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The role of azurocidin and its implications in periodontal and peri-implant disease: A systematic review

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ABSTRACT

Objectives: This systematic review aimed to explore the role of Azurocidin (Azu) in the pathogenesis of periodontal and peri-implant disease and its potential use as an inflammatory biomarker.
Materials and methods: Four electronic databases were used for study identification: PubMed, Google Scholar, ScienceDirect, and Scopus from Oct 10, 1991 to Jul 15, 2024. Study selection and data extraction were performed in a blinded and independent manner. The Joanna Briggs Institute (JBI) tool was used to assess the quality of cross-sectional articles, and the Newcastle-Ottawa scale was used to assess cohort studies.
Results: Out of 222 identified articles, nine studies met the inclusion criteria. These studies included 462 participants: 156 with healthy teeth and implants and 306 with periodontal conditions such as gingivitis, periodontitis, apical periodontitis, peri-implant mucositis, and peri-implantitis. A total of 1313 samples were analyzed (163 saliva, 118 PICF, 1003 GCF, 11 gingival tissue, and 18 infected root canals). ELISA was the most common method for azurocidin analysis (66.6 %), followed by LC-MS/MS (33.3 %), nLC-MS/MS (11.1 %), and Western Blot (11.1 %). Azu levels were consistently elevated in individuals with periodontitis compared to periodontally healthy subjects.
Conclusions: Azu may contribute to the inflammatory processes in periodontal and peri-implant diseases. Although elevated levels are observed in periodontitis, its diagnostic value remains unclear due to limited and heterogeneous data.

1. Introduction

Periodontal disease (PD) continues to be a major public health problem that impacts the oral and systemic health of the world's population (Lin et al., 2023). PD in dentate individuals includes conditions such as gingivitis, periodontitis, and apical periodontitis, while in subjects with dental implants (DI), it includes peri-implant mucositis and peri-implantitis (Heitz-Mayfield et al., 2024a; b; Ye et al., 2023). The etiopathogenesis of PD is a multifactorial process that begins with a picture of inflammation around the teeth and/or DI, which can progress

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in the absence of adequate treatment to a destructive condition with a significant impact in terms of oral morbidity, tooth loss, or DI (Rakhewar et al., 2023) (Fig. 1).

The sequence starts with the formation of a biofilm on the tooth or DI surface, specifically associated with the gingival sulcus. This biofilm is composed of a community of microorganisms, mainly anaerobic Gram-negative bacteria such as *Aggregatibacter Actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, whose virulence factors trigger an inflammatory immune response in the host (Didilescu et al., 2024). This first polymicrobial challenge results in the release of proinflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) and prostaglandins, together with proteolytic enzymes such as matrix metalloproteinases (MMPs) and some other chemokines such as fractalkine (CX3CL1) and monocyte chemoattractant protein (MCP-1/CCL2). These molecules regulate different functions, including I) increased expression of other proinflammatory cytokines (IL-17A, IL-18, IL-33) that mediate osteoclastogenesis, II) vasodilation, III) destruction of the extracellular matrix, and IV) migration of neutrophils (first line of defense) to the site of the infectious process (Liu et al., 2024). When neutrophils reach periodontal tissues, they become activated and phagocytize microbes, as well as promote the release of extracellular traps and lysosomal enzymes, including azurophil/primary granule proteins such as azurocidin (Azu).

The AZU gene is located on the short arm of chromosome 19, region 1, band 3, subband 3 (19p13.3). In relation to its structure, it has its promoter, 5 exons, 4 introns, and 5′UTR and 3′UTR regions with a total of 907 nucleotides. The full-length mRNA initiates translation in exon 1 and ends in exon 5. There is an isoform of AZU (Azurocidin protein 2024; Azurocidin gen 2024) (https://www.uniprot.org; https://www.ncbi.nlm.nih.gov/gene/566). Regarding its processing, the precursor protein of Azu (prepro-azurocidin) consists of 251 amino acids (aa), with a molecular weight of 26.88 kDa. After removal of the signal peptide (19 aa), a 232 aa protein (pro-azurocidin) is formed. The zymogen is then transferred from the endoplasmic reticulum to the Golgi apparatus via vesicles, where the pro-azurocidin is converted to Azu (removal of 10 aa by the action of peptidases) thus the mature form of Azu is characterized by 222 aa (24 kDa), 3 glycosylation units, 1 chloride ion, 15 precipitating ethanol molecules, 323 water molecules and 4 disulfide bridges (Almeida et al., 1991; Gullberg et al., 1999; Karlsen et al., 1998; McGuire et al., 1993; Morgan et al., 1991; Pohl et al., 1990). Azu is a protein that has antimicrobial activity, enhances vascular permeability, and thereby acts as a chemokine by recruiting monocytes circulating in peripheral blood to sites of inflammation, so it is also implicated in the pathogenesis of PD (Zhang et al., 2022).

The diagnosis of PD continues to be clinical and radiographic; however, the identification of biomarkers in PD that allow early and accurate detection of either of these conditions is critical to improving the prognosis of the teeth or ID involved (Gürsoy et al., 2024). Biological samples that have been used for the analysis of inflammatory markers are at the systemic level serum and blood plasma, while at the local level, non-invasive access samples such as saliva, gingival tissue, peri-implant crevicular fluid (PICF), gingival crevicular fluid (GCF) and samples from infected root canals have been collected (Fadli et al., 2024).

High-throughput proteomic analysis such as liquid chromatography with mass spectrometry (LC-MS/MS) and nano-scale liquid chromatography with mass spectrometry (nLC-ESI-MS/MS), together with immunoassays such as western blot and enzyme-linked immunosorbent assay (ELISA) have the potential to identify molecules that could be used as complementary tools to clinical and radiographic parameters in the follow-up of PD (Tsuchida & Nakayama, 2022).

In relation to gingivitis and periodontitis, studies have shown that the presence of both conditions is associated with elevated levels of IL-23/IL-17 and CX3CL1/CX3CR1 axes, as well as proinflammatory cytokines such as IL-18 and IL-33 (Alarcón-Sánchez et al., 2024a; b; c; d). In relation to apical periodontitis, a recent systematic review found that the presence of this condition is associated with certain inflammatory markers such as IL-1, IL-2, and IL-6, oxidative markers such as nitric oxide and superoxide anions, as well as IgG and IgM immunoglobulins (Matos-Sousa et al., 2024). In relation to peri-implant mucositis and peri-implantitis, studies have shown that the presence of such conditions is associated with elevated levels of cytokines such as IL-1β, IL-6, TNF-α and MMPs, specifically MMP-8 (Ghassib et al., 2019; Lumbikananda et al., 2024) (Fig. 2). In the case of peri-implant conditions, the identification of biomarkers is equally critical due to the similar yet distinct immune-inflammatory responses. Peri-implant mucositis and peri-implantitis involve microbial dysbiosis and neutrophil-driven inflammation that may resemble periodontitis, but with differences in tissue architecture and healing response (Ghassib et al., 2019; Lumbikananda et al., 2024). Therefore, evaluating biomarkers such as azurocidin in peri-implant crevicular fluid (PICF) is crucial for understanding their diagnostic potential in implant-related diseases.

Up-regulation of these proinflammatory mediators can trigger neutrophil degranulation and, with it the release of Azu,

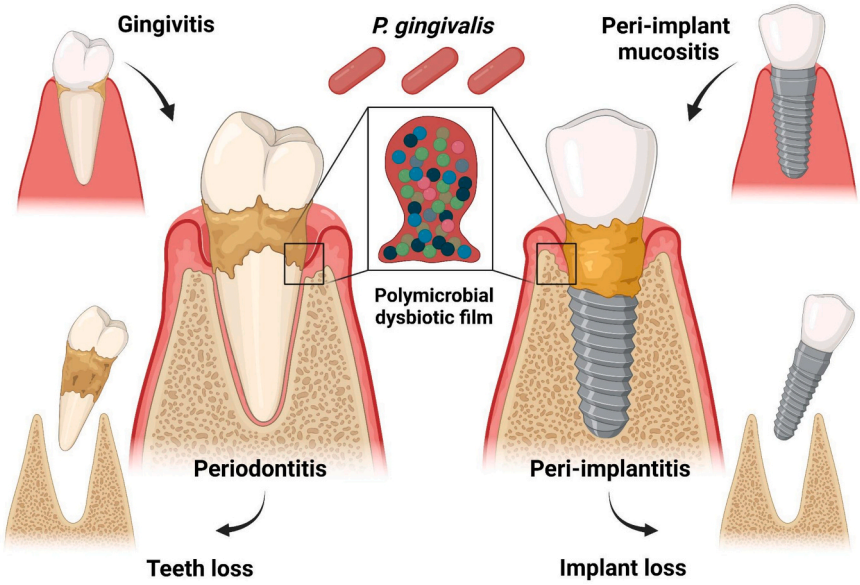

Fig. 1. Etiopathogenesis of periodontal disease.

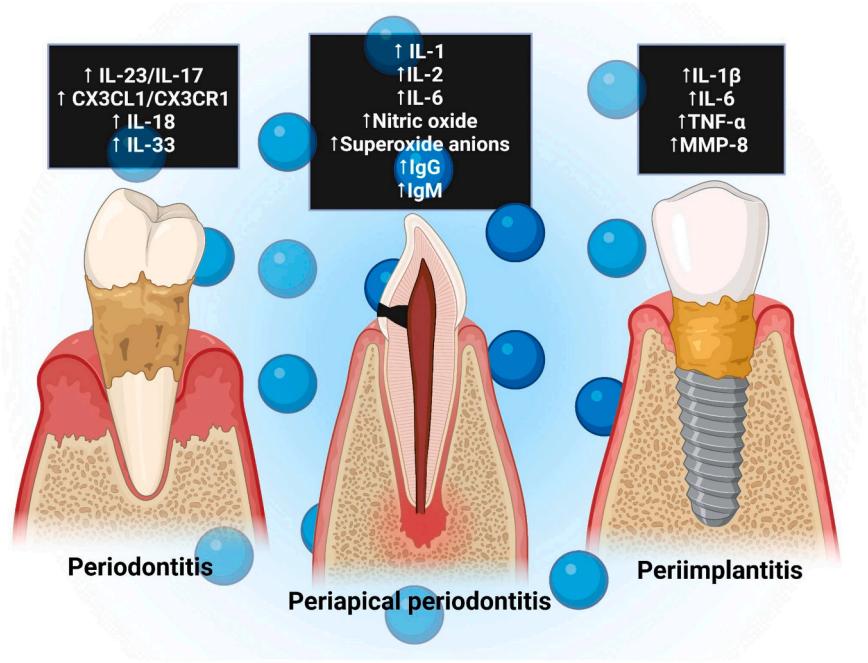

Fig. 2. Biomarkers present in the different biofluids of subjects with periodontal disease.

myeloperoxidase (MPO), neutrophil elastase (NE), cathepsin G, proteinase 3, alpha and beta-defensins, lysozymes, proteolytic enzymes, bactericidal/permeability-increasing protein and other proteins from secondary, tertiary and secretory granules that could intensify the inflammatory chain reaction linked to periodontal tissue destruction (Othman et al., 2022).

Despite recent findings, there is currently no systematic review that analyzes and summarizes the results of clinical studies evaluating the levels of Azu and other azurophilic granule proteins in the different biofluids of PD subjects. Therefore, the present study's main purpose was to explore the role of Azu in the pathogenesis of periodontal and peri-implant disease and its potential use as an inflammatory biomarker.

2. Materials and methods

This project followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Page et al., 2021). Its registration can be accessed at https://doi.org/10.17605/OSF.IO/NFTX4.

2.1. PECOS system

- P [Population]: Subjects with PD (Gingivitis, periodontitis, apical periodontitis, peri-implant mucositis, and peri-implantitis).
- E [Exposure]: Azu expression in saliva, GCF, PICF, gingival tissue, and infected root canals.
- C [Comparator]: Healthy subjects
- O [Outcomes]: Differences in Azu expression levels according to periodontal condition.
- S [Studies]: Observational clinical studies of cross-sectional and cohort type.

2.2. Research question

What is the role of Azu in the pathogenesis of PD and its potential use as an inflammatory biomarker?

2.3. Eligibility criteria

Original cross-sectional and cohort studies were included that examined AZU expression using methodologies such as ELISA, Western Blot, LC/MS-MS, and nLC-ESI-MS/MS in various biological samples such as plasma, serum, saliva, gingival tissue, PICF, and GCF from adults with gingivitis, periodontitis, apical periodontitis, mucositis and/or peri-implantitis. These subjects comprised the PD group. In addition, these individuals should not have received pharmacological treatments (antibiotics, anti-inflammatory drugs, or immunosuppressants) or non-surgical periodontal treatments (prophylaxis, scaling, and root planing) for at least three months prior to the study. On the other hand, the control group consisted of periodontally and systemically healthy individuals. In both groups, studies had to include data on periodontal clinical parameters such as plaque index, probing depth, clinical attachment level, and radiographic bone loss for disease diagnosis. Research measuring Azu levels in subjects with systemic diseases, alcoholism, and pregnancy, as well as letters to the editor, conference abstracts, theses, review articles, meta-analyses, book chapters, and short communications, were excluded.

2.4. Information sources

For this review, the following electronic databases were consulted: PubMed, Google Scholar, ScienceDirect, and Scopus from Oct 10, 1991 to Jul 15, 2024.

2.5. Search strategy

The search strategy consisted of using medical subject headings (MeSH): "AZU1 protein, human," "heparin-binding protein receptor," "periodontal diseases," "gingivitis," "gingivitis, necrotizing ulcerative," "periodontitis," "chronic periodontitis," "aggressive periodontitis," "periapical periodontitis," "mucositis," and "peri-implantitis," in combination with the use of Boolean operators' AND' and" OR." Finally, with the main purpose of enriching this first process, a manual search was performed in the following Journals: *Journal of Periodontal Research, International Journal of Periodontics & Restorative Dentistry, Journal of Periodontal and Implant Science, Journal of Periodontology, Journal of*

Clinical Periodontology, and *Periodontology 2000*. In addition, the reference lists of existing studies were reviewed to identify any additional articles that had not been detected by the initial search strategy (Table 1).

2.6. Study selection

The study selection process was carried out independently by M.A.A.S and A.H. To ensure consistency in the selection process, a calibration exercise was conducted using a random sample of 10 % of the records. The inter-reviewer agreement was assessed using Cohen's kappa statistic, which revealed a substantial level of agreement (κ=0.91), confirming the reliability of the screening criteria. The procedure involved reading titles and abstracts, excluding duplicates and irrelevant topics, and evaluating articles according to pre-defined inclusion and exclusion criteria. Subsequently, a complete reading of potentially eligible articles was performed. Any inconsistencies that arose during the selection process were resolved by consulting two additional investigators (R.R.M and S.M.L.M). All records were managed and extracted using EndNote V.9 software.

2.7. Data collection

C.H.M.B and M.A.A.S independently extracted the following data: first author's name and year of publication, country, gender, age, number of cases (including teeth with gingivitis, periodontitis, and apical periodontitis, as well as implants with mucositis and/or peri-implantitis), number of controls (healthy teeth and/or implants), total population, number of samples analyzed, periodontal condition, type of biological sample analyzed, biomarker evaluated, methodology used, numerical values obtained by ELISA test, and main results. All variables were organized into pre-defined tables using Microsoft Word software.

2.8. Quality evaluation and applicability

The quality of the analytical cross-sectional studies was evaluated in blind using the Joanna Brigs Institute (JBI) tool (Moola et al., 2020). The checklist consists of eight questions to assess responses such as "No," "Yes," "Not applicable," and "Unclear." A score > 7 is considered high quality (León-Figueroa et al., 2024 Aug 9). On the other hand, cohort studies were evaluated using the Newcastle-Ottawa scale (Stang et al., 2010). In this case, a score > 6 is considered good quality, whereas the perfect score would be 9 (Xiao et al., 2024). The analyses were performed independently by two authors (S.M.L.M and M.A.A.S); in case of discrepancies, a third author (A.H) intervened and resolved by consensus with the whole group of investigators.

Table 1
The full search strategy used in PubMed, Google Scholar, ScienceDirect, and Scopus.

Database	Search Strategy
PubMed	(("AZU1 protein, human" [Supplementary Concept]) AND "Biomarkers"[Mesh]) AND "Periodontal Diseases"[Mesh]
Google Scholar	TITLE-ABS-KEY (Azurocidin AND Gingival Crevicular Fluid OR Saliva OR Serum OR Plasma OR Gingival Tissue OR Periimplant Crevicular Fluid AND Periodontal Disease OR Gingivitis OR Periodontitis OR Apical Periodontitis OR Mucositis OR Periimplantitis)
ScienceDirect	TITLE-ABS-KEY (Azurocidin AND biomarkers AND periodontal disease)
Scopus	TITLE-ABS-KEY (Azurocidin AND biomarkers AND periodontal disease)

3. Results

3.1. Study selection

Initially, 222 articles were found in four electronic databases, including PubMed ($n = 4$), Google Scholar ($n = 195$ articles), ScienceDirect ($n = 15$), and Scopus ($n = 8$). In the manual search, no additional articles of interest related to the main topic of this research were found. In the identification phase, 22 duplicates were discarded. Subsequently, in the screening phase and based on the title and abstract, 191 studies were excluded. After the full-text review of the remaining articles, nine were selected for qualitative analysis (Afacan & Atmaca İlhan, 2020; Choi et al., 2011 Jul 28; Guzman et al., 2018; Halstenbach et al., 2023; Jasim et al., 2024; Loureiro et al., 2021; Leppilahti et al., 2014; Nalmpantis et al., 2020; Xanthopoulou et al., 2024) and of these, two were used for meta-analysis (Fig. 3).

3.2. Quality assessment of observational studies

Of the cross-sectional studies, 57.1 % were of moderate quality (Loureiro et al., 2021; Nalmpantis et al., 2020; Leppilahti et al., 2014; Choi et al., 2011 Jul 28). The rest (42.9 %) were of high quality (Jasim et al., 2024; Xanthopoulou et al., 2024; Afacan & Atmaca İlhan, 2020). One hundred percent of the cohort studies were of good quality (Halstenbach et al., 2023; Guzman et al., 2018) (Tables 2 and 3).

3.3. Azurocidin in periodontal and peri-implant disease: Outcomes of clinical studies

This systematic review analyzed a total of 9 articles (Afacan & Atmaca İlhan, 2020; Choi et al., 2011 Jul 28; Guzman et al., 2018; Halstenbach et al., 2023; Jasim et al., 2024; Loureiro et al., 2021; Leppilahti et al., 2014; Nalmpantis et al., 2020; Xanthopoulou et al., 2024), of which 7 were cross-sectional studies (Afacan & Atmaca İlhan, 2020; Choi et al., 2011 Jul 28; Jasim et al., 2024; Loureiro et al., 2021; Leppilahti et al., 2014; Nalmpantis et al., 2020; Xanthopoulou et al., 2024) and 2 were cohort studies (Halstenbach et al., 2023; Guzman et al., 2018). Most of the articles (66.6 %) were published after 2020 (Afacan & Atmaca İlhan, 2020; Guzman et al., 2018; Halstenbach et al., 2023; Jasim et al., 2024; Loureiro et al., 2021; Leppilahti et al., 2014; Nalmpantis et al., 2020; Xanthopoulou et al., 2024). The oldest study was from 2011 (Choi et al., 2011 Jul 28), and the most recent was published in 2024 (Jasim et al., 2024; Xanthopoulou et al., 2024). The 9 studies were published in seven different countries (Afacan & Atmaca İlhan, 2020; Choi et al., 2011 Jul 28; Guzman et al., 2018; Halstenbach et al., 2023; Jasim et al., 2024; Loureiro et al., 2021; Leppilahti et al., 2014; Nalmpantis et al., 2020; Xanthopoulou et al., 2024). Three (33.3 %) studies were conducted in Greece (Guzman et al., 2018; Nalmpantis et al., 2020; Xanthopoulou et al., 2024), the rest (11.1 %) were conducted in Iraq (Jasim et al., 2024), Germany (Halstenbach et al., 2023), Brazil (Loureiro et al., 2021), Turkey (Afacan & Atmaca İlhan, 2020), Chile (Leppilahti et al., 2014) and Korea (Choi et al., 2011 Jul 28) (Table 4).

The number of subjects ranged from 6 to 59 in the included studies, with a total of 462 participants, of which 156 represented the control group (healthy teeth and implants), and 306 represented the exposure group (teeth and implants with gingivitis, periodontitis, apical periodontitis, peri-implant mucositis, and peri-implantitis). 24.5 % of the participants were male, the other 24.5 % were female, and in 51 % of the cases, the sex was not specified (Choi et al., 2011 Jul 28; Jasim et al., 2024; Loureiro et al., 2021; Xanthopoulou et al., 2024). The age range of the subjects varied from 37.2 to 85 years, with a mean age ± standard deviation of 44.9 ± 5.9 years. A total of 1313 samples were collected in the included studies, of which 163 were saliva, 118 were PICF, 1003 were GCF, 11 were gingival tissue, and 18 were infected root canals (Table 4). The most commonly used method of analysis to determine

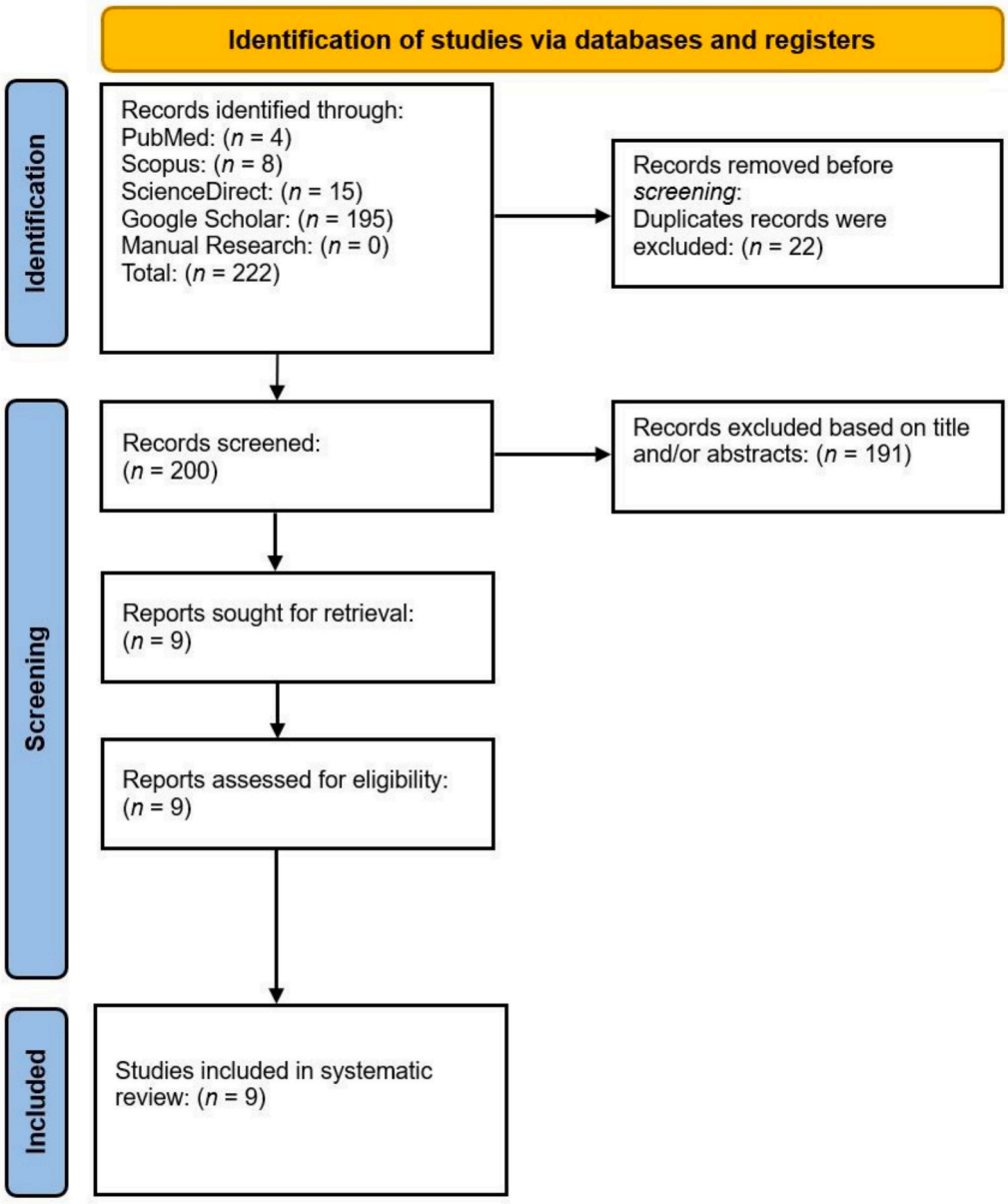

Fig. 3. PRISMA flow diagram. Abbreviations: PRISMA Preferred Reporting Items for Systematic and Meta-Analyses.

Azu expression was ELISA (66.6 %), followed by LC-MS/MS (33.3 %), nLC-MS/MS (11.1 %) and Western Blot (11.1 %) (Table 5).

In this systematic review, it was found that subjects with gingivitis and periodontitis presented increased levels of Azu in saliva, gingival tissue, and GCF compared to healthy individuals (Afacan & Atmaca İlhan, 2020; Choi et al., 2011 Jul 28; Guzman et al., 2018; Jasim et al., 2024; Leppilahti et al., 2014; Nalmpantis et al., 2020).

On the other hand, Azu expression was also found to be up-regulated in root canal samples from individuals with asymptomatic apical periodontitis compared to individuals with symptomatic apical periodontitis (Loureiro et al., 2021).

Regarding subjects with peri-implant mucositis and peri-implantitis, two studies evaluated Azu levels in PICF. (Xanthopoulou et al., 2024) found increased Azu levels in implants with peri-implant mucositis compared to healthy implants, although this difference was not statistically significant. (Halstenbach et al., 2023) reported a significant up-regulation of Azu in peri-implantitis compared to healthy implants, suggesting a potential role for Azu in the inflammatory response around dental implants. These findings indicate that while Azu is detectable in PICF, its diagnostic utility may vary depending on disease severity and methodology (Table 5).

4. Discussion

This systematic review aimed to evaluate the potential role of azurocidin (Azu) as an inflammatory biomarker in different forms of periodontal and peri-implant diseases. Given the heterogeneity in disease pathogenesis and tissue response, the findings are discussed separately for periodontal diseases, apical periodontitis, and peri-implant conditions. Azu, a cationic antimicrobial protein stored in azurophil granules of neutrophils, has been implicated in inflammatory processes and vascular permeability and is therefore of interest in oral infectious diseases (Choi et al., 2011 Jul 28; Othman et al., 2022; Halstenbach et al., 2023).

Initially, in the innate immune response, bacteria present in the dental biofilm interact with receptors on host cells, such as epithelial cells and gingival fibroblasts, through binding between pathogen-associated molecular patterns and pattern recognition receptors. This

Table 2
JBI assessment for cross-sectional studies.

Items	Authors / Reference						
	(Jasim et al., 2024)	(Xanthopoulou et al., 2024)	(Loureiro et al., 2021)	(Nalmpantis et al., 2020)	(Afacan & Atmaca İlhan, 2020)	(Leppilahti et al., 2014)	(Choi et al., 2011)
Were the criteria for inclusion in the sample clearly defined?	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Unclear**
Were the study subjects and the setting described in detail?	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Was the exposure measured in a valid and reliable way?	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Were objective, standard criteria used for measurement of the condition?	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Was confounding factors identified?	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**
Were strategies to ideal with confounding factors stated?	**No**	**No**	**No**	**No**	**No**	**No**	**No**
Were the outcomes measured in a valid and reliable way?	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Was appropriate statistical analysis used?	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Quality	**High**	**High**	**Moderate**	**Moderate**	**High**	**Moderate**	**Moderate**

Answers: Yes, No, Unclear and Not applicable.
Score: Low → 1–3; Moderate → 4–6; High → $\geq$ 7 [30]

Table 3
Newcastle-Ottawa assessment for cohort studies.

Study	Selection				Comparability		Results			Total Score
	S1	S2	S3	S4	C1	C2	R1	R2	R3	
(Halstenbach et al., 2023)	⋆	⋆	⋆	⋆	⋆	0	⋆	⋆	⋆	**8 (Good)**
Guzmán *et al.*, 2018	⋆	⋆	⋆	⋆	⋆	0	⋆	⋆	⋆	**8 (Good)**

Table 4
Features of included studies.

Author's & year	**Country**	Study type	**Sex** Male / Female	Age (Mean or Range)	***n*** (CG/ EG)	Subjects total / Samples total
(Jasim et al., 2024)	Iraq	Cross-Sectional	Data not shown	Data not shown	25 HT; 30 G; 30 P	85 / 85
(Xanthopoulou et al., 2024)	Greece	Cross-Sectional	Data not shown	52.5–62	27 HI; 41 PM; 12 PI	8 / 80
(Halstenbach et al., 2023)	Germany	Cohort	8 / 6	52–85	9 HT; 12 HI; 17 PI	14 / 38
(Loureiro et al., 2021)	Brazil	Cross-Sectional	Data not shown	Data not shown	9 APs; 9APa	18 / 18
(Nalmpantis et al., 2020)	Greece	Cross-Sectional	51 / 50	50.4	48 HT; 53 CP	101 / 101
(Afacan & Atmaca İlhan, 2020)	Turkey	Cross-Sectional	38 / 40	37.2	38 HT; 40 P	78 / 78
Guzmán et al., 2018	Greece	Cohort	5 / 5	48.8	10 CP	10 / 10
(Leppilahti et al., 2014)	Chile	Cross-Sectional	11 / 12	43.3	9 HT; 6 G; 8 P	23 / 23
(Choi et al., 2011)	Korea	Cross-Sectional	Data not shown	Data not shown	36 G; 59 MP; 30 SP	125 / 880

Abbreviations: *HT* Healthy teeth; *HI* Healthy implant; *G* Gingivitis; *P* Periodontitis; *CP* Chronic periodontitis; *MP* Moderate periodontitis; *SP* Severe periodontitis; *APs* Apical periodontitis symptomatic; *APa* Apical periodontitis asymptomatic; *PM* Peri-implant mucositis; *PI* Peri-implantitis.

process triggers the production of proinflammatory cytokines, such as IL-1β, TNF-α, and prostaglandins, which promote the recruitment and activation of inflammatory cells, especially neutrophils and macrophages, thereby increasing tissue destruction (Liu et al., 2024; Loos & Van Dyke, 2020). In relation to neutrophils, different stimuli from proinflammatory cytokines and chemokines, complement fragments, adhesion molecules, and extracellular Ca^{2+}, as well as bacterial formyl peptides interact and activate G protein-coupled receptors, FCγ receptors and integrin β2/ Mac-1, which triggers the degranulation response. When Azu is released from the azurophil granules, it interacts directly with endothelial cells by binding to glycosaminoglycans and activating protein kinase C and Rho kinase (Othman et al., 2022). This triggers Ca^{2+} influx, leading to cytoskeleton reorganization and cell contraction, creating spaces between endothelial cells that result in vascular leakage and facilitate neutrophil extravasation. Azu has also been shown to induce MCP-1 expression in endothelial cells through the FAK/PI3K/AKT and p38 MAPK/NF-kB signaling pathways, promoting monocyte migration (Chang et al., 2018; Chen et al., 2024). In this regard, *in vitro* studies have evaluated the exposure of lipopolysaccharide and Azu in peripheral blood monocytes and revealed that a dual exposure increases the production of PGE_2, IL-8, MIP-1α, IL-1β and TNF-α (Heinzelman et al., 1998; 2001). These last two proinflammatory mediators up-regulate the expression of receptor activator ligands for nuclear factor κ B (RANKL), producing osteoclastogenesis ((Almeida-Junior et al., 2023)); thus, Azu would contribute to alveolar bone destruction in periodontitis (Fig. 4).

The immunopathogenesis of PD cannot be restricted to a single cytokine profile, as multiple microbial and host-related factors modulate disease expression and progression (Gürsoy et al., 2024). Azu is one such mediator, whose release reflects neutrophil activation and endothelial interaction in response to proinflammatory signaling (Othman et al., 2022). Therefore, understanding its behavior in various biofluids is essential to distinguish disease-specific inflammatory patterns.

The involvement of Azu in gingivitis and periodontitis is due to its ability to modulate the inflammatory response. In gingivitis, this protein acts in the innate immune response, being released by neutrophils in the

Table 5
Azurocidin as a potential inflammatory biomarker in periodontal disease = Results of clinical studies.

Author's & year	Periodontal condition	Sampling	Biomarker	Methods	Azu value EG	Azu value CG	Outcomes
(Jasim et al., 2024)	Periodontitis Gingivitis	Saliva	Azurocidin	ELISA	178.188 ng/mL 156.510 ng/mL	66.614 ng/mL	Azu levels increased in teeth with periodontitis and gingivitis compared to healthy teeth ($p < 0.001$)
(Xanthopoulou et al., 2024)	Peri-implant mucositis Peri-implantitis	PICF	Azurocidin	ELISA	52.86 (61.00) pg/30 s 35.53(58.86) pg/30 s	33.16(52.88) pg/30 s	Azu levels increased in implants with peri-implant mucositis compared to healthy implants and implants with peri-implantitis, but without statistically significant differences ($p > 0.05$)
(Halstenbach et al., 2023)	Peri-implantitis	PICF	Azurocidin	LC-MS/MS	NR	NR	Azu and MPO were found to be up-regulated in implants with peri-implantitis compared to healthy implants
(Loureiro et al., 2021)	Apical periodontitis	Infected root canals	Azurocidin	nLC-ESI-MS/MS	NR	NR	Azu was upregulated in periodontitis apical asymptomatic compare to apical periodontitis symptomatic
(Nalmpantis et al., 2020)	Periodontitis	GCF	Azurocidin	ELISA	359.13 (178.89) pg/30 s	99.42(86.85) pg/30 s	Azu levels increased in teeth with periodontitis compared to healthy teeth ($p < 0.001$)
(Afacan & Atmaca İlhan, 2020)	Periodontitis	Saliva	Azurocidin	ELISA	NR	NR	Azu levels increased in teeth with periodontitis compared to healthy teeth ($p < 0.05$)
Guzmán et al., 2018	Periodontitis	GCF	Azurocidin	LC-MS/MS	NR	NR	Downregulation of Azu after mechanical periodontal treatment
(Leppilahti et al., 2014)	Periodontitis Gingivitis	GCF	Azurocidin	ELISA	18/20 = (94.7 %) 19/19 = (100 %)	13/20 = (65 %)	Azu levels increased in teeth with periodontitis and gingivitis compared to healthy teeth ($p < 0.016$)
(Choi et al., 2011)	Periodontitis Gingivitis	GCF Gingival tissue	Azurocidin	LC-MS/MS ELISA → Western blotting	118.3 pg/mL 136.6 pg/mL	40.8 pg/mL	Azu levels increased in teeth with gingivitis, followed by teeth with periodontitis compared to healthy teeth ($p < 0.004$)

Abbreviations: *Azu* Azurocidin; *NR* Not reported; *PICF* Peri-implant crevicular fluid; *GCF* Gingival crevicular fluid; *LC-MS/MS* Liquid chromatography- tándem mass spectometry; *ELISA* Enzyme-linked Immunosorbent assay; *nLC-ESI-MS/MS* Nano-scale liquid chromatography tándem mass spectometry; *EG* Exposure group; *CG* Control group.

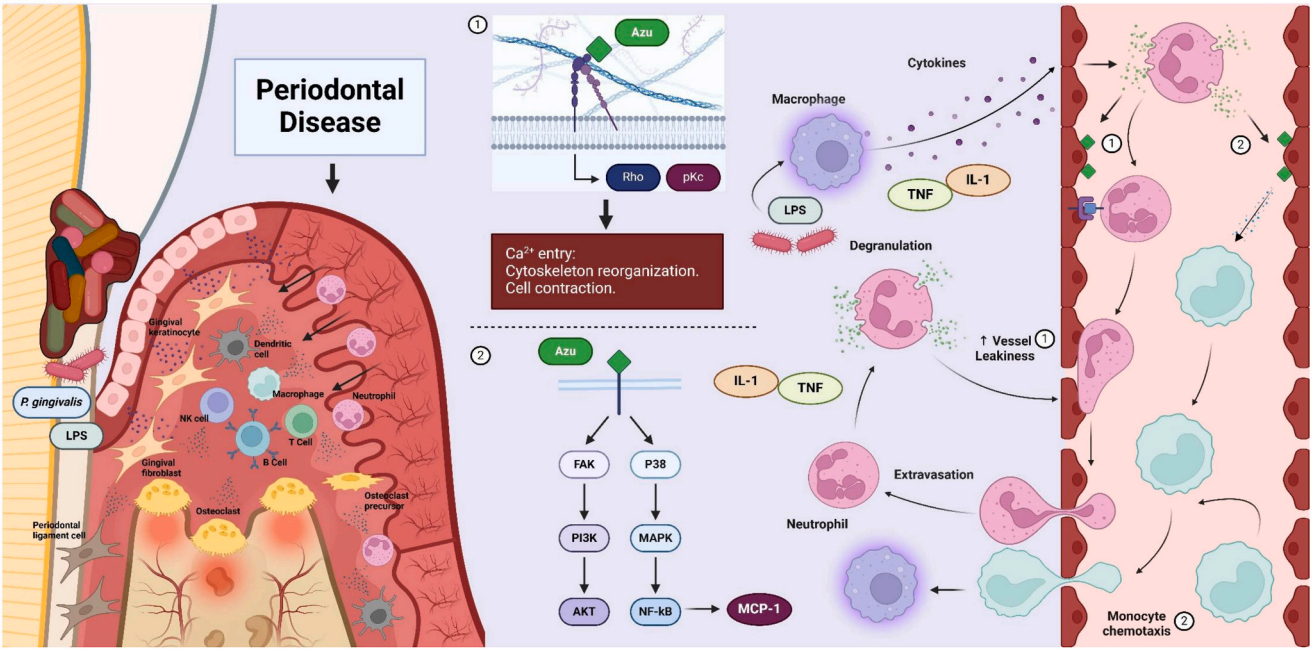

Fig. 4. Involvement of azurocidin in the inflammatory response. Bacterial aggression induces the production of cytokines, which enter the circulation and activate neutrophil degranulation. 1) Immediately after Azu is released, it binds to glycosaminoglycans of the vascular endothelium. This activates protein kinase C and Rho kinase, which triggers Ca2 + entry, and this, in turn, reorganizes the cytoskeleton and cell contraction, creating spaces between endothelial cells, leading to vascular leakage, and facilitating neutrophil extravasation. 2) In addition, Azu up-regulates MCP-1 expression by activating FAK/PI3K/AKT and p38 MAPK/NF-kB signaling pathways, promoting monocyte chemotaxis.

areas of the inflammatory process in the presence of periodontopathogenic bacteria. On the other hand, its increase in the periodontitis picture reflects a more severe inflammation associated with the destruction of the periodontium (Afacan & Atmaca İlhan, 2020; Choi, Heo, Lee, & Cho, 2011; Jasim, Al-Ghurabi, & Abdulameer, 2024; Leppilahti et al., 2014). Thus, AZU not only participates in the defense against microorganisms but can also contribute to the activation and migration of immune cells, exacerbating inflammation and tissue damage (Nisha & Annie, 2017). Therefore, its regulation has a dual role: protective in the early stages of PD (gingivitis) but destructive in advanced cases (periodontitis) (Choi et al., 2011 Jul 28; Jasim et al., 2024; Leppilahti et al., 2014; Nisha & Annie, 2017). Although gingivitis and periodontitis are indeed two diseases that differ in their severity and clinical repercussions, neutrophils play a fundamental role in the inflammatory response in both periodontal conditions. Azu has been studied as a potential biomarker of inflammation in gingivitis and periodontitis. Azu levels have been found to be elevated in GCF and saliva of patients with periodontitis compared to levels in patients with gingivitis or periodontally healthy individuals (Halstenbach et al., 2023; Xanthopoulou et al., 2024). In addition to Azu, the role of other azurophil granule proteins, such as NE, MPO, beta-defensin 2, cathepsin G, and proteinase 3, have been studied and contribute to pathogen destruction but may also exacerbate tissue damage in periodontal diseases. Both Azu and other neutrophil azurophil granule proteins have been identified as key biomarkers in periodontal inflammation, particularly in periodontitis (Gul et al., 2016; Soldati et al., 2022; Türkoğlu et al., 2014).

In apical periodontitis, the identification of Azu has shown that its overexpression at involved sites may be a key marker of neutrophil activation. Its role as a proinflammatory mediator stands out in the perpetuation of tissue damage in the periapical area, suggesting that in addition to being a marker of the immune response, it may also contribute to the pathogenesis of the disease. This emphasizes the relevance of neutrophils and their azurophilic granules in the development and progression of apical periodontitis (Hussain et al., 2024; Loureiro et al., 2021).

The identification of biomarkers in peri-implant mucositis and peri-implantitis that enable early and accurate detection of these conditions is crucial for improving the prognosis of the affected implants. Several key proteins have been identified, including MMP-8 and Azu, the latter being a crucial component of neutrophil azurophil granules. While Azu has been validated as a biomarker in periodontitis (Zuo et al., 2024), its expression in peri-implant diseases appears more variable. Unlike gingival tissues, peri-implant tissues lack a periodontal ligament and have reduced vascularity, which may influence neutrophil recruitment and degranulation dynamics. This tissue-specific immune response may partly explain the limited diagnostic performance of Azu in PICF, despite its biological relevance (Halstenbach et al., 2023; Xanthopoulou et al., 2024). Thus, a panel of markers may be necessary to enhance diagnostic accuracy in peri-implant disease. Among the most relevant clinical studies is the one performed by (Xanthopoulou et al., 2024), in which azurocidin levels were compared between healthy implants, implants with peri-implant mucositis, and implants with peri-implantitis. The findings showed that, although Azu was present in all groups, no significant differences were found between them, suggesting a limited diagnostic utility of Azu as an independent biomarker to discriminate between health states and peri-implant conditions. Additionally, although the sensitivity of the method for Azu identification was 80 %, the specificity was low at 40 %, increasing the likelihood of error in its use as a biomarker. These findings are consistent with a pilot study, which determined that Azu does not appear to be a suitable biomarker for the detection of these conditions. This research suggests that Azu, despite being involved in the immune and inflammatory response, is not a sufficiently specific indicator to identify the various peri-implant disease states (Halstenbach et al., 2023). Despite these results, Azu continues to be a molecule of interest due to its role in the immune response and its potential to complement other markers, such as MMP-8, in the diagnosis of peri-implant disease. It would be interesting to continue exploring Azu, with the hope that it could be used as part of a biomarker panel for the comprehensive assessment of periodontal and peri-implant health.

4.1. Limitations and future

This study had some limitations, the most significant limitation lying in the inability to perform a meta-analysis due to methodological differences between the included studies. These included discrepancies in the method of sample collection, the type of biofluid/sample analyzed (saliva, PICF, GCF, gingival tissue, and infected root canals), as well as the use of multiple methodologies (ELISA, Western Blot, LC-MS/MS, and nLC-MS/MS). Furthermore, there is a lack of numerical data in the publications, and it is impossible to acquire them by contacting the authors. Additionally, different units of measurement were used for Azu expression that could not be homogenized.

Further clinical studies in humans, as well as experimental investigations with cell lines and animal models, are needed to elucidate the role of Azu in PD further. The role of Azu in individuals with dental caries and aggressive periodontitis also remains to be explored. On the other hand, systemic levels of Azu in serum and blood plasma of PD subjects have not yet been evaluated. Also, it would be important to study how Azu levels vary in the different stages/grades according to the new classification of periodontal and peri-implant diseases, as well as to follow up on the behavior of this molecule after basic periodontal therapy. Finally, we encourage researchers to perform genetic studies to determine if there is an association between any genetic variant of the *AZU* gene and individuals with PD.

5. Conclusions

The available clinical evidence suggests a potential overexpression of Azu in periodontal disease, particularly in periodontitis, compared to periodontally healthy individuals. However, due to the small number of studies, methodological heterogeneity, and inconsistent reporting, definitive conclusions regarding the diagnostic value of Azu cannot yet be established.

Abbreviations

Azu: Azurocidin; PD: Periodontal disease; DI: Dental implant; TNF-α: Tumor necrosis factor alpha; MCP-1: Monocyte chemoattractant protein; IL-1β: Interleukin 1 beta; RANKL: Receptor activator ligand for nuclear factor κ B; MMPs: Matrix metalloproteinases; MMP-8: Matrix metalloproteinase 8; CX3CL1: Fractalkine; IL-17A: Interleukin 17 A; IL-18: Interleukin 18; IL-33: Interleukin 33; IL-2: Interleukin 2; IL-6: Interleukin 6; IgG: Immunoglobulin G; IgM: Immunoglobulin M; MPO: Myeloperoxidase; NE: Elastase; PGE2: Prostaglandin E2; IL-8: Interleukin 8; MIP-1α: Macrophage inflammatory protein 1 alpha; IL-4: Interleukin 4; IL-5: Interleukin 5; IL-13: Interleukin 13; ELISA: Enzyme-linked immunosorbent assay; LC-MS/MS: Liquid chromatography with mass spectrometry; nLC-MS/MS: Nano-scale liquid chromatography with mass spectrometry; PICF: Peri-implant crevicular fluid; GCF: Gingival crevicular fluid; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-analysis; JBI: Joanna Briggs Institute.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None

Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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