

SALIVARY AND SERUM CYSTATINS (S/SN/C) AS SENTINELS OF THE PROTEASE–ANTIPROTEASE IMBALANCE LINKING PERIODONTITIS TO RHEUMATOID ARTHRITIS: A PERSPECTIVE FROM PILOT Q-TOF PEPTIDOMIC SCREENING

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ABSTRACT

Periodontitis and rheumatoid arthritis (RA) are both, at core, diseases of unchecked proteolysis: cysteine cathepsins degrade collagen, cartilage, and connective tissue in the periodontium and the synovium alike, held in check physiologically by an endogenous cystatin superfamily. We revisit the cystatin–cathepsin balance as a candidate convergent 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 for association with clinical group, the cystatin superfamily (cystatin S, SN, and C) was the single strongest discriminator in serum, with complete separation between groups (Fisher's exact $p = 2.13 \times 10^{-7}$, Cramér's $V = 1.00$): detection rose from 0% in Control to 100% in RA-without-periodontitis (odds ratio 323, 95% CI 5.75–18,131.4), then fell back to 0% in RA-with-periodontitis. We situate this striking but small-sample pattern against an established, and sometimes contradictory, literature on salivary cystatins in periodontitis and on cystatin C as a marker of RA-associated cardiovascular and renal risk. Rather than asserting a validated biomarker, we use the pilot data to argue that the cystatin superfamily deserves systematic, isoform-resolved, quantitatively calibrated study across both compartments of the RA–periodontitis axis — and we are explicit about why a nine-sample pilot group cannot settle that question on its own.

KEYWORDS: cathepsin; cystatin; periodontitis; protease–antiprotease balance; proteomics; rheumatoid arthritis; 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]. A substantial body of evidence now links RA bidirectionally to periodontitis, with both diseases converging on innate-immune activation and progressive, protease-driven destruction of connective tissue — cartilage and bone in the joint, the periodontal ligament and alveolar bone in the mouth [2]. Much of the biomarker literature on this axis has focused on what proteolysis produces — citrullinated peptides, degraded collagen fragments, acute-phase reactants. Comparatively less attention has gone to what physiologically restrains it: the endogenous cystatin superfamily of cysteine protease inhibitors, whose principal targets, the cysteine cathepsins, are directly implicated in both periodontal and synovial tissue breakdown.

This perspective considers the cystatin superfamily — specifically the salivary S-type cystatins (cystatin S and cystatin SN) and the more broadly distributed cystatin C — as a candidate convergent marker of the protease–antiprotease imbalance that underlies both diseases. We do so alongside a pilot untargeted Q-TOF peptidomic screen of serum and saliva across Control, RA-without-periodontitis, and RA-with-periodontitis groups, in which cystatin-family peptides emerged, by a clear margin, as the most statistically differentiating signal among sixteen candidate protein/peptide families screened. We treat that result as an invitation to a more rigorous study, not as a conclusion in itself.

2. The Cystatin Superfamily: Guardians of the Protease Balance

Human saliva contains a distinct family of type-2 (S-type) cystatins — cystatin S, cystatin SA, and cystatin SN — secreted by the submandibular and sublingual glands. Functional characterisation of these purified peptides showed that cystatin SN potently inhibits the lysosomal cathepsins B, H, and L, and cystatin SA inhibits cathepsin L, while cystatin S itself binds calcium and contributes to enamel pellicle formation rather than acting principally

as a protease inhibitor — the three S-type cystatins are functionally distinct despite their shared nomenclature [3]. Cathepsins B, K, L, and S are, in turn, directly implicated in the pathophysiology of joint destruction in RA: acting at the acidic pH of the inflamed synovial and periarticular microenvironment, where matrix metalloproteinases are less active, cysteine cathepsins degrade aggrecan and collagen and drive osteoclast-mediated bone resorption, placing the cystatins that restrain them mechanistically upstream of structural joint damage [4]. Cystatin C, structurally related but more widely distributed than the S-type cystatins, has an independent evidence base in RA that extends beyond periodontal tissue. Serum cystatin C concentrations are higher in RA patients than in matched controls, correlate positively with erythrocyte sedimentation rate, C-reactive protein, and the 28-joint Disease Activity Score, and are associated with the Framingham cardiovascular risk score — findings interpreted as reflecting both systemic inflammation and subclinical renal impairment in RA [5]. A subsequent study specifically linked serum cystatin C, but not cathepsin S, to subclinical carotid atherosclerosis in RA, reinforcing cystatin C's candidacy as a marker that bridges joint inflammation and the excess cardiovascular risk that characterises the disease [6].

3. Salivary Cystatins at the Periodontal Frontline

Within periodontal research, the cystatin superfamily has been investigated as a candidate biomarker with a genuinely mixed evidentiary record: some studies report salivary cystatins depleted in periodontitis, others report them elevated, and the balance of evidence has been described as controversial even within a single review. The most methodologically rigorous recent contribution used untargeted proteomics and MALDI-TOF mass spectrometry to identify cystatin SN (CST1) as markedly depleted in patients with severe periodontitis compared with periodontally healthy controls, with expression restored towards healthy levels after periodontal treatment — a pattern consistent with progressive consumption or proteolytic degradation of the inhibitor as periodontal disease advances [7]. Because cystatin SN's principal targets — cathepsins B, H, and L — are themselves implicated in periodontal collagen breakdown, its depletion in active disease is mechanistically coherent: as the inhibitor falls, the proteases it restrains are proportionally freer to act, a self-reinforcing cycle rather than a passive biomarker signal [3,7].

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 a companion perspective on calprotectin arising from this project: serum and saliva samples from Control, RA-without-periodontitis, and RA-with-periodontitis groups (Table 1), with detection of a cystatin-family peptide (cystatin S, SN, or C, pooled at the superfamily level because the recorded mass-to-charge values did not permit confident isoform-level discrimination) 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; the cystatin superfamily ranked first by statistical strength.

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 cystatin-superfamily detection frequency. In serum, cystatin-family peptides were detected in every RA-without-periodontitis sample (9/9, 100%; 95% CI 70.1–100%) but in no Control sample (0/8) and no RA-with-periodontitis sample (0/10) — complete separation between the RA-without-periodontitis group and both other groups, yielding the strongest omnibus association of any marker screened from this dataset (Fisher's exact $p = 2.13 \times 10^{-7}$, Cramér's $V = 1.00$; Table 3). The odds ratio for RA-without-periodontitis versus Control, calculated with Haldane–Anscombe continuity correction, was 323 (95% CI 5.75–18,131.4); saliva showed a directionally consistent but far less extreme gradient (Control 50%, RA-without-periodontitis 80%, RA-with-periodontitis 100%, all descriptive given $n \leq 5$ per group).

Biofluid	Group	Detected / n	Frequency % (95% CI)
Serum	Control	0 / 8	0.0 (0.0–32.4)
Serum	RA without periodontitis	9 / 9	100.0 (70.1–100.0)
Serum	RA with periodontitis	0 / 10	0.0 (0.0–27.8)
Saliva	Control	1 / 2	50.0 (9.5–90.5)
Saliva	RA without periodontitis	4 / 5	80.0 (37.6–96.4)
Saliva	RA with periodontitis	1 / 1	100.0 (20.7–100.0)

Table 2. Cystatin superfamily (S/SN/C) detection frequency by biofluid and clinical group. CI: Wilson score 95% confidence interval.

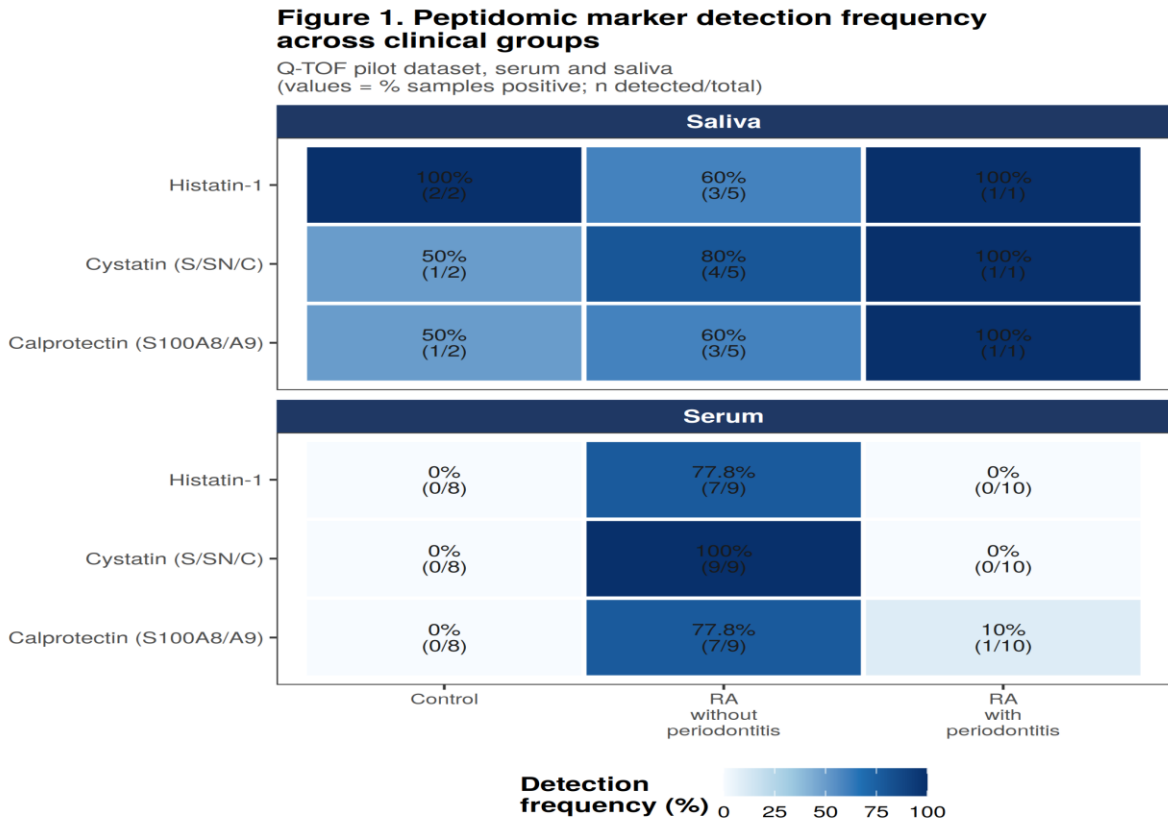


Figure 1. Detection frequency (%) of the three candidate markers screened from the pilot Q-TOF dataset. The cystatin superfamily (middle row of each panel) shows the sharpest serum contrast of the three: absolute presence in RA-without-periodontitis against absolute absence in both Control and RA-with-periodontitis.

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

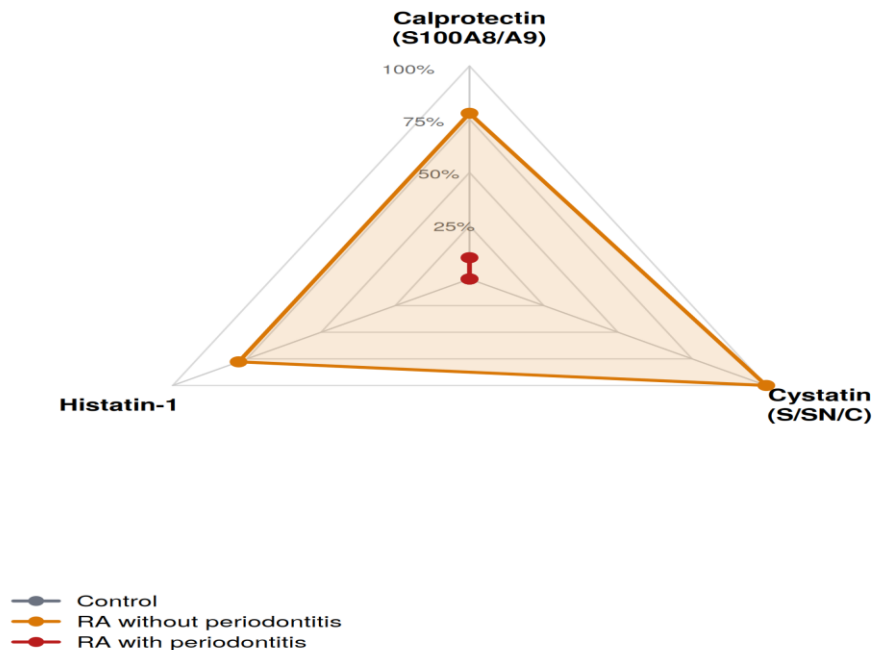


Figure 2. Serum detection-frequency profile of the three screened markers by clinical group. The RA-without-periodontitis vertex (amber) reaches 100% on the cystatin axis, the widest excursion of any marker-group combination in the panel.

A Cramér's V of 1.00 denotes perfect statistical association within this sample — every serum sample can be correctly classified as RA-without-periodontitis or not, purely from cystatin-family detection status. We flag this explicitly as a small-sample phenomenon rather than evidence of a biologically deterministic switch: with group sizes of 8–10, a single differently classified sample would materially change the statistic, and complete separation of this kind is a recognised artefact of small, unreplicated pilot data rather than a hallmark of robust biomarker performance. The more informative observation, in our view, is not the size of the statistic but its direction and its asymmetry: cystatin-family detection was effectively absent in RA-with-periodontitis as well as in Control, a pattern that would be consistent with the depletion mechanism reported for salivary cystatin SN in periodontitis [7] — if periodontal inflammation consumes or suppresses the inhibitor regardless of concurrent RA status, that alone could explain why the RA-with-periodontitis group resembles Control rather than RA-without-periodontitis on this axis.

Figure 3. Cystatin (S/SN/C) detection frequency by clinical group

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

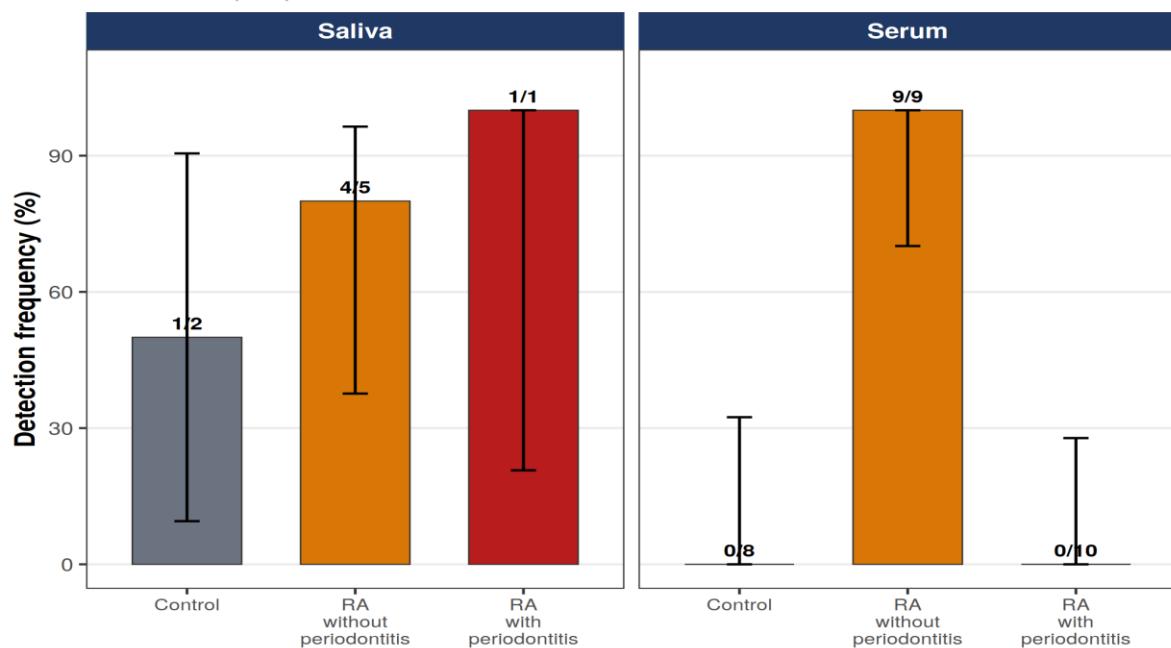


Figure 3. Cystatin superfamily (S/SN/C) detection frequency by clinical group, faceted by biofluid. Bars show % samples positive; error bars are 95% Wilson score confidence intervals; labels show detected/total samples.

Figure 4. Cystatin (S/SN/C)—odds of detection vs Control (serum)

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

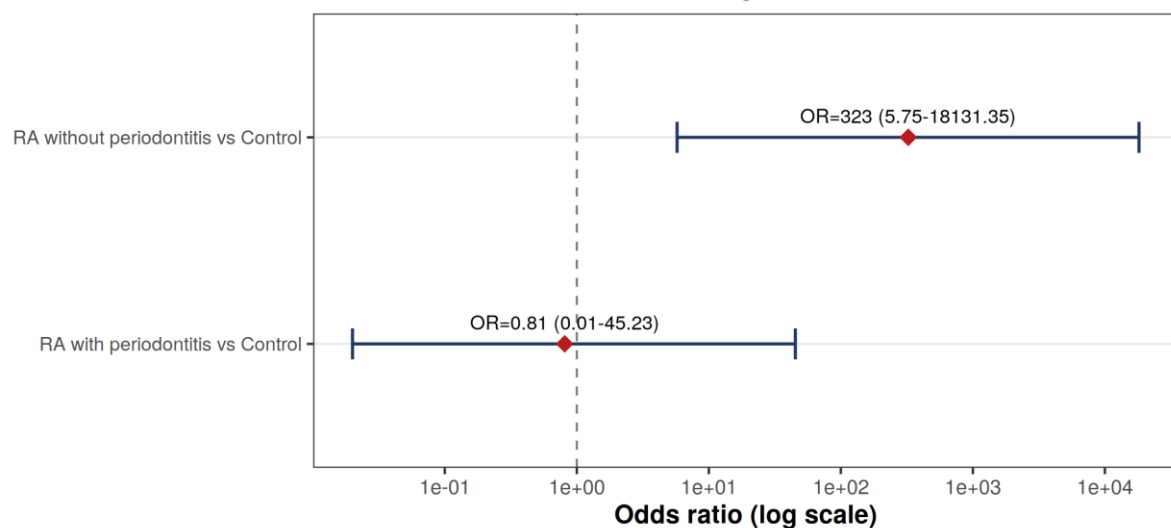


Figure 4. Odds of cystatin-family detection in RA-without-periodontitis and RA-with-periodontitis relative to Control (serum), with Haldane–Anscombe corrected 95% confidence intervals on a log scale. Note the markedly wider interval for the RA-without-periodontitis comparison, reflecting complete separation in a small sample.

Test	Statistic	95% CI / p-value
Fisher's exact, serum (3-group omnibus)	Cramér's V = 1.00	p = 2.13×10^{-7}
Fisher's exact, saliva (3-group omnibus)	Cramér's V = 0.37	p = 1.00 (descriptive)
OR, RA-without-periodontitis vs Control (serum)	OR = 323.0	95% CI 5.75–18,131.4
OR, RA-with-periodontitis vs Control (serum)	OR = 0.81	95% CI 0.01–45.2
Logistic trend, ordinal severity (serum)	$\beta = -0.17$	p = 0.738 (ns)

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

5. Perspective: Reading a Small-Sample ‘Perfect’ Signal Honestly

The pilot dataset's headline statistic — complete separation of RA-without-periodontitis from both other groups — is exactly the kind of result that should invite scrutiny rather than celebration. Sample sizes are small (8–10 per serum group, 1–5 per saliva group), the assay records qualitative detection rather than calibrated concentration, the recorded mass-to-charge values do not allow confident discrimination between cystatin S, SN, and C, and no clinical covariates (ACPA/RF status, DAS28, periodontal staging, medication exposure) were available for adjustment. We also note, without over-interpreting it, that this same non-monotonic pattern — a strong RA-without-periodontitis signal that attenuates in RA-with-periodontitis — recurred independently across all three markers screened from this dataset (cystatin, calprotectin, and histatin-1), which argues against a marker-specific artefact but does not rule out a shared, dataset-level explanation such as a systematic difference in how the RA-with-periodontitis subgroup was sampled or processed.

What the pilot data do contribute is a sharper version of an existing open question in the periodontal-cystatin literature: does active periodontal inflammation deplete the cystatin superfamily regardless of coexisting systemic disease, or does RA-associated systemic inflammation modulate that relationship? The salivary CST1-depletion evidence in periodontitis alone [7] and the serum cystatin C elevation evidence in RA alone [5,6] point in opposite directions when periodontitis and RA occur separately; a dataset in which both conditions co-occur, and cystatin detection falls to Control-like levels, hints that the periodontitis-associated depletion mechanism may dominate over the RA-associated elevation mechanism when both are present — a specific, falsifiable hypothesis that isoform-resolved, quantitative, adequately powered sampling could test directly.

A validated dual-compartment cystatin panel would have practical appeal precisely because the underlying biology — restraint of the same cysteine cathepsins implicated in both periodontal and joint destruction — offers a mechanistic rationale that a purely correlative biomarker would lack [3,4]. Isoform-specific immunoassay or targeted mass spectrometry (distinguishing cystatin S, SN, and C rather than pooling them, as the present untargeted screen was obliged to do) would be a necessary next step, alongside matched sampling of RA-with-periodontitis and RA-without-periodontitis cohorts of adequate size.

6. CONCLUSION

The cystatin superfamily sits mechanistically upstream of the protease-driven tissue destruction shared by periodontitis and RA, with independent evidence implicating cystatin C in RA disease activity and cardiovascular risk, and cystatin SN in periodontal tissue preservation. A pilot Q-TOF peptidomic screen identifies cystatin-family detection as the single strongest discriminator among sixteen candidate markers, with a pattern — elevated in RA-without-periodontitis, silent in RA-with-periodontitis — that generates a specific, testable hypothesis about how coexisting periodontal disease might modulate this signal. We present it as exactly that: a hypothesis, sharpened by pilot data, awaiting isoform-resolved and adequately powered confirmation.

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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.

REFERENCES

1. GBD 2021 Rheumatoid Arthritis Collaborators. Global, regional, and national burden of rheumatoid arthritis, 1990–2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. *Lancet Rheumatol.* 2023;5(10):e594–e610.
2. Malcolm J, Culshaw S. Aberrant immunity in the oral cavity—a link with rheumatoid arthritis? *Front Oral Health.* 2024;5:1430886.
3. Baron AC, DeCarlo AA, Featherstone JDB. Functional aspects of the human salivary cystatins in the oral environment. *Oral Dis.* 1999;5(3):234–240.
4. Behl T, Chadha S, Sehgal A, Singh S, Sharma N, Kaur R, et al. Exploring the role of cathepsin in rheumatoid arthritis. *Saudi J Biol Sci.* 2022;29(1):402–410.
5. Lertnawapan R, Bian A, Rho YH, Kawai VK, Raggi P, Oeser A, et al. Cystatin C, renal function, and atherosclerosis in rheumatoid arthritis. *J Rheumatol.* 2011;38(11):2297–2300.
6. Tejera-Segura B, de Vera-González A, López-Mejías R, González-Gay MA, Ferraz-Amaro I. Serum cathepsin S and cystatin C: relationship to subclinical carotid atherosclerosis in rheumatoid arthritis. *Clin Exp Rheumatol.* 2016;34(2):230–235.
7. Romano F, Franco F, Corana M, Abbadessa G, Di Scipio F, Pergolizzi B, et al. Cystatin SN (CST1) as a novel salivary biomarker of periodontitis. *Int J Mol Sci.* 2023;24(18):13834.