the present study by using both clinical and transcriptomic approaches. The goals were to compare levels of circulating inflammatory markers among the various categories of periodontal severity; to determine the relationships among probing pocket depth, clinical attachment loss, CRP, hematological markers and derived inflammatory measures; to assess the association between periodontal burden with CRP; to characterize cell-specific inflammatory and immune signatures in chronic apical periodontitis; and to examine whether the resulting clinical and molecular data would identify convergent inflammatory domains that could be relevant to oral-systemic inflammation.

2. METHODOLOGY

2.1 Research Design

The study adopted a secondary data analytical design for the analysis of the interrelationship between periodontal and endodontic infections and systemic inflammatory responses. The analysis consisted of two complementary components: clinical evaluation of the periodontal disease, and transcriptomic evaluation of inflammatory signatures of chronic apical periodontitis, in relation to the presence or absence of the circulating inflammatory markers. The clinical and transcriptomic data sets were independent of each other, which is why they were analyzed separately, but interpreted together at the level of inflammatory patterns.

2.2 Data Source

Periodontal examination data were gathered during the national health survey cycle in 2009-2010 and comprised site-level probing pocket depth (PPD) and clinical attachment loss (CAL) data [13]. Systemic inflammatory measurements [14,15] were obtained from CRP and complete blood count data for the corresponding cycle. The endodontic component used processed single-cell transcriptomic data from a sample of tissues from human chronic apical periodontitis [16].

2.3 Periodontal and Systemic Inflammatory Assessment

Records of periodontal, CRP and complete blood count were matched by the unique participant identifier (UID). Those who had a full periodontal exam were kept. Participant-level mean and maximum PPD and CAL were computed after any invalid periodontal measurement codes were treated as missing. The periodontal status was defined as no periodontitis, mild, moderate, and severe based on CAL and interproximal PPD. Systemic inflammatory markers were CRP, leucocyte count, neutrophils, lymphocytes, monocytes, and platelets. Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and systemic immune-inflammation index (SII) were further calculated based on blood cell counts. The clinical inflammatory profile assessed and compared in the periodontal severity categories consisted of these variables. The final analysis consisted of 4086 subjects who had full periodontal examinations, with most having both CRP and complete blood count determinations.

2.4 Endodontic Transcriptomic Analysis

To analyze the processed chronic apical periodontitis (CAP) data set, average log fold change, the proportion of expression within clusters, and the proportion of expression outside clusters, as well as adjusted P values, were evaluated. Robust cluster-associated markers were identified by the combination of the following criteria: adjusted $P < 0.05$, average log fold change ≥ 0.5 , and expression in at least 25% of the cells in each of the corresponding clusters. The cell populations were defined by discriminative markers and inflammatory and immune-associated genes identified. Markers were chosen for their magnitude and cellular specificity and subdivided into 5 mechanistically relevant groups: acute-phase/IL-6 signaling, IL-1/TNF inflammatory, lymphocyte chemotaxis/activation, monocyte/macrophage inflammation, and neutrophil

recruitment/activation. Because of the lack of available comparator without a history of complications, molecular analysis was limited to cluster-associated inflammatory signatures and not to case-control differential expression.

2.5 Statistical Analysis

Medians and interquartile ranges were used to summarise continuous inflammatory markers. The Kruskal–Wallis test was used to compare differences between the different severity groups of the periodontia. Mann–Whitney U tests with Holm correction for multiple comparisons were used for pairwise CRP comparisons. Spearman rank correlations were performed to determine the relationships between continuous periodontal measures and systemic inflammatory markers. For regression analysis, CRP was log-transformed ($\ln(1 + \text{CRP})$). Linear regression models were used to separately evaluate the relationship of transformed CRP to mean PPD, maximum PPD, mean CAL, maximum CAL, and periodontal severity, respectively, with 95% confidence intervals based on HC3 robust standard errors. A P value of < 0.05 , two-sided, was considered statistically significant. The clinical and transcriptomic data were not combined on the participant level, but the results were only combined by comparing inflammatory profiles.

3. RESULTS

3.1 Periodontal Status and Systemic Inflammatory Markers

5,037 periodontal examination records were available; 4,086 of those represented participants who had a complete periodontal examination. Of these, 1,990 (48.70%) had no periodontitis, 357 (8.74%) had mild periodontitis, 1,301 (31.84%) had moderate periodontitis, and 438 (10.72%) had severe periodontitis. The number of patients with complete periodontal examinations was 3,911, and the number of patients with CBC measurements was approximately 3,930. The distributions of systemic inflammatory markers based on periodontal status are presented in Table 1. Significant overall differences among periodontal groups were observed for CRP ($P = 0.048$), WBC ($P = 0.001$), neutrophils ($P = 0.005$), lymphocytes ($P = 0.010$), monocytes ($P = 0.002$), platelets ($P < 0.001$), and PLR ($P < 0.001$). The median values of CRP were 0.19, 0.20, 0.20 and 0.21 mg/dL for no-periodontitis, mild, moderate and severe groups, respectively. Corresponding median WBC counts were 6.80, 7.00, 6.80, and $7.30 \times 10^9/\text{L}$. NLR ($P = 0.201$) and SII ($P = 0.106$) did not differ significantly among periodontal categories.

Table 1. Systemic inflammatory markers according to periodontal disease severity

Marker	No periodontitis, median (IQR)	Mild, median (IQR)	Moderate, median (IQR)	Severe, median (IQR)	P value
CRP (mg/dL)	0.19 (0.07–0.43)	0.20 (0.08–0.47)	0.20 (0.08–0.47)	0.21 (0.10–0.50)	0.048
WBC ($10^9/\text{L}$)	6.80 (5.60–8.10)	7.00 (5.90–8.40)	6.80 (5.70–8.10)	7.30 (5.70–8.80)	0.001
Neutrophils ($10^9/\text{L}$)	4.00 (3.10–5.00)	3.90 (3.20–5.20)	3.90 (3.00–5.00)	4.20 (3.10–5.53)	0.005
Lymphocytes ($10^9/\text{L}$)	2.00 (1.60–2.50)	2.10 (1.70–2.70)	2.00 (1.60–2.50)	2.00 (1.68–2.60)	0.010
Monocytes ($10^9/\text{L}$)	0.50 (0.40–0.60)	0.50 (0.40–0.60)	0.50 (0.40–0.60)	0.50 (0.40–0.70)	0.002
Platelets ($10^9/\text{L}$)	237.00 (200.00–283.00)	233.00 (199.00–275.00)	229.00 (192.00–271.00)	225.00 (196.00–272.00)	<0.001
NLR	2.00 (1.50–2.55)	1.97 (1.46–2.75)	1.92 (1.45–2.65)	2.07 (1.51–2.84)	0.201
PLR	119.85 (94.78–150.45)	109.33 (87.42–142.67)	113.54 (90.47–145.00)	106.74 (86.97–138.18)	<0.001
SII	473.36 (338.03–641.85)	445.76 (311.11–653.25)	440.60 (318.73–620.97)	452.34 (318.84–669.03)	0.106

Note: Values are median (IQR). CRP, C-reactive protein; WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.

As seen in Figure 1, the distributions of CRP and WBC according to periodontal category are as follows. CRP levels were plotted by severity of periodontitis (Figure 1A), and medians were computed for each group, which ranged from 0.19 mg/dL in patients with no periodontitis to 0.21 mg/dL in patients with severe periodontitis. The median WBC in the severe-periodontitis group (Figure 1B) was $7.30 \times 10^9/\text{L}$, whereas the median in participants without periodontitis was $6.80 \times 10^9/\text{L}$.

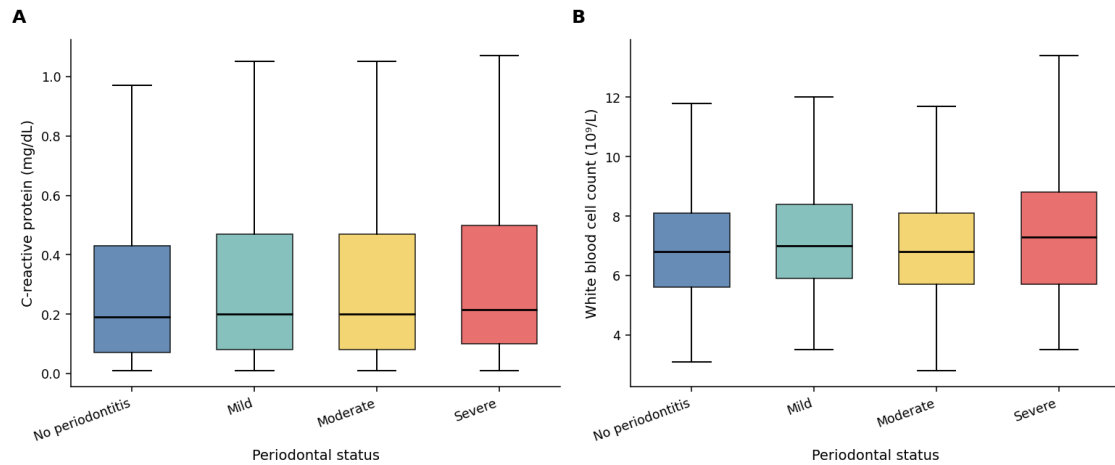


Figure 1. Systemic inflammatory markers according to periodontal disease severity. (A) Distribution of C-reactive protein concentrations across periodontal severity categories. (B) Distribution of white blood cell counts across periodontal severity categories.

3.2 Correlations Between Periodontal Burden and Systemic Inflammatory Markers

Four continuous periodontal parameters: mean PPD, maximum PPD, mean CAL, and maximum CAL were analyzed by correlation. The correlation coefficients for these periodontal measures with the systemic inflammatory markers are presented in Table 2 using the Spearman method. Mean PPD was positively correlated with CRP ($\rho = 0.069$, $P < 0.001$), WBC ($\rho = 0.076$, $P < 0.001$), neutrophils ($\rho = 0.069$, $P < 0.001$), monocytes ($\rho = 0.056$, $P < 0.001$), and NLR ($\rho = 0.039$, $P = 0.021$). Negative correlations were observed with platelets ($\rho = -0.087$, $P < 0.001$) and PLR ($\rho = -0.098$, $P < 0.001$). Maximum PPD was correlated with CRP ($\rho = 0.073$, $P < 0.001$), WBC ($\rho = 0.071$, $P < 0.001$), neutrophils ($\rho = 0.049$, $P = 0.003$), lymphocytes ($\rho = 0.055$, $P < 0.001$), and monocytes ($\rho = 0.063$, $P < 0.001$). Mean CAL was positively correlated with CRP ($\rho = 0.069$, $P < 0.001$), WBC ($\rho = 0.056$, $P < 0.001$), neutrophils ($\rho = 0.056$, $P < 0.001$), monocytes ($\rho = 0.082$, $P < 0.001$), and NLR ($\rho = 0.053$, $P = 0.001$). Maximum CAL was significantly correlated with the following parameters: CRP, monocytes, platelets and PLR. There were no significant correlations between any of the four periodontal measures and SII.

Table 2. Correlations between periodontal disease burden and systemic inflammatory markers

Periodontal measure	Inflammatory marker	N	Spearman ρ	P value
Mean PPD	CRP	3594	0.069	<0.001
Mean PPD	WBC	3607	0.076	<0.001
Mean PPD	Neutrophils	3600	0.069	<0.001
Mean PPD	Lymphocytes	3600	0.028	0.096
Mean PPD	Monocytes	3600	0.056	<0.001
Mean PPD	Platelets	3607	-0.087	<0.001
Mean PPD	NLR	3600	0.039	0.021
Mean PPD	PLR	3600	-0.098	<0.001
Mean PPD	SII	3600	-0.017	0.313
Maximum PPD	CRP	3594	0.073	<0.001
Maximum PPD	WBC	3607	0.071	<0.001
Maximum PPD	Neutrophils	3600	0.049	0.003
Maximum PPD	Lymphocytes	3600	0.055	<0.001
Maximum PPD	Monocytes	3600	0.063	<0.001
Maximum PPD	Platelets	3607	-0.051	0.002
Maximum PPD	NLR	3600	0.002	0.913
Maximum PPD	PLR	3600	-0.097	<0.001
Maximum PPD	SII	3600	-0.028	0.095
Mean CAL	CRP	3594	0.069	<0.001

Mean CAL	WBC	3607	0.056	<0.001
Mean CAL	Neutrophils	3600	0.056	<0.001
Mean CAL	Lymphocytes	3600	-0.011	0.497
Mean CAL	Monocytes	3600	0.082	<0.001
Mean CAL	Platelets	3607	-0.104	<0.001
Mean CAL	NLR	3600	0.053	0.001
Mean CAL	PLR	3600	-0.075	<0.001
Mean CAL	SII	3600	-0.008	0.611
Maximum CAL	CRP	3594	0.050	0.002
Maximum CAL	WBC	3607	0.026	0.119
Maximum CAL	Neutrophils	3600	0.024	0.148
Maximum CAL	Lymphocytes	3600	-0.017	0.313
Maximum CAL	Monocytes	3600	0.060	<0.001
Maximum CAL	Platelets	3607	-0.097	<0.001
Maximum CAL	NLR	3600	0.028	0.089
Maximum CAL	PLR	3600	-0.067	<0.001
Maximum CAL	SII	3600	-0.027	0.106

Note: PPD, probing pocket depth; CAL, clinical attachment loss; CRP, C-reactive protein; WBC, white blood cell count; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index. Systemic inflammatory markers are correlated with periodontal measurements in the direction and magnitude as presented in Figure 2. The range of correlation coefficient from -0.104 to 0.082. The highest positive correlation was seen between mean CAL and monocytes ($\rho = 0.082$), while the highest negative correlation was seen between mean CAL and platelets ($\rho = -0.104$).

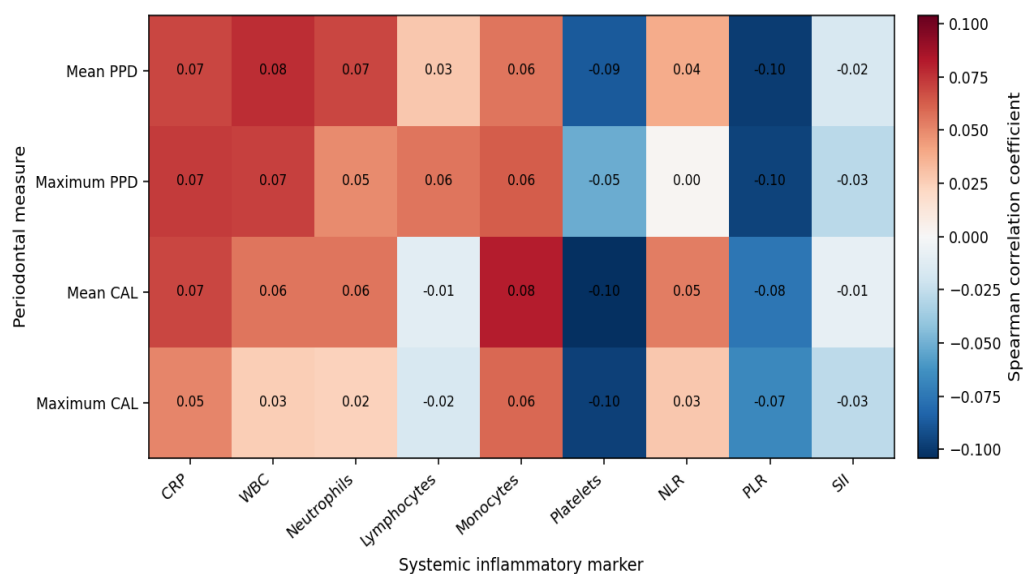


Figure 2. Correlation Heatmap between periodontal disease burden and systemic inflammatory markers.

3.3 Regression Analysis of Periodontal Measures and CRP

Log-transformed CRP was then regressed on the periodontal measurements to further explore the associations. The regression coefficients, 95% confidence intervals, and model R^2 values for continuous periodontal measures and periodontal severity categories are displayed in Table 3. Mean PPD was positively associated with log-transformed CRP ($\beta = 0.031$, 95% CI: 0.012–0.050; $P = 0.001$). Significant positive coefficients were also obtained for maximum PPD ($\beta = 0.011$, 95% CI: 0.004–0.018; $P = 0.002$), mean CAL ($\beta = 0.017$, 95% CI: 0.007–0.027; $P < 0.001$), and maximum CAL ($\beta = 0.007$, 95% CI: 0.002–0.012; $P = 0.009$). Using no periodontitis as the reference category, mild periodontitis was not significantly associated with log-transformed CRP ($\beta = 0.015$, 95% CI: -0.021–0.051; $P = 0.419$), nor was moderate periodontitis ($\beta = 0.011$, 95% CI: -0.011–0.033; $P = 0.312$). Severe periodontitis showed a positive coefficient of 0.042 (95% CI: 0.006–0.078; $P = 0.022$). The R^2 values for the models were 0.0042 for the mean PPD, 0.0032 for the maximum PPD, 0.0047 for the mean CAL, 0.0026 for the maximum CAL, and 0.0017 for the categorical severity model.

Table 3. Regression analysis of periodontal disease measures in relation to log-transformed C-reactive protein

Model	Predictor	N	β (95% CI)	P value	R ²
Continuous periodontal measure	Mean PPD	3594	0.031 (0.012 to 0.050)	0.001	0.0042
Continuous periodontal measure	Maximum PPD	3594	0.011 (0.004 to 0.018)	0.002	0.0032
Continuous periodontal measure	Mean CAL	3594	0.017 (0.007 to 0.027)	<0.001	0.0047
Continuous periodontal measure	Maximum CAL	3594	0.007 (0.002 to 0.012)	0.009	0.0026
Periodontal severity	Mild vs no periodontitis	3911	0.015 (−0.021 to 0.051)	0.419	0.0017
Periodontal severity	Moderate vs no periodontitis	3911	0.011 (−0.011 to 0.033)	0.312	0.0017
Periodontal severity	Severe vs no periodontitis	3911	0.042 (0.006 to 0.078)	0.022	0.0017

Note: The outcome variable was log-transformed CRP. β , regression coefficient; CI, confidence interval; PPD, probing pocket depth; CAL, clinical attachment loss; CRP, C-reactive protein.

Pairwise CRP comparisons were subsequently performed across periodontal severity groups.. The unadjusted comparison between no periodontitis and severe periodontitis was $P = 0.009$, although the adjusted value was $P = 0.056$ when corrected for multiple comparisons. All other pairwise comparisons had adjusted P values > 0.476 , and none were < 0.05 .

3.4 Endodontic Transcriptomic Inflammatory Profile

The processed endodontic data set included 17,914 records, 7,454 unique genes, and 13 clusters. 2,500 strong cluster-associated marker records were left after filtering on markers, which represent 1,734 unique genes. The number of significant markers ranged between 20 and 398 markers, from cluster 2 to cluster 1, respectively. The median log fold changes for the clusters were 0.663 to 0.956, and the maximum values were 1.259 to 5.778. 1,798 marker records (1,427 unique genes) remained after cell-type annotation. The annotated clusters comprised B cells, plasma/B-lineage cells, T cells, cytotoxic T/NK-like lymphocytes, myeloid cells/macrophages, mast cells, fibroblast/stromal cells, endothelial cells, pericytes/vascular smooth muscle cells, epithelial cells and proliferating/cycling cells. A total of 109 marker records (67 unique genes) were identified by inflammatory/immune filtering. There were 25 inflammatory markers in myeloid cells/macrophages, 16 in fibroblast/stromal cells, 15 in B cells, 11 in cytotoxic T/NK-like lymphocytes, 10 in epithelial cells, 9 in endothelial cells, 6 in proliferating/cycling cells, 6 in pericytes/vascular smooth muscle cells, 3 in mast cells, and 2 in plasma/B-lineage cells.

Focusing on the most highly ranked inflammatory/immune genes, CCL5, S100A8, HLA-DRA, HLA-DRB1, HLA-DPB1, S100A9, HLA-DPA1, CCL4, HLA-DQA1, and CXCL12 were chosen. The highest integration score was observed for CCL5 (4.420), and the average log fold change for CCL5 was 3.292 in the group of cytotoxic T/NK-like lymphocytes. The maximum log fold changes were 3.397 for S100A8 and 3.189 for S100A9. There were several clusters of HLA class II genes including HLA-DRA, HLA-DRB1, HLA-DPB1, HLA-DPA1, HLA-DQA1, and HLA-DQB1.

To display the distribution of the selected inflammatory/immune genes by the annotated cell population, the data were plotted on a cell-by-cell basis in Figure 3A. S100A8 and S100A9 had the largest log fold changes in epithelial cells, and CCL5 was most abundant in cytotoxic T/NK-like lymphocytes. The number of unique genes in each of the pre-defined mechanistic domains is shown in Figure 3B. There were 7 counts for monocyte/macrophage inflammation, 5 counts for neutrophil recruitment/activation, 5 counts for lymphocyte chemotaxis/activation, 4 counts for IL-1/TNF inflammatory signaling, and 2 counts for acute-phase/IL-6 signaling.

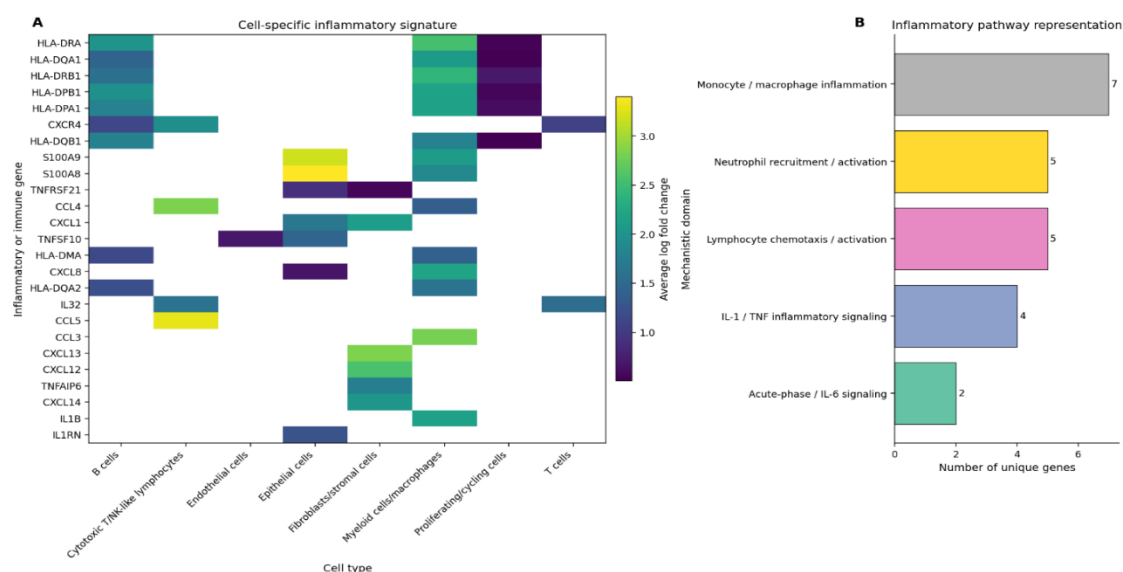


Figure 3. Inflammatory molecular profile of chronic apical periodontitis. (A) Cell-specific inflammatory and immune gene signature across annotated cell populations, displayed according to average log fold change. (B) Number of unique genes represented within the predefined inflammatory mechanistic domains.

3.5 Mechanistic Domain Analysis of Endodontic Markers

Table 4 lists the chosen endodontic markers by the five pre-defined inflammatory domains, the cell types, the average log fold change, the proportion of expression and the adjusted P value. The average log fold changes for epithelial S100A8 and S100A9 were 3.397 and 3.189, and for myeloid cells/macrophages, CXCL8 were 2.191. Within the domain of lymphocyte chemotaxis/activation, CCL5 (AVG logFC = 3.292), CXCL13 (2.834) and CXCL12 (2.562) were found. Among the monocyte/macrophage domain, CCL4 was found in the cytotoxic T/NK-like lymphocytes (2.821) and in myeloid cells/macrophages (2.808), as well as in myeloid cells/macrophages (1.385). The average log fold changes were 2.159 for IL1B in myeloid cells/macrophages, 1.248 for IL1RN in epithelial cells, and 0.666 for IL1R1 in fibroblast/stromal cells for the inflammatory signaling pathway of IL-1/TNF. The SOCS3 gene in pericytes/vascular smooth muscle cells (VSM) and IL6ST in endothelial and fibroblast/stromal cells were both in the acute-phase/IL-6 category (average logFC = 0.925 and 0.620 and 0.528, respectively). P values were adjusted for all markers presented in the table and were < 0.001 for each marker.

Table 4. Cell-specific endodontic inflammatory markers classified according to mechanistic domain

Mechanistic domain	Gene	Predominant cell type	Average logFC	Expression in cluster	Expression outside cluster	Adjusted P value
Acute-phase signaling / IL-6	SOCS3	Pericytes/vascular smooth muscle cells	0.925	0.701	0.501	<0.001
Acute-phase signaling / IL-6	IL6ST	Endothelial cells	0.620	0.533	0.195	<0.001
Acute-phase signaling / IL-6	IL6ST	Fibroblasts/stromal cells	0.528	0.495	0.180	<0.001
IL-1 / TNF inflammatory signaling	IL1B	Myeloid cells/macrophages	2.159	0.372	0.026	<0.001
IL-1 / TNF inflammatory signaling	IL1RN	Epithelial cells	1.248	0.573	0.030	<0.001
IL-1 / TNF inflammatory signaling	IL1R1	Fibroblasts/stromal cells	0.666	0.402	0.054	<0.001
Lymphocyte chemotaxis/activation	CCL5	Cytotoxic T/NK-like lymphocytes	3.292	0.893	0.100	<0.001
Lymphocyte chemotaxis/activation	CXCL13	Fibroblasts/stromal cells	2.834	0.653	0.248	<0.001
Lymphocyte chemotaxis/activation	CXCL12	Fibroblasts/stromal cells	2.562	0.918	0.275	<0.001
Monocyte/macrophage inflammation	CCL4	Cytotoxic T/NK-like lymphocytes	2.821	0.695	0.179	<0.001
Monocyte/macrophage inflammation	CCL3	Myeloid cells/macrophages	2.808	0.570	0.163	<0.001
Monocyte/macrophage inflammation	CCL4	Myeloid cells/macrophages	1.385	0.459	0.201	<0.001
Neutrophil recruitment/activation	S100A8	Epithelial cells	3.397	0.940	0.429	<0.001
Neutrophil recruitment/activation	S100A9	Epithelial cells	3.189	0.950	0.482	<0.001
Neutrophil recruitment/activation	CXCL8	Myeloid cells/macrophages	2.191	0.409	0.094	<0.001

4. DISCUSSION

Based on clinical findings, there is a slight relationship between periodontal disease burden and systemic inflammatory activity. CRP was significantly different between the different periodontal severity categories, with the highest median CRP and white blood cell counts among the participants with severe periodontitis. Pocket depths were also correlated with continuous measures of probing depth and clinical attachment loss and were positively related to CRP, leukocyte count, neutrophils and monocytes with small correlation coefficients. Results of regression analyses confirmed the pattern, as mean and maximum periodontal measurements were significantly positively associated with log-transformed CRP. Mild and moderate periodontitis were not significant, but severe periodontitis was in comparison to no periodontitis. The low R² values suggest that only a small portion of the variability of CRP was accounted for by periodontal status. Also, the systemic effect that was observed was not strong, since there was no significant difference between any two pairwise comparisons after Holm correction. Not all of the hematological parameters were consistent for all inflammatory indices. The PLR was found to decrease with the increasing severity of periodontal disease, while platelet count was also found to decrease in this regard; no significant difference was found between the NLR and SII in the various categories. Weak inverse correlations were also identified between the periodontal burden and platelet measures by correlation analysis and

between SII and periodontal burden. Based on these findings, individual blood-derived inflammatory markers may not all react similarly to periodontal disease, and therefore, cannot be viewed as equivalent markers of systemic inflammation. The CRP pattern observed is in agreement with the reports that periodontal therapy has been found to decrease the circulating CRP, which suggests a relationship between the inflammatory burden of the periodontal disease and the systemic acute-phase activity [17]. Increased levels of hs-CRP have also been found as the severity of chronic periodontitis increased from previous studies, in a similar order to the present findings [18]. In another study of patients with acute myocardial infarction (AMI), an observational study was performed that revealed a link between the severity of periodontal disease and CRP [19]. There is less consensus about the evidence for composite inflammatory indices. An NHANES study reported the correlation of SII and systemic inflammation response index with periodontitis [20], but the correlation of SII was not significant in the present analysis. Blood-biomarker analysis of NHANES data also has recently confirmed relationships between periodontal disease and systemic inflammatory markers derived from leukocytes [21]. The endodontic transcriptomic component exhibited a pattern that was inflammatory, but different from the others. Prominent signatures for myeloid/macrophage, epithelial, stromal and lymphoid tissue were evident in the chronic apical periodontitis tissue, including high-ranking markers such as S100A8, S100A9, CCL5, CCL3, CCL4, CXCL8, CXCL12, CXCL13, and IL1B. Proteomic analysis of the inflammatory and host-response proteins has identified that there is a significant difference between acute apical abscess and chronic apical periodontitis, indicating disease-specific molecular activity in periapical tissues [22]. The chemokine signaling pathway is also well-known in periodontal inflammation, in which a complex network of leukotactic and regional immune-cell trafficking molecules is involved [23]. The discovery of IL1B and other inflammatory markers in the endodontic component is also confirmed by the evidence that the proinflammatory cytokine activity is increased in endo-periodontal lesions at both local and systemic levels [24]. It is clear from the clinical and molecular observations that there has to be an inflammatory linkage between periodontal diseases and endodontic disease, but it is not clear whether these are the same individuals or the same compartment of the body. Small, but measurable increases in circulating inflammatory markers were associated with periodontal disease, and the increased transcription of neutrophil recruitment, monocyte/macrophage activation, lymphocyte chemotaxis, and IL-1/TNF-related signaling was strong in the case of chronic apical periodontitis. Convergence is therefore greatest at the inflammatory pathways level than at the association level of the patients.

The present results suggest that chronic oral infections are a source of inflammatory burden and have implications for understanding them as sources. PPD and CAL could be clinically available markers correlated with systemic inflammatory status, and the transcriptomic characterisation of apical periodontitis could identify certain cellular pathways that could play a role in the perpetuation of local inflammation. Combining clinical and molecular data could aid in the further study of the oral-systemic inflammatory relationship and target pathways for validation.

There are some restrictions to be taken into account. The clinical analysis is cross-sectional, thus not being able to prove temporal or causal relationships. The periodontal and endodontic datasets were independent datasets, so there was no direct comparison of the participants. The endodontic transcriptomic dataset also did not have a healthy comparator, meaning that the molecular results are cluster-associated signatures, and not case-control differential expression. Future research should evaluate the relationship between periodontal and endodontic disease in the same individuals over time and add matched healthy controls for comparison of transcriptomic changes. Longitudinal integration of periodontal examination, endodontic status, circulating biomarkers, and molecular profiles of the tissues would allow treatment-related changes to be evaluated and help define the possible mechanisms of inflammatory convergence, and whether this is due to common mechanisms, severity, or host susceptibility among oral inflammatory conditions.

5. CONCLUSION

The inflammatory profiles on the clinical and molecular aspects were complementary in periodontal and endodontic infections. Small differences in systemic inflammatory markers and modest associations with CRP were observed with greater periodontal burden, with severe periodontitis showing the clearest association with CRP. Elevated expression of the genes S100A8, S100A9, CXCL8, IL1B, CCL3, CCL4 and CCL5 showed a distinct local inflammatory signature in chronic apical periodontitis. The overall conclusions suggest convergence of the inflammation of periodontal and endodontic disease, but do not suggest that there was any direct participant-level or causal relationship. The modest systemic effect sizes suggest that oral inflammatory burden is not a major contributor to systemic inflammation, but rather an element of a larger inflammatory milieu. A model that integrates the clinical approach to periodontal measurements with tissue molecular profiling is useful for understanding the link between various inflammatory conditions in the oral cavity and elsewhere, and can be used in future longitudinal studies involving both the oral and systemic disease state measured in the same set of patients.

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