

SERUM AND SALIVARY CALPROTECTIN (S100A8/A9) AS A CONVERGENT BIOMARKER ACROSS THE RHEUMATOID ARTHRITIS–PERIODONTITIS AXIS: A PERSPECTIVE FROM PILOT Q-TOF PEPTIDOMIC SCREENING

Anitha Krishnan Pandarathodiyil^{1,2}, Ramya Ramadoss³, Saranya Chinnadurai⁴, Sivapathasundharam B⁵

¹Faculty of Dentistry, SEGi University, Petaling Jaya, Selangor, Malaysia

²Department of Oral Pathology, Saveetha Dental College and Hospitals, Adjunct Faculty, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

³Department of Oral Biology, Saveetha Dental College and Hospitals, Saveetha Institute Medical and Technical Science, Saveetha University, Chennai, Tamil Nadu, India

⁴Department of Rheumatology, Saveetha Medical College and Hospital, Saveetha Institute Medical and Technical Science, Saveetha University, Chennai, Tamil Nadu, India

⁵Pro Vice-Chancellor, Sri Venkateswara University, Tamil Nadu, India

ABSTRACT

Rheumatoid arthritis (RA) and periodontitis are increasingly recognised as bidirectionally linked inflammatory diseases that converge on shared innate-immune effectors. Calprotectin (S100A8/A9), a neutrophil- and monocyte-derived alarmin, is among the most consistently validated soluble biomarkers of RA disease activity, frequently outperforming C-reactive protein and the erythrocyte sedimentation rate, and it is independently implicated in periodontal tissue inflammation. We revisit this evidence alongside a pilot untargeted Q-TOF peptidomic screen of serum and saliva from Control, RA-without-periodontitis, and RA-with-periodontitis groups. Calprotectin-family peptides were the third most differentially detected signal among sixteen screened protein/peptide families (Fisher's exact $p = 3.85 \times 10^{-4}$, Cramér's $V = 0.75$, serum), rising from 0% detection in Control to 77.8% in RA-without-periodontitis (odds ratio 51, 95% CI 2.1–1240.3). Detection was markedly lower in the RA-with-periodontitis subgroup (10%), a non-monotonic pattern we interpret cautiously given the small pilot sample. We present this as a data-anchored perspective, not a validated diagnostic claim: the findings reinforce calprotectin's candidacy as a dual-fluid, treat-to-target biomarker for RA, while surfacing an open question — whether concurrent periodontitis alters detectable calprotectin signal — that merits investigation in adequately powered, quantitatively calibrated cohorts.

KEYWORDS: calprotectin; disease-activity biomarker; periodontitis; proteomics; rheumatoid arthritis; S100A8/A9; saliva; good health and well-being

1. INTRODUCTION

Rheumatoid arthritis (RA) affects an estimated 17.6 million people worldwide, with global age-standardised prevalence having risen by 14.1% since 1990 and projected to reach 31.7 million cases by 2050 [1]. Alongside this rising burden, a substantial body of epidemiological and mechanistic evidence has established a bidirectional relationship between RA and periodontitis, two chronic inflammatory diseases that share innate-immune activation pathways, cytokine profiles, and, in susceptible individuals, a common downstream product: citrullinated self-protein [2]. Periodontal pathogens, most notably *Porphyromonas gingivalis*, express a bacterial peptidylarginine deiminase capable of citrullinating host and bacterial proteins, offering a plausible mucosal origin for the anti-citrullinated protein antibody response that characterises seropositive RA [3]. This convergence has intensified interest in biomarkers that are legible in both the joint and the periodontium — signals that might, in principle, be sampled non-invasively from saliva as readily as from serum.

Among candidate markers, calprotectin (S100A8/A9) occupies a distinctive position. It is not a bystander acute-phase reactant but a direct effector of innate immunity, released by activated neutrophils and monocytes at sites of inflammation, and its blood concentration has repeatedly tracked RA disease activity more closely than C-reactive protein (CRP) or the erythrocyte sedimentation rate (ESR) [4]. It is also detectable in saliva and periodontal tissue, where it participates in the same neutrophil-driven tissue-destructive programme implicated in periodontitis. This perspective revisits calprotectin's candidacy as a convergent RA–periodontitis biomarker in light of a pilot untargeted Q-TOF peptidomic screen of serum and saliva, and asks what a small, hypothesis-generating dataset can — and cannot — add to an already substantial literature.

2. Calprotectin (S100A8/A9): From Bench to Bedside in Rheumatoid Arthritis

Calprotectin is a heterodimeric complex of the S100 calcium-binding proteins S100A8 and S100A9, constituting up to 40% of neutrophil cytosolic protein and released extracellularly during activation, degranulation, or cell death. Once outside the cell, it signals through Toll-like receptor 4 and the receptor for advanced glycation end-products, amplifying phagocyte recruitment and pro-inflammatory cytokine release — placing it mechanistically

upstream of, rather than merely secondary to, synovial inflammation [4]. In patients with established RA, serum and plasma calprotectin concentrations correlate with ultrasound-detected synovitis more closely than any other routinely available biomarker, and independently predict radiographic progression [5]. Calprotectin's clinical value becomes particularly apparent when conventional acute-phase markers are unreliable: in patients treated with interleukin-6-receptor blockade (tocilizumab), CRP and ESR are pharmacologically suppressed irrespective of true disease activity, yet serum calprotectin continues to distinguish active from inactive disease, with reported sensitivity and specificity both approximating 80% at a cut-off near 1900 ng/mL [6]. Calprotectin's biology is, however, genuinely dual-natured: beyond its pro-inflammatory alarmin function, it also participates in antimicrobial defence and tissue repair, a duality recently summarised as its having ‘two sides of the same coin’ — a caution against treating it as a simple linear readout of disease severity [7].

3. Calprotectin at the Periodontal–Systemic Interface

Calprotectin's relevance is not confined to the joint. It is among the most extensively studied inflammatory proteins in periodontal research, where it is released locally by neutrophils infiltrating the gingival crevice and is measurable in gingival crevicular fluid, whole saliva, and serum; a scoping review has catalogued its consistent elevation across stages of periodontal inflammation [8]. A cross-sectional screen using saliva, blood, and gingival crevicular fluid confirmed S100A8/A9 as a discriminator of established periodontitis across all three compartments, reinforcing its candidacy as a shared, matrix-flexible signal [9]. Most directly relevant to the present perspective, a quasi-experimental study in women with concurrent periodontitis and RA measured calprotectin in both serum and saliva before and after non-surgical periodontal therapy: periodontal treatment reduced periodontal indices, and serum calprotectin concentrations fell in parallel with a reduction in RA disease activity (DAS28) over twelve weeks, in the same patients, sampled in both fluids [10]. Calprotectin has additionally been linked to periodontitis-associated cardiovascular comorbidity, a further point of convergence given the excess cardiovascular risk that characterises RA [11]. Together, this literature frames calprotectin not as two separate disease markers that happen to share a name, but as a single innate-immune signal legible across the oral–systemic axis.

4. Pilot Evidence from Q-TOF Peptidomic Screening

4.1 Dataset and analytic approach

To examine whether this convergence is reflected in an independent, untargeted dataset, we screened an exploratory Q-TOF peptidomic dataset comprising serum and saliva samples from three clinical groups: Control, RA without periodontitis, and RA with periodontitis (Table 1). Because the dataset records qualitative peptide/protein identifications by mass-to-charge (m/z) value rather than calibrated abundances, we treated detection — the presence of a calprotectin-family peptide (S100A8, S100A9, or the S100A8/A9 heterocomplex, including the calgranulin B nomenclature) in a given sample — as the analytic unit, analogous to spectral-count-based semiquantitative approaches used in label-free discovery proteomics. Sixteen candidate protein/peptide families detected in the dataset were screened by Fisher's exact test for association with clinical group in serum; calprotectin ranked third by statistical strength (Cramér's $V = 0.75$), behind the cystatin superfamily and histatin-1, each the subject of a companion perspective arising from the same dataset.

Biofluid	Control	RA without periodontitis	RA with periodontitis
Serum	8	9	10
Saliva	2	5	1

Table 1. Distinct samples analysed per biofluid and clinical group, pilot Q-TOF peptidomic dataset. Values are counts of distinct samples.

4.2 Detection frequency across clinical groups

Table 2 summarises calprotectin-family detection frequency by biofluid and clinical group. In serum, calprotectin was undetected in any Control sample (0/8) but present in 7 of 9 samples from the RA-without-periodontitis group (77.8%; 95% CI 45.3–93.7%) — an omnibus association strong enough to be statistically robust despite the small sample (Fisher's exact $p = 3.85 \times 10^{-4}$; Table 3). The corresponding odds of detection in RA-without-periodontitis relative to Control was 51-fold (95% CI 2.1–1240.3), calculated with Haldane–Anscombe continuity correction given the zero cell in the Control group; the very wide confidence interval reflects quasi-complete separation in a small sample and should be read as indicating a strong signal, not a precise point estimate. Saliva showed a numerically similar gradient (Control 50%, RA-without-periodontitis 60%, RA-with-periodontitis 100%), but with only two to five samples per group this comparison did not reach statistical significance and is reported descriptively (Fisher's exact $p = 1.00$).

Biofluid	Group	Detected / n	Frequency % (95% CI)
Serum	Control	0 / 8	0.0 (0.0–32.4)
Serum	RA without periodontitis	7 / 9	77.8 (45.3–93.7)
Serum	RA with periodontitis	1 / 10	10.0 (1.8–40.4)

Biofluid	Group	Detected / n	Frequency % (95% CI)
Saliva	Control	1 / 2	50.0 (9.5–90.5)
Saliva	RA without periodontitis	3 / 5	60.0 (23.1–88.2)
Saliva	RA with periodontitis	1 / 1	100.0 (20.7–100.0)

Table 2. Calprotectin (S100A8/A9) detection frequency by biofluid and clinical group. CI: Wilson score 95% confidence interval.

Figure 1. Peptidomic marker detection frequency across clinical groups

Q-TOF pilot dataset, serum and saliva
(values = % samples positive; n detected/total)

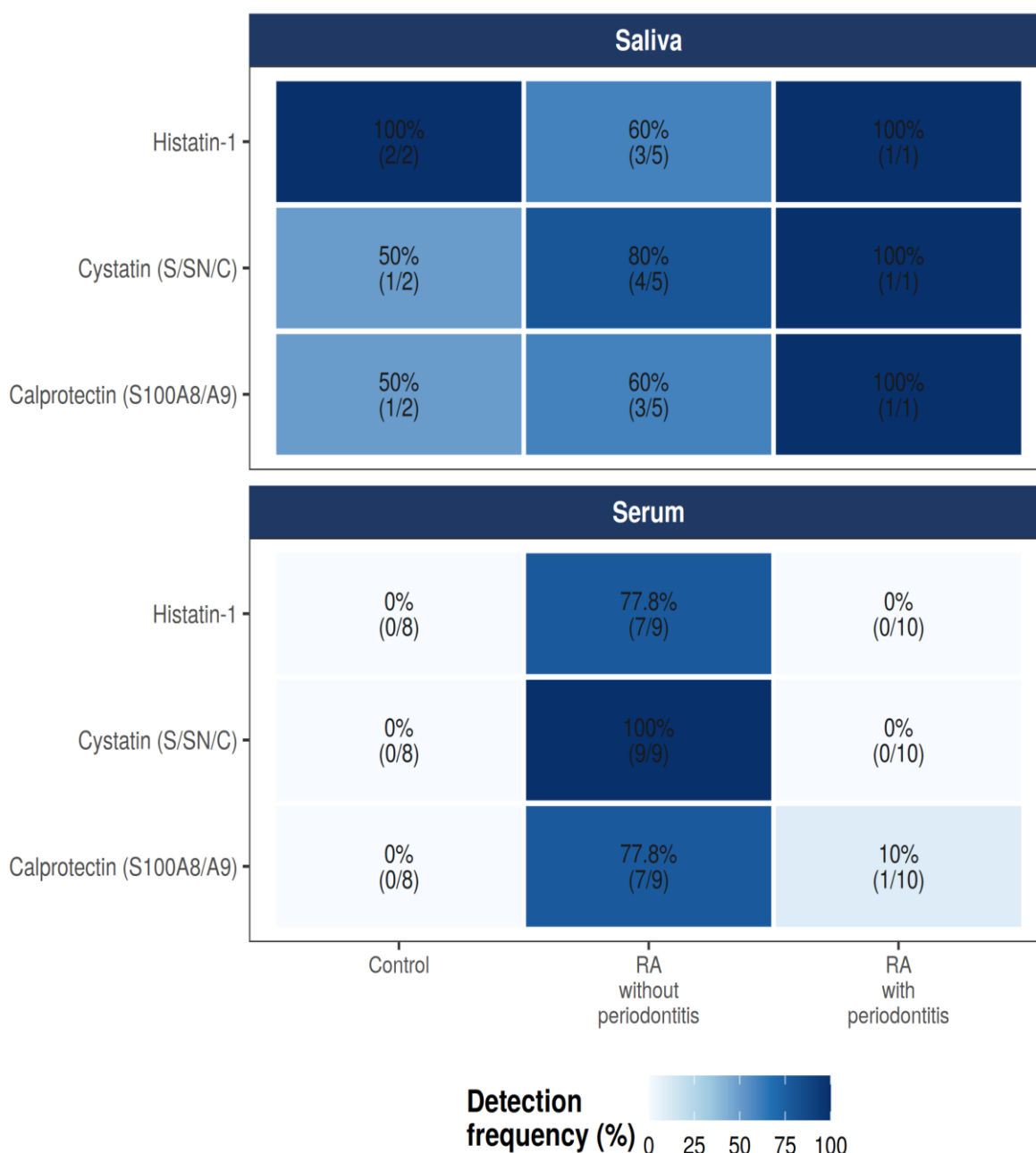


Figure 1. Detection frequency (%) of the three candidate markers screened from the pilot Q-TOF dataset — calprotectin, the cystatin superfamily, and histatin-1 — across biofluid and clinical group. Calprotectin's pattern (bottom row of each panel) mirrors the other two screened markers, with detection concentrated in the RA-without-periodontitis group.

Figure 2. Serum detection-frequency profile by clinical group (Q-TOF pilot dataset)

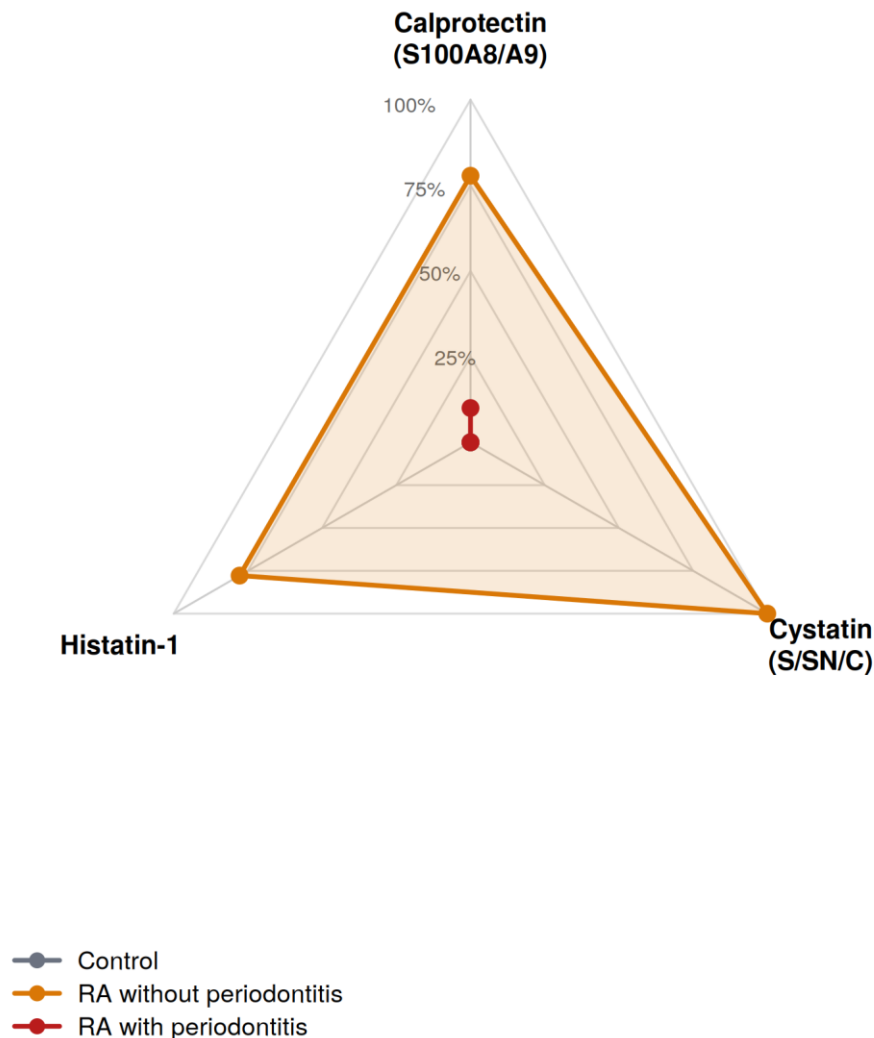


Figure 2. Serum detection-frequency profile of the three screened markers by clinical group. Control (grey) collapses to the origin (0% detection of all three markers); RA-without-periodontitis (amber) shows near-complete positivity; RA-with-periodontitis (red) remains close to Control except for a partial calprotectin signal (10%).

Detection in the RA-with-periodontitis serum subgroup was unexpectedly low (1/10, 10%; odds ratio 2.7 versus Control, 95% CI 0.1–75.1) — closer to Control than to the RA-without-periodontitis pattern — and a logistic trend test across the ordinal sequence Control → RA-without-periodontitis → RA-with-periodontitis was not significant ($\beta = 0.11$, $p = 0.833$; Table 3), meaning that detection did not simply track increasing clinical burden. This non-monotonic pattern departs from the intuitive expectation that concurrent periodontitis would compound systemic inflammation and further elevate a shared alarmin, and it is worth naming candidate explanations rather than leaving the observation unexplained: ion suppression or matrix complexity in a more heterogeneous inflammatory milieu could reduce calprotectin's detectability by untargeted mass spectrometry even if its true concentration were unchanged or higher; the RA-with-periodontitis subgroup may differ systematically in medication exposure, disease duration, or periodontal severity in ways the dataset does not capture; or the small per-group sample size ($n = 10$) may simply have produced an unrepresentative draw. We regard each of these as testable hypotheses rather than conclusions.

Figure 3. Calprotectin (S100A8/A9) detection frequency by clinical group

Bars = % samples positive; error bars = 95% Wilson score CI; labels = detected/total

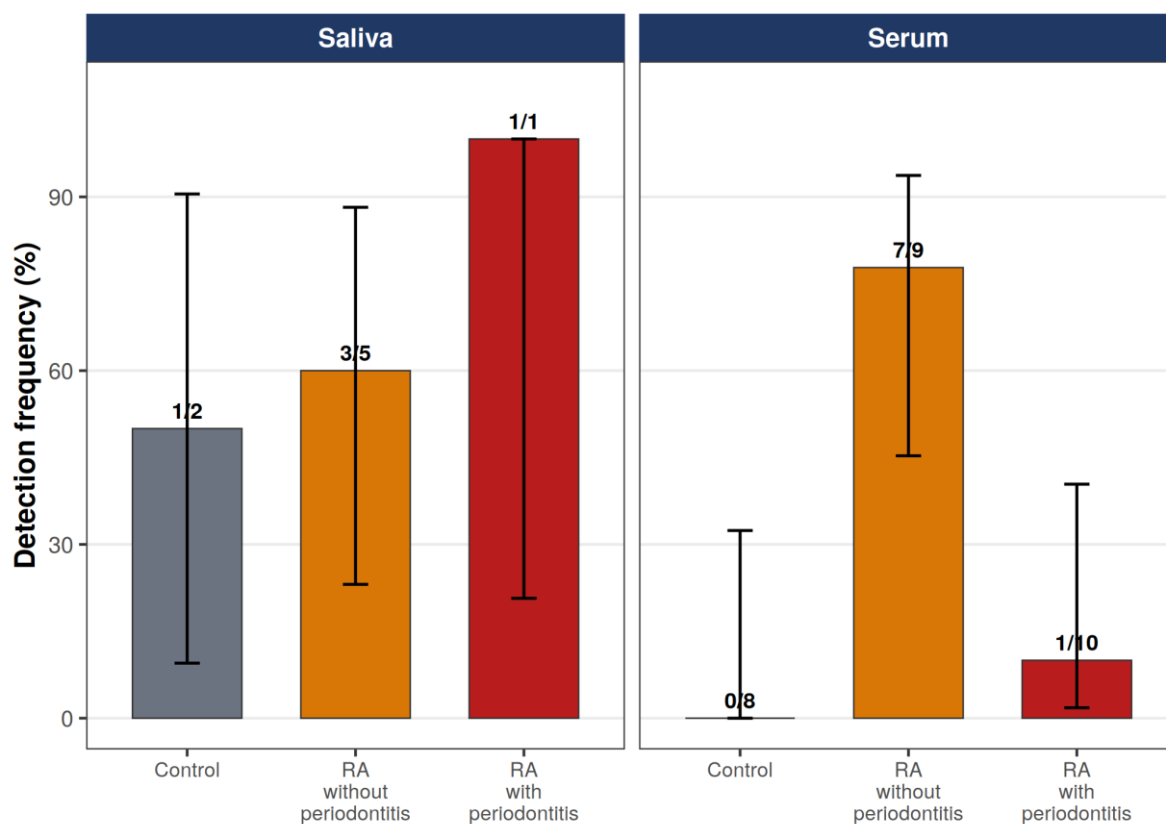


Figure 3. Calprotectin (S100A8/A9) detection frequency by clinical group, faceted by biofluid. Bars show % samples positive; error bars are 95% Wilson score confidence intervals; labels show detected/total samples.

Figure 4. Calprotectin (S100A8/A9)—odds of detection vs Control (serum)

Haldane-Anscombe corrected OR, 95% CI, log scale

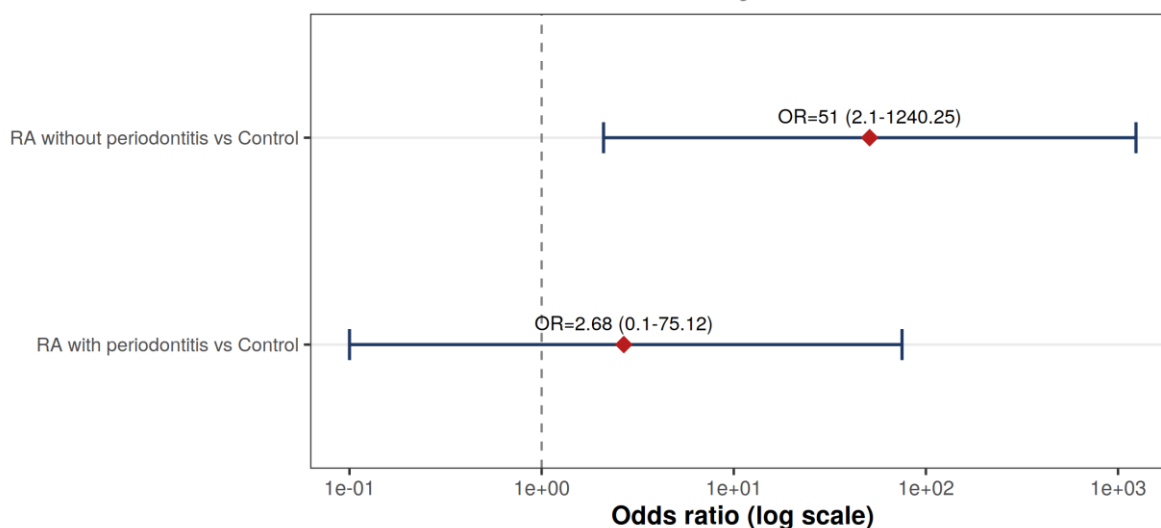


Figure 4. Odds of calprotectin detection in RA-without-periodontitis and RA-with-periodontitis relative to Control (serum), with Haldane–Anscombe corrected 95% confidence intervals on a log scale.

Test	Statistic	95% CI / p-value
Fisher's exact, serum (3-group omnibus)	Cramér's V = 0.75	p = 3.85×10^{-4}
Fisher's exact, saliva (3-group omnibus)	Cramér's V = 0.31	p = 1.00 (descriptive)
OR, RA-without-periodontitis vs Control (serum)	OR = 51.0	95% CI 2.1–1240.3

Test	Statistic	95% CI / p-value
OR, RA-with-periodontitis vs Control (serum)	OR = 2.7	95% CI 0.1–75.1
Logistic trend, ordinal severity (serum)	$\beta = 0.11$	p = 0.833 (ns)

Table 3. Statistics table: serum calprotectin detection versus clinical group. OR: odds ratio, Haldane–Anscombe corrected. ns: not significant.

5. Perspective: What This Adds, and Its Limits

This pilot dataset cannot, and should not, be read as a validation of calprotectin as a diagnostic biomarker: sample sizes per group are small (as few as one saliva sample in the RA-with-periodontitis arm), detection is qualitative rather than quantitatively calibrated, clinical covariates (disease activity scores, ACPA/RF status, medication exposure, periodontal staging) are not available for adjustment, and the dataset derives from a single analytical pipeline without independent replication. The composite pattern across all three markers screened from this dataset — near-universal multi-marker positivity in RA-without-periodontitis (89% of serum samples positive for two or more of calprotectin, the cystatin superfamily, and histatin-1) alongside near-complete negativity in both Control and RA-with-periodontitis — is internally consistent, which argues against simple random artefact, but internal consistency within one pipeline is not external validity.

What the pilot data usefully do is sharpen a specific, testable question raised by the established literature: does periodontitis alter the detectability or concentration of circulating calprotectin in RA, and if so, in which direction? The intervention literature showing that periodontal therapy lowers serum calprotectin in RA-with-periodontitis patients [10] implies that active periodontal inflammation should, if anything, add to the systemic calprotectin pool — the opposite of the low detection observed here in the untreated RA-with-periodontitis subgroup. Reconciling that discrepancy will require a study designed for the purpose: matched RA-with-periodontitis and RA-without-periodontitis cohorts, quantitative calprotectin measurement (ELISA or targeted mass spectrometry, e.g., multiple-reaction monitoring) in paired serum and saliva, and full capture of periodontal staging, DAS28, and treatment exposure.

If confirmed, a dual-fluid calprotectin assay would be attractive precisely because it does not require joint-specific sampling and remains informative when interleukin-6-pathway inhibitors render CRP and ESR uninterpretable [6]. Embedding periodontal status as a covariate — rather than an incidental comorbidity — in future calprotectin biomarker studies would test whether the oral cavity is a confounder to be adjusted for, or a second, clinically actionable compartment in its own right.

6. CONCLUSION

Calprotectin (S100A8/A9) remains among the best-supported soluble biomarkers of RA disease activity, with an independent evidence base linking it to periodontal inflammation through shared neutrophil-driven biology. A pilot Q-TOF peptidomic screen reproduces the expected serum elevation in RA relative to Control with a strong statistical signal, but also surfaces an unexplained attenuation of detection in the presence of concurrent periodontitis. We present this as a call for quantitatively calibrated, adequately powered dual-fluid studies that treat periodontal status as a first-class variable in RA biomarker research, rather than as a resolved conclusion in itself.

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Data Availability Statement: The pilot peptidomic dataset analysed in this perspective, together with the R analysis scripts and CSV outputs underlying Tables 2–3 and Figures 1–4, are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare no competing conflict of interests.

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