

SALIVARY HISTATIN-1 AS A NON-INVASIVE SENTINEL OF ORAL–SYSTEMIC DYSREGULATION IN RHEUMATOID ARTHRITIS: A PERSPECTIVE FROM PILOT Q-TOF PEPTIDOMIC SCREENING

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ABSTRACT:

Histatin-1 is a histidine-rich antimicrobial peptide secreted almost exclusively by the salivary glands, where it also drives oral wound healing and angiogenesis — functions of particular interest in rheumatoid arthritis (RA), an autoimmune disease independently associated with delayed wound healing. We examine histatin-1's candidacy as a non-invasive, saliva-accessible marker of the RA–periodontitis axis using a pilot untargeted Q-TOF peptidomic screen of serum and saliva across Control, RA-without-periodontitis, and RA-with-periodontitis groups. Among sixteen candidate protein/peptide families screened, histatin-1 was the second most differentially detected signal in serum (Fisher's exact $p = 4.95 \times 10^{-5}$, Cramér's $V = 0.84$), rising from 0% detection in Control to 77.8% in RA-without-periodontitis (odds ratio 51, 95% CI 2.1–1240.3) before falling back to 0% in RA-with-periodontitis — the same non-monotonic pattern observed for two companion markers screened from the same dataset. In saliva, by contrast, histatin-1 was detected in every Control sample (2/2) and in most RA samples, a markedly different shape from its serum profile. We use these pilot findings to motivate, rather than conclude, a case for treating saliva as a legitimate primary sampling matrix in RA biomarker discovery, and we detail specifically why the present dataset falls short of that standard on its own.

KEYWORDS: histatin-1; oral–systemic axis; periodontitis; proteomics; rheumatoid arthritis; saliva; wound healing; 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]. Much of the biomarker interest generated by the well-established bidirectional link between RA and periodontitis has focused on molecules shared between blood and saliva — inflammatory mediators and proteolytic products detectable in either compartment [2]. Histatin-1 occupies a different, and in some ways more interesting, position: it is secreted almost exclusively by the salivary glands, so its presence outside the oral cavity is not simply a matter of a systemic protein happening to diffuse into saliva, but potentially informative in its own right about oral-mucosal and periodontal barrier status.

Histatin-1 also carries biological relevance to RA beyond its salivary origin. RA is an autoimmune disease independently associated with delayed cutaneous and mucosal wound healing, a clinical observation that has motivated interest in wound-healing-promoting peptides as both RA-relevant biology and potential therapeutic leads. Histatin-1 is one of the principal wound-closure-stimulating factors identified in human saliva, and separately promotes endothelial adhesion, migration, and angiogenesis — functions plausibly relevant to a disease in which wound repair is impaired. This perspective examines histatin-1's candidacy as a saliva-accessible marker of the RA–periodontitis axis using a pilot untargeted Q-TOF peptidomic screen, and asks what a small, hypothesis-generating dataset can add to that case.

2. Histatin-1: Salivary Biology, Antimicrobial and Regenerative Functions

Histatin-1 belongs to a small family of histidine-rich cationic peptides secreted by the parotid, submandibular, and sublingual glands, with well-characterised antimicrobial and antifungal activity that helps constrain the oral microbiome. Beyond host defence, histatin-1 and its fragment histatin-2 were identified as the major wound-closure-stimulating factors present in human saliva, promoting keratinocyte and fibroblast migration in cell-culture wound assays [3]. More recent work has extended this regenerative role: histatin-1 promotes endothelial cell adhesion, migration, and angiogenesis, establishing it as a genuine pro-angiogenic factor relevant to oral

mucosal wound repair, not merely an antimicrobial peptide with an incidental secondary function [4]. This dual antimicrobial–regenerative biology is precisely what makes histatin-1 mechanistically interesting in an RA context: patients with RA and other autoimmune disorders display delayed wound healing, a clinical problem serious enough to motivate the repurposing of RA-therapeutic peptides such as AESIS-1 as wound-healing agents in experimental models [5]. Whether endogenous salivary wound-healing peptides such as histatin-1 are themselves dysregulated in RA — rather than merely biologically plausible candidates — has not, to our knowledge, been directly tested.

3. Histatin-1 at the Periodontal Frontline: A Contested Direction

The periodontal literature on histatin and disease severity is notably less settled than the corresponding literature on, for example, salivary cystatins. A cross-sectional study comparing periodontally healthy, gingivitis, and periodontitis groups found that salivary histatin levels increased numerically from health through to periodontitis, though the difference did not reach statistical significance, and gingival crevicular fluid histatin showed no clear relationship with disease severity at all — the authors concluded that histatin's utility as a severity biomarker was, on their evidence, questionable [6]. This stands in contrast to host-defence proteins such as cystatin SN, which is consistently depleted as periodontal disease advances. A plausible reading is that histatin-1 behaves as a reactive, compensatorily upregulated antimicrobial peptide — secreted in proportion to microbial challenge rather than consumed by it — which would predict elevation, not depletion, with worsening periodontal dysbiosis, broadly consistent with cross-sectional screening work identifying salivary host-defence peptides, calprotectin among them, as compartment-flexible discriminators of periodontal status [7].

4. Pilot Evidence from Q-TOF Peptidomic Screening

4.1 Dataset and analytic approach

We screened the same exploratory Q-TOF peptidomic dataset described in companion perspectives on calprotectin and the cystatin superfamily arising from this project: serum and saliva samples from Control, RA-without-periodontitis, and RA-with-periodontitis groups (Table 1), with detection of a histatin-1-family peptide (including named fragments) treated as the analytic unit per sample. Sixteen candidate protein/peptide families were screened by Fisher's exact test for association with clinical group in serum; histatin-1 ranked second by statistical strength, immediately behind the cystatin superfamily.

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 histatin-1 detection frequency. The serum pattern mirrored the two companion markers screened from this dataset: undetected in Control (0/8), present in 7 of 9 RA-without-periodontitis samples (77.8%; 95% CI 45.3–93.7%), and undetected again in RA-with-periodontitis (0/10) — a strong omnibus association (Fisher's exact $p = 4.95 \times 10^{-5}$, Cramér's $V = 0.84$; Table 3) and an odds ratio of 51 (95% CI 2.1–1240.3) for RA-without-periodontitis versus Control. The saliva pattern was materially different in shape: histatin-1 was detected in both Control samples (2/2, 100%), in 3 of 5 RA-without-periodontitis samples (60%), and in the single RA-with-periodontitis sample (100%). Unlike serum, where detection was essentially absent in Control, saliva showed histatin-1 present at baseline in healthy individuals — consistent with its role as a constitutively secreted component of oral innate defence rather than a purely inflammation-induced signal.

Table 2. Histatin-1 detection frequency by biofluid and clinical group. CI: Wilson score 95% confidence interval.

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	0 / 10	0.0 (0.0–27.8)
Saliva	Control	2 / 2	100.0 (34.2–100.0)
Saliva	RA without periodontitis	3 / 5	60.0 (23.1–88.2)
Saliva	RA with periodontitis	1 / 1	100.0 (20.7–100.0)

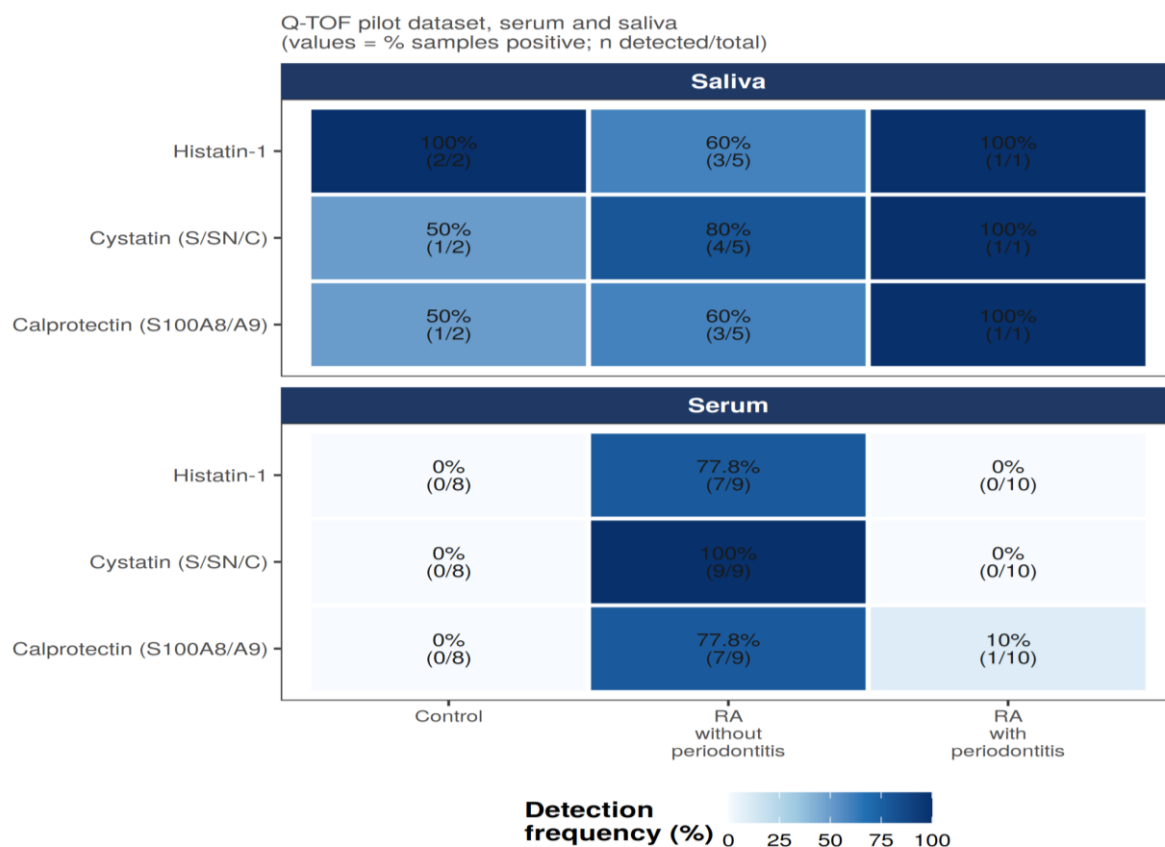


Figure 1. Detection frequency (%) of the three candidate markers screened from the pilot Q-TOF dataset. Histatin-1 (top row of each panel) is the only marker of the three detected in every Control saliva sample, contrasting with its complete absence from Control serum.

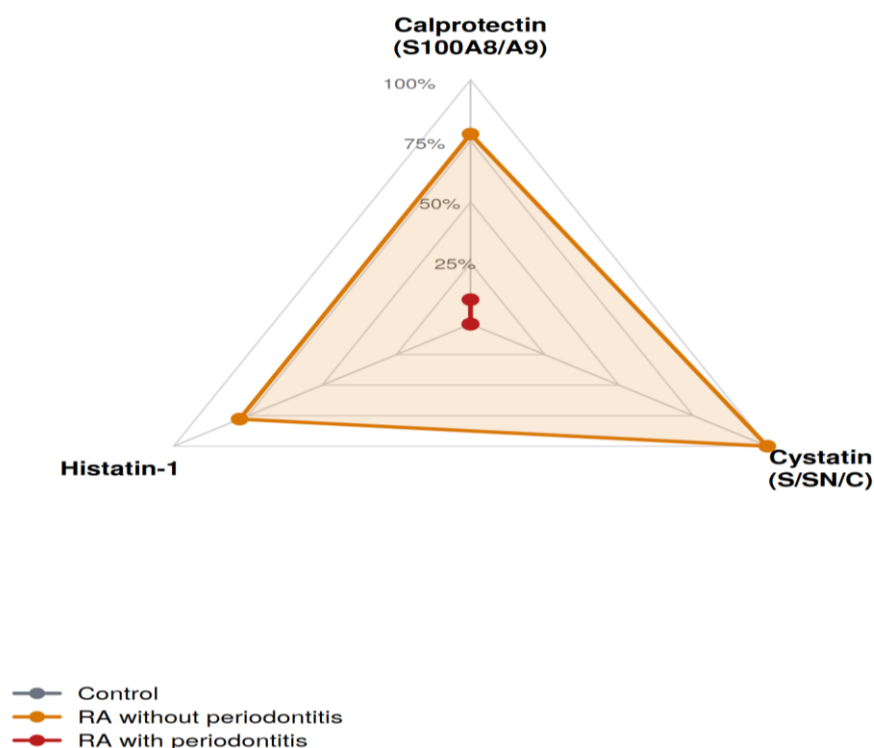


Figure 2. Serum detection-frequency profile of the three screened markers by clinical group. Histatin-1's RA-without-periodontitis value (77.8%) closely tracks calprotectin on the same axis, while Control and RA-with-periodontitis remain at or near the origin.

The contrast between the serum and saliva profiles is, in our view, the most substantive observation to emerge from this marker. If histatin-1 is essentially undetectable in the serum of healthy individuals but reliably present in their saliva, then its appearance in RA-without-periodontitis serum may represent something more specific than ‘general inflammation’: a translocation event, in which a peptide normally confined to the oral cavity becomes detectable in the circulation — whether through heightened mucosal or periodontal barrier permeability, altered systemic neutrophil trafficking, or another route not captured by this dataset. That reframing would make serum histatin-1 a candidate marker of barrier integrity or oral-to-systemic spillover, distinct in interpretation from a marker such as calprotectin that is plausibly synthesised systemically in the first place. We offer this as a hypothesis prompted by the pattern, not a claim it establishes.

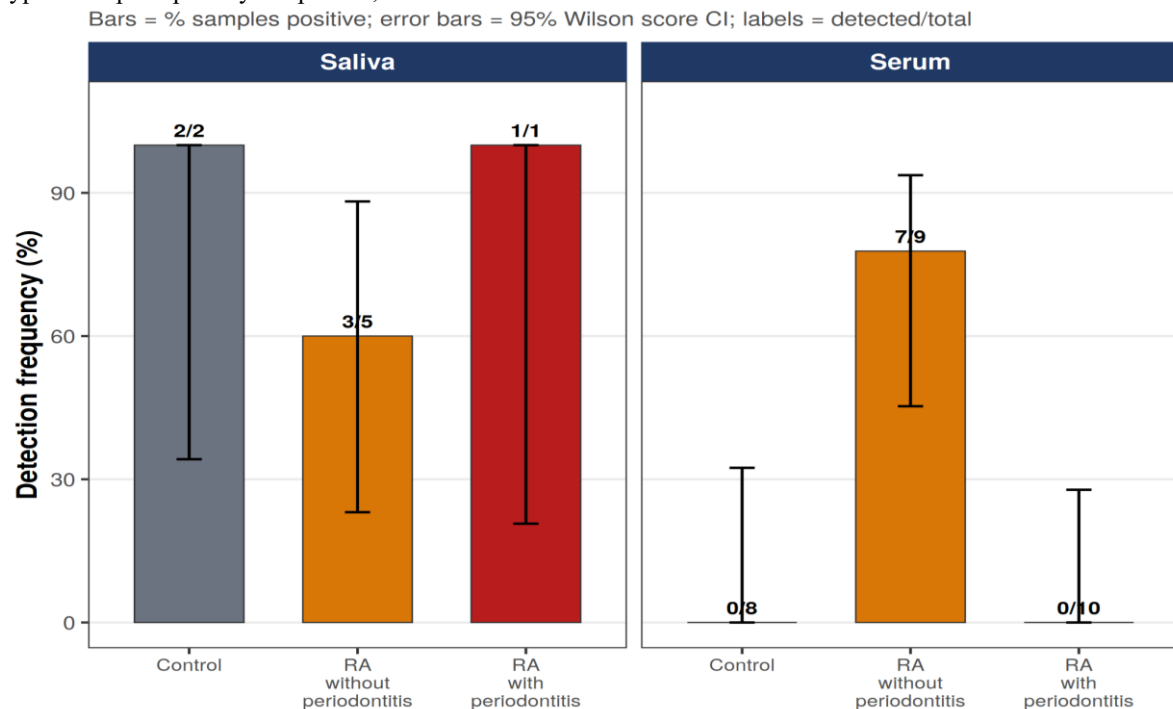


Figure 3. Histatin-1 detection frequency by clinical group, faceted by biofluid. Bars show % samples positive; error bars are 95% Wilson score confidence intervals; labels show detected/total samples.

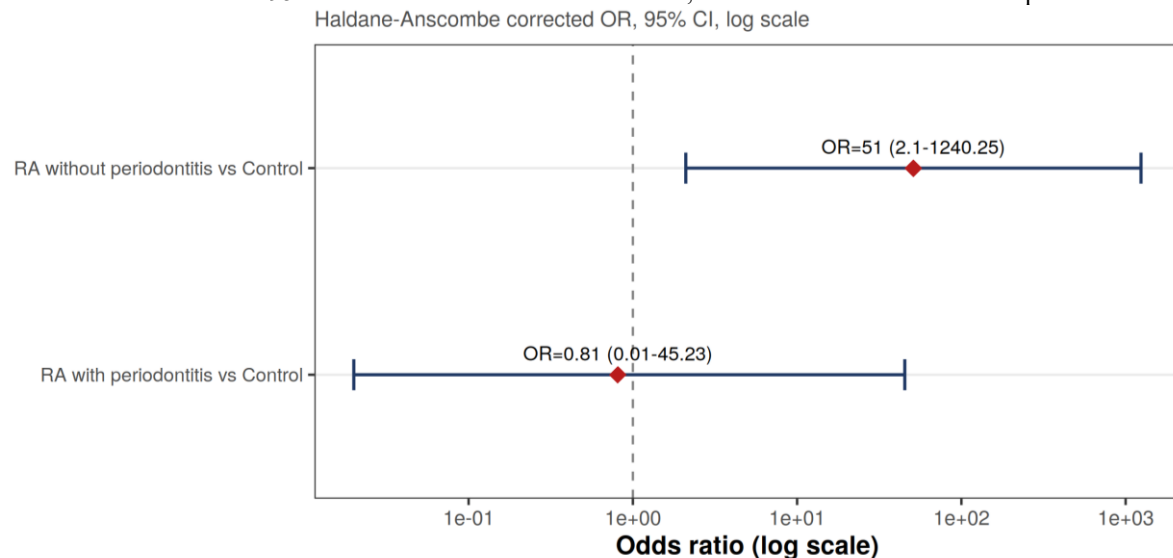


Figure 4. Odds of histatin-1 detection in RA-without-periodontitis and RA-with-periodontitis relative to Control (serum), with Haldane–Anscombe corrected 95% confidence intervals on a log scale.

Test	Statistic	95% CI / p-value
Fisher's exact, serum (3-group omnibus)	Cramér's V = 0.84	p = 4.95×10^{-5}
Fisher's exact, saliva (3-group omnibus)	Cramér's V = 0.45	p = 1.00 (descriptive)
OR, RA-without-periodontitis vs Control (serum)	OR = 51.0	95% CI 2.1–1240.3
OR, RA-with-periodontitis vs Control (serum)	OR = 0.81	95% CI 0.01–45.2
Logistic trend, ordinal severity (serum)	$\beta = -0.15$	p = 0.780 (ns)

Table 3. Statistics table: serum histatin-1 detection versus clinical group. OR: odds ratio, Haldane–Anscombe corrected. ns: not significant.

5. Perspective: Saliva as a Primary, Not Secondary, Sampling Matrix

A recurring assumption in RA biomarker research is that saliva is, at best, a convenient proxy for blood. Histatin-1's biology argues against that framing: it is not a systemic protein that leaks into saliva, but a salivary protein whose appearance in serum is the exception requiring explanation. That distinction matters for how the present pilot findings should be read. The saliva data here are consistent, if weakly so given the small samples, with the existing periodontal literature's suggestion that whole-saliva histatin is not strongly severity-graded [6] — our RA-without-periodontitis saliva detection (60%) was, if anything, numerically lower than Control (100%), which does not replicate the (non-significant) increase reported by Bhadbhade and colleagues. Given that both comparisons rest on small samples with wide, overlapping confidence intervals, we read this as two independent pilot datasets each consistent with a weak or condition-independent salivary histatin-1 signal, rather than as genuinely contradictory findings — but it is a caution against extrapolating confidently from either study alone. The serum picture is where this dataset adds something more specific: an unexplained, statistically strong elevation in RA-without-periodontitis that is absent in both Control and RA-with-periodontitis. As with the two companion markers screened from this dataset, we cannot distinguish, on the present evidence, between a genuine biological signal specific to RA without concurrent periodontal disease, a technical artefact of mass spectrometric detection in a more complex RA-with-periodontitis matrix, or an unmeasured clinical difference between the two RA subgroups. Resolving this will require quantitative, isoform-specific measurement of histatin-1 by ELISA or targeted mass spectrometry in matched serum and saliva from adequately sized RA-with-periodontitis and RA-without-periodontitis cohorts, ideally paired with a functional readout of mucosal or periodontal barrier permeability.

6. CONCLUSION

Histatin-1's near-exclusive salivary origin, combined with its established antimicrobial and pro-angiogenic wound-healing functions, makes it a biologically distinctive candidate marker for the RA–periodontitis axis — one whose appearance outside saliva is itself informative. A pilot Q-TOF peptidomic screen finds histatin-1 strongly and specifically elevated in RA-without-periodontitis serum, while its salivary profile remains close to the weak, non-significant pattern reported in the existing periodontal literature. We present this as motivation for treating saliva as a primary sampling matrix in RA biomarker discovery, and as a specific, falsifiable question — whether serum histatin-1 reflects oral-to-systemic barrier spillover — for future quantitative studies to test.

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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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