Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

MICROBIOMA AND METATRANSCRIPTOMA AS BIOMARKERS FOR PERIIMPLANTITIS

Madrid, academic year 2025/2026

Identification number: 186

ABSTRACT

Introduction: Peri-implantitis is a plaque-associated inflammatory disease characterized by progressive bone loss around dental implants and represents a major cause of implant failure. Conventional diagnostic methods rely mainly on clinical and radiographic parameters, which often detect the disease only after irreversible tissue destruction has occurred. Recent advances in omics technologies, particularly microbiome and metatranscriptome analyses, provide new opportunities to identify microbial and functional biomarkers associated with peri-implant disease; **Objective:** The primary objective was to determine whether microbiome and metatranscriptome analyses can identify specific microbial and functional signatures associated with peri-implantitis in adults with dental implants compared with clinically healthy peri-implant sites; the secondary objective was to evaluate the potential of these signatures as diagnostic biomarkers; **Material and Methods:** A research question based on the PICO framework was formulated and a systematic review was conducted according to PRISMA 2020 guidelines. Bibliographic searches were performed in PubMed, Medline Complete, EBSCO and Google Scholar using combinations of the terms “microbiome”, “metatranscriptome”, “peri-implantitis” and “biomarkers”. Articles published between 2015 and 2025 in English, French or Spanish were screened according to predefined eligibility criteria; **Results:** After the application of inclusion and exclusion criteria, 12 in vivo studies were included. The studies consistently reported microbial dysbiosis in peri-implantitis, with enrichment of anaerobic taxa such as *Porphyromonas*, *Treponema*, *Prevotella* and *Fusobacterium*, together with depletion of health-associated genera such as *Streptococcus* and *Rothia*. Metatranscriptomic analyses also revealed functional changes including increased amino-acid catabolism, virulence-associated pathways and host inflammatory activation; **Conclusions:** Microbiome and metatranscriptome analyses identify distinct microbial and functional signatures associated with peri-implantitis and show promising potential as diagnostic biomarkers, although longitudinal studies and standardized methodologies are required for its clinical application.

KEYWORDS

Dentistry, Biomarker(s), Metatranscriptome, Microbiome, Peri-implantitis

RESUMEN

Introducción: La periimplantitis es una enfermedad inflamatoria asociada a la placa bacteriana caracterizada por la pérdida progresiva de hueso alrededor de los implantes dentales y constituye una causa importante de fracaso implantario. Los métodos diagnósticos convencionales se basan principalmente en parámetros clínicos y radiográficos, que a menudo detectan la enfermedad en fases avanzadas cuando el daño tisular ya es irreversible. Los avances recientes en tecnologías ómicas, especialmente los análisis del microbioma y del metatranscriptoma, ofrecen nuevas perspectivas para identificar biomarcadores microbianos y funcionales asociados a la enfermedad periimplantaria; **Objetivo:** El objetivo principal fue determinar si los análisis del microbioma y del metatranscriptoma permiten identificar firmas microbianas y funcionales específicas asociadas con la periimplantitis en adultos con implantes dentales en comparación con sitios periimplantarios clínicamente sanos; el objetivo secundario fue evaluar su potencial como biomarcadores diagnósticos; **Material y métodos:** Se formuló una pregunta de investigación basada en el método PICO y se realizó una revisión sistemática siguiendo las directrices PRISMA 2020. La búsqueda bibliográfica se llevó a cabo en PubMed, Medline Complete, EBSCO y Google Scholar utilizando los términos “microbiome”, “metatranscriptome”, “peri-implantitis” y “biomarkers”. Se seleccionaron artículos publicados entre 2015 y 2025 en inglés, francés o español; **Resultados:** Se incluyeron 12 estudios in vivo. Los resultados mostraron disbiosis microbiana en la periimplantitis, con aumento de taxones anaerobios como *Porphyromonas*, *Treponema*, *Prevotella* y *Fusobacterium*, y disminución de géneros asociados con la salud como *Streptococcus* y *Rothia*. Los análisis metatranscriptómicos evidenciaron además una reprogramación funcional caracterizada por mayor catabolismo de aminoácidos, activación de factores de virulencia y respuesta inflamatoria del huésped; **Conclusiones:** Los análisis del microbioma y del metatranscriptoma permiten identificar firmas microbianas y funcionales asociadas con la periimplantitis y muestran potencial como biomarcadores diagnósticos, aunque se requieren estudios longitudinales y metodologías estandarizadas para su aplicación clínica.

PALABRAS CLAVE

Odontología, Biomarcador(es), Metatranscriptoma, Microbioma, Periimplantitis

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ABBREVIATIONS

16S rDNA: 16S ribosomal deoxyribonucleic acid

16S rRNA: 16S ribosomal ribonucleic acid

IL-1β: Interleukin-1 beta

IL-6: Interleukin-6

IL-8: Interleukin-8

ML: Machine learning

MMP-8: Matrix metalloproteinase-8

NGS: Next-generation sequencing

PI: Peri-implantitis

PICF: Peri-implant crevicular fluid

PT: Periodontitis

RNA-seq: RNA sequencing

TNF-α: Tumor necrosis factor-alpha

1. INTRODUCTION

1.1 General background

1.1.1 Dental implants and their clinical relevance

Dental implants have been widely used since the last few decades as an effective and common prosthodontic solution to treat edentulism with long term survival rates at 5 years exceeding 90%. This high success rate underscores the clinical relevance of implant placement (1,2).

Consequently, as dental implant use increases, with an annual growth rate reaching up to 23% in 2026, the prevalence of potential complications such as peri-implantitis is also likely to increase (3).

1.1.2 Peri-implantitis: definition, prevalence and clinical relevance

Peri-implantitis (PI) can be defined as a plaque induced inflammatory condition affecting patients with dental implants. It is characterized by progressive destruction of the supporting tissue and includes processes such as bone remodelling and immune reactions. Clinically, PI firstly manifests itself with redness, swelling and bleeding on probing, until the process extends apically, resulting in progressive loss of osseointegration and bone attachment around the implant, eventually leading to implant failure (4–6).

PI represents the advanced stage of peri implant disease, following peri implant mucositis, which according to the 2017 World Workshop classification of periodontal and peri-implant diseases and conditions is characterized by "*bleeding on probing and visual signs of inflammation*" without bone destruction. It corresponds to a limited reversible inflammation of soft tissue. Whereas, still according to the 2017 World Workshop consensus, PI is defined as "*a plaque-associated pathological condition occurring in tissues around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone*" (7–9). The shift from mucositis to PI marks the onset of permanent and irreversible bone loss surrounding the implant (10,11).

The prevalence of PI varies significantly across studies, mainly due to differences in diagnostic criteria and population characteristic (12). However, according to Astolfi *et al.,* 2022 28% to 51% of patients with one or more implants suffer from biological complications such as PI (13).

According research, PI and periodontitis (PT) show shared similarities which can help us to address this situation (14). Nevertheless, PI tends to develop more rapidly and with greater bone destruction over a shorter period than PT making it a consequent problem for the implant's long-term survival (4,6). This difference is attributed to the unique anatomical and histological characteristics of peri-implant tissues: that is, unlike natural teeth, implants lack a periodontal ligament and have a reduced vascular supply, which compromises host defence and healing capacity. Consequently, once inflammation reaches the bone, the progression becomes more severe, and treatment outcomes are less predictable (8,14).

Similarly to PT, PI includes risk factors such as oral hygiene, smoking, diabetes and inadequate maintenance and as well periodontitis history. The presence of one or more of these risk factors significantly increases the susceptibility to inflammation and subsequent bone loss (2,10,15,16).

Understanding the microbial, immunological, and molecular basis of PI is essential for the development of reliable biomarkers and early diagnostic tools. Through metagenomic and metatranscriptomic analyses, personalized prevention and intervention strategies in implantology may become possible (3,17,18).

1.2 Pathophysiology of peri-implantitis

1.2.1 Biological mechanisms of tissue destruction

As mentioned, PI is distinguished by progressive loss of the surrounding and supporting bone around the dental implant(s) accompanied by general inflammation including redness, swelling of the adjacent soft tissues (4–6). Although often compared to PT, its biological basis presents both similarities and its own distinct mechanisms (19–21).

Gene expression analysis shows that PI tissue presents distinct mRNA signatures while PT is mainly characterized by bacterial-response pathways that indicates that PI displays a heightened activation of innate immune and defence response genes (21).

Although the aetiology of PI is still controversial and not fully understood (22). It has been established that the destruction process begins when a biofilm forms on the implant's surface and the peri-implant sulcus (5,10,15).

As shown in figure 1, the following inflammatory host response involves activation of immune cells such as macrophages, neutrophilic granulocytes and dendritic cells. The acute and recovery phases of inflammation are mainly dominated by M1 and M2 phenotypes of macrophages.

M1 cells release pro-inflammatory cytokines like interleukin-1β (IL-1β), interleukin-1 (IL-6), interleukin-8 (IL-8), tumor-necrosis factor α (TNF-α) and matrix metalloproteinase-8 (MMP-8) among others. That stimulate the endothelium contributing to inflammation, localized deterioration of tissue, and enhance the entrance of IgG. In recent research, macrophage polarization towards the M1 phenotype has been identified as a potential factor in the exacerbation of the inflammatory environment and in promoting bone resorption around implants. These cytokines act as inflammatory signals and stimulate osteoclastogenesis whilst inhibiting osteoblastic bone formation. In the context of a dental implant the "*race for the surface*" between bone cells and bacteria is an important concept: if bacteria predominate, osseointegration is compromised and inflammatory bone loss may happen. On the other hand, macrophages expressing the M2 phenotype act as an anti-inflammatory conducting tissue repair. (23–30).

Additionally, the area surrounding dental implants does not have the same natural defences found in natural teeth, such as the periodontal ligament, cementum, and Sharpey's fibers. Consequently, the bones and tissues surrounding implants might react differently to damage or infection. This could explain why PI often leads to quicker, crater-shaped bone loss compared to the more even, horizontal bone loss pattern seen in PT (31,32).

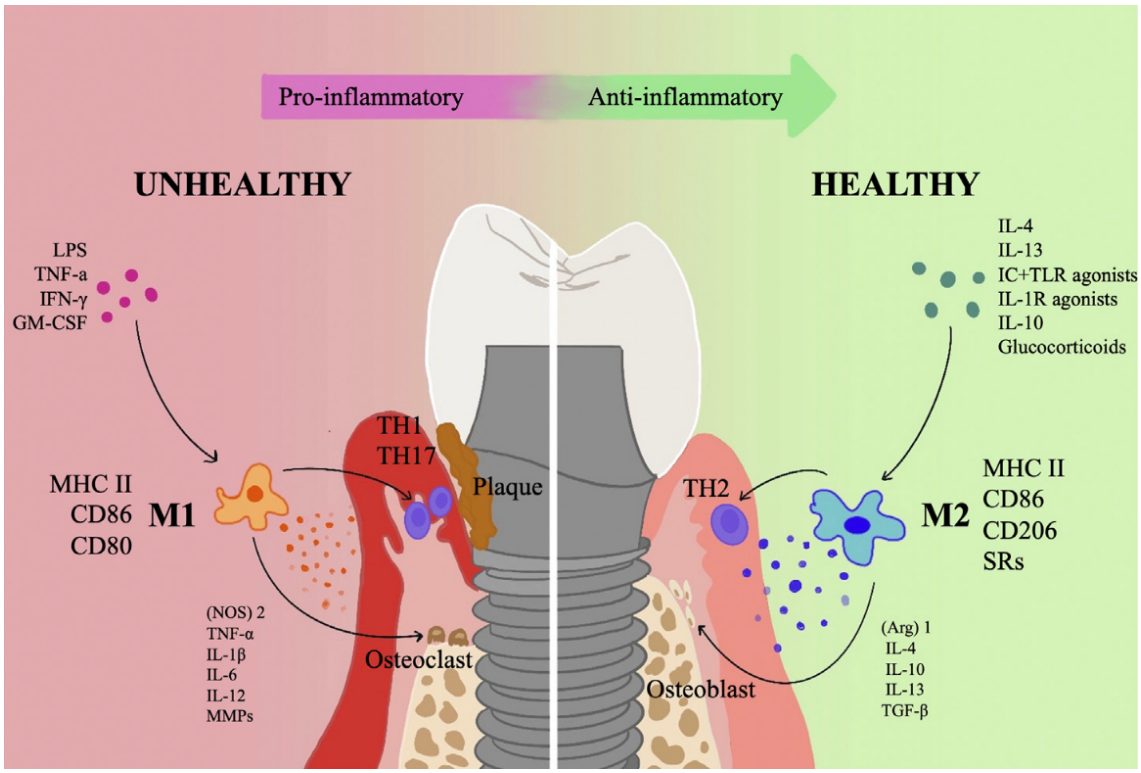


Figure 1. An overview of how polarized macrophages contribute to the incidence and growth of peri-implantitis (26).

1.2.2 Microbiological aspect of peri-implantitis

From a microbiological point of view, we can note a particular profile associated from health to PI, involving a succession of microbial shifts (3,5,14,19).

Healthy periodontal areas typically support a varied bacterial community mainly composed of gram-positive facultative anaerobes such as *Streptococcus* and *Actinomyces* species. These bacteria are crucial for the health of peri-implant tissue, as they inhibit the colonization of pathogenic species via competitive exclusion and through the production of antimicrobial compounds. However, when environmental or host-related factors promote excessive biofilm accumulation, this balance may be disrupted, leading to tissue inflammation (3,19,33). Indeed, systematic reviews have identified higher counts of pathogenic species such as *Porphyromonas gingivalis, Mogibacterium timidum, Prevotella intermedia, Treponema denticola* and *Fusobacterium nucleatum* in implants affected by PI whereas in implants of healthy patients *Actinomyces naeslundii, Haemophilus parainfluenzae […]* were more prevalent (3,22,34).

Meta-analyses have shown significantly higher total bacterial loads at the implant-abutment interface in implants with PI compared to healthy implants. However, studies using next-generation sequencing have highlighted that a complex and distinct microbial signature for PI has not yet been reliably identified. Nonetheless, there is clear evidence of increased microbial diversity and the presence of opportunistic taxa such as *Enterobacteriaceae* and *Staphylococcus* species in affected peri-implant areas (34–36).

Mechanically, microbes adhering to the implant surface form biofilms that produce virulence factors such as proteases and fimbriae triggering host-cell responses which then damage the peri-implant tissues (35–37).

PI emerges from a complex interaction between an altered microbiological setting, a dysbiotic biofilm, and a destructive host inflammatory, immune response that disrupts the typical balance of peri-implant tissues. This will ultimately lead to bone loss and if not treated, to implant failure (3,33–38).

1.3 Understanding the oral microbiome

The oral microbiome is one of the most well studied microbiomes. It's a complex ecosystem of microorganisms including viruses, fungi, archaea etc., living in the human oral cavity (3,5). It represents the second largest microbial ecosystem of the body after the gut (39).

The oral cavity is continuously exposed to over 700 distinct species. The varied structures of the oral environment including the tongue, cheeks, teeth, gingival sulcus etc., create numerous microhabitats, also known as niches, that provide favourable conditions for the establishment and maintenance of these diverse bacterial communities. Each have their own "microbial identity," defined by a characteristic and individualized microbial composition. Despite interindividual variability, the main function of the oral microbiome remains largely conserved. Fundamentally, the oral microbiota plays an active role in maintaining physiological, defensive and nutritional development in humans.

A dynamic, mutually beneficial relationship exists among the microorganisms residing in the oral cavity, a state referred to as *eubiosis*. On the other hand, disruption of this balanced microbial community leads to *dysbiosis*, promoting the proliferation of pathogens and the onset of oral or systemic diseases.

Recent improvements in bioinformatics have permitted the study of the human oral microbiome, through genomic and metagenomic studies, to deepen the understanding of each role pathogens play in this ecosystem (5,39,40).

1.4 From Metagenomics to Metatranscriptomics: gene expression and functional activity

1.4.1 Metagenomic profiling of peri-implant microbiome

One of the most used approaches to investigate a microbiome is by analysing its constituent genomes through metagenomics. The primary objective of metagenomic analysis is to determine the taxonomic composition and potential functional capabilities of a microbial community. It involves examining the collective genetic material obtained from entire communities of microorganisms. This process includes next-generation sequencing (NGS) following DNA extraction from the samples. NGS generates vast amounts of short-read data, that are computationally assembled to produce a representative profile of the microbial community. A widely used molecular marker gene in metataxonomic research is the 16S ribosomal DNA (16S rDNA) and for a more precise identification of bacterial taxa full-length 16s ribosomal RNA (16S rRNA) gene sequencing can also be done (17,41).

Recent advancements in NGS technologies provide a promising future for microbiome-based diagnostics. As previously discussed, several studies utilizing 16S rDNA or 16s rRNA sequencing and metagenomics have demonstrated that peri-implant sites represent unique ecological niches characterized by distinct bacterial communities (17).

It is important to note that the definition of the microbiome, initially described as a microbial community inhabiting a uniquely defined environment, has recently evolved to include the combined influences of environmental and host factors. This broader perspective has proven valuable for understanding how these dynamic interactions shape microbial ecosystems (41).

Several studies have further investigated microbial activity in PI using comparative metagenomic analyses and have successfully narrowed down potential causative bacteria. Within co-occurrence network analyses, *Solobacterium moorei* and *Prevotella denticola* were identified as highly active and central taxa, representing core species specific to PI. These findings provide novel insights into potential biomarkers that may aid in risk prediction and offer analytical support for therapeutic management (6,38).

As highlighted in recent reviews, the application of next-generation sequencing technologies in microbiological research holds significant potential to help clarify the microbial pathogenesis of PI. When combined with metatranscriptomic analyses, these approaches may contribute to the development of more effective preventive, diagnostic, and therapeutic strategies (6,38).

The initial investigations in this field primarily focused on metagenomic analysis of PI to explore the microbial and genetic potential through DNA sequencing. This was subsequently complemented by metatranscriptomic studies aimed at identifying differences in microbial and gene activity (38).

1.4.2 Metatranscriptomics: assessing gene expression and activity

Whilst metagenomics identify the taxonomic profile of the microbial community, metatranscriptomics provide functional profile, reflecting real-time microbial activity. This approach offers a clearer understanding of the genes actively expressed during PI (18,41).

Several studies have applied RNA sequencing (RNA-seq) to peri-implant biofilms by collecting samples from both healthy and PI patients. These investigations identified multiple transcriptional changes in the samples from diseased individuals. The most distinct disparities were observed in amino acid metabolism, particularly in the catabolism of histidine, lysine and tryptophane, which was significantly upregulated in PI. These metabolic

pathways generate inflammatory byproducts such as ammonia, hydrogen sulfide, and polyamines, contributing to tissue destruction and dysbiosis. In contrast, healthy biofilms exhibited greater expression of anabolic and carbohydrate-metabolic pathways, consistent with a balanced microbial activity (17,18,42).

Integrating metagenomic and metatranscriptomic datasets has enabled the identification of functional biomarkers with strong diagnostic potential (17). Key enzymes such as urocanate hydratase, tripeptide aminopeptidase, NADH:ubiquinone reductase, and phosphoenolpyruvate carboxykinase were repeatedly associated with PI and achieved high predictive accuracy (average AUC ≈ 0.85) in machine learning models based on both DNA- and RNA-derived data. These RNA-based enzyme activities represent metabolic reprogramming within pathogenic biofilms, highlighting their potential as potential molecular indicators for disease detection (17,19,43).

Similarly, Komatsu *et al.* (2020) demonstrated that the expression of virulence-associated genes, such as plasmin receptor/glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was significantly elevated in PI compared to PT. These enzymes enhance bacterial attachment and invasion, thereby linking microbial gene expression directly to specific clinical manifestations (38).

Collectively, these findings highlight how the integration of metagenomic and metatranscriptomic analyses can deepen the current understanding and diagnosis capability in PI. DNA-based metagenomics defines the microbial composition and its functional potential, while RNA-based metatranscriptomics reveals the active metabolic pathways driving the disease. The combination of these complementary approaches identifies both taxonomic and functional biomarkers, ranging from specific bacterial taxa to enzyme expression profiles, paving the way towards personalized, microbiome-guided diagnostics and early detection of peri-implant disease (17,38,43).

1.5 Biomarkers: concept, classification and clinical relevance

According to the U.S Food and Drug Administration (FDA) and the National Institutes of Health (NIH) Biomarker Working Group department, a biomarker is defined as: a characteristic that is objectively measured and elevated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention. It must be measurable accurately and reproducibly. In simpler terms, a biomarker is a measurable biological variable providing information about health or disease status. Biomarkers can take

various forms, including molecular, radiographic, histological or physiological parameters (44–46).

Recent advances in omics technologies, such as metagenomics and metatranscriptomics, have broadened the concept of biomarkers. These approaches now encompass microbial and gene expression profiles, offering deeper insight into host–microbe interactions and their role in diseases such as PI (43).

Biomarkers can be classified according to their functions, biological source, or clinical application. In 2016, the FDA and NIH Biomarkers Working Group introduced the BEST (Biomarkers, Endpoints, and other Tools) Resource, to provide a standardized framework for biomarker terminology and classification (46,47). Table 1 summarizes the main categories of biomarkers based on their clinical purpose and functional role.

In the field of peri-implant conditions, biomarkers have emerged as valuable tools for early detection, risk assessment and the development of personalized treatment strategies. Conventional clinical and radiographic assessments often identify PI only after substantial tissue destruction has occurred. In contrast, biochemical and molecular biomarkers found in peri-implant crevicular fluid (PICF) or saliva may enable earlier, non-invasive diagnosis (23,48).

Recent systematic reviews have highlighted those cytokines such as IL-1β, IL-6 and TNF-α, as well as enzymes like MMP-8, are consistently elevated in PI (48–50). Moreover, metagenomics and metatranscriptomics biomarkers are uncovering specific microbial signatures and gene expression profiles that may serve as next-generation diagnostic indicators (43).

Table 1. Classification resume of biomarkers according to their clinical application (43–50).

References	Category	Definition	Examples: general and PI context
Lumbikananda *et al.* (48) Sachelarie *et al.* (50)	Diagnostic	Identify the presence of a disease or condition.	IL-1β, MMP-8 for PI.
Radu *et al.* (49) Ardila *et al*. (43)	Prognostic	Predict the likely course or outcome of a disease.	Elevated TNF-α or IL-1β predicting implant loss risk.
Shah *et al.* (47) Lumbikananda *et al.*(48)	Predictive	Indicate likelihood of response to a given treatment.	IL-6 levels predicting anti-inflammatory therapy response.
Lumbikananda *et al.* (48) Strimbu & Tavel (45)	Risk	Identify increased risk of developing a disease in otherwise healthy individuals.	High IL-1 genotype risk for PI.
Ahmad *et al.* (46) Califf (44)	Pharmacodynamic	Reflect biological response to a therapeutic intervention.	Decreased MMP-8 post local or systemic antimicrobial treatment.
Ardila *et al.* (43) Radu *et al*. (49)	Metagenomics and Metatranscriptomics	Reflect microbial composition and gene activity associated with disease.	Upregulation of *Fusobacterium nucleatum* and *Prevotella intermedia* transcripts in PI biofilm.

1.6 The Microbiome as a source of diagnosis biomarkers in peri-implantitis

Peri-implant microbiome offers a rich and dynamic source of diagnostic biomarkers that reflect both the ecological state of the implant surface and the biological activity of the surrounding tissues. Rather than serving solely as an etiological factor, the microbial community itself can act as a quantifiable indicator of disease status. Distinct alterations in its composition, diversity, and functional output have been consistently correlated with PI, supporting its role as a reservoir of potential diagnostic signatures (17,38,51)

In PI, a reproducible microbial shift toward anaerobic and proteolytic species has been documented. Comparative analyses have shown increased abundance of *Fusobacterium nucleatum*, *Prevotella intermedia* and *Campylobacter rectus*, while health-associated genera such as *Rothia* and *Streptococcus* become depleted (6,49,52). The quantification of these taxa in PICF or submucosal biofilms has therefore been proposed as a diagnostic criterion capable of distinguishing healthy from diseased implants with high accuracy (4,23)

However, diagnostic analysis extends beyond taxonomic composition. The peri-implant microbiome exhibits distinct metabolic and virulence pathways correlated with clinical inflammation. Functional analyses have revealed enrichment of transcripts associated with lipopolysaccharide synthesis, protease activity and oxidative stress tolerance in PI lesions (43). These functional markers allow for real-time assessment of microbial activity, identifying not only the organisms present but also those that are metabolically active and contribute to tissue destruction. This information has diagnostic value, as it enables differentiation between active disease and simple colonization (17–19,53)

From a clinical perspective, microbial biomarkers can be detected using minimally invasive sampling techniques, such as PICF analysis or saliva collection and could be integrated into chairside diagnostic platforms. When combined with host inflammatory mediators such as IL-1β or MMP-8, microbial markers form multi-biomarker panel with enhanced predictive performance for early detection (48,50,54).

Overall, the peri-implant microbiome plays a crucial role in disease pathogenesis and serves as a sensitive biosensor of peri-implant health. The identification of specific microbial taxa and gene expression profiles associated with inflammation lays the foundation for microbiome-based diagnostics, enabling earlier, more accurate and personalized detection of PI (17).

1.7 The Metatranscriptome as functional biomarker source

The metatranscriptome represents the active functional state of the peri-implant microbiome, providing important insight into the molecular mechanisms underlying pathological processes. By evaluating the collective RNA expression profiles of microbial communities, this approach identifies actively transcribed genes directly involved in peri-implant inflammation and tissue destruction. Unlike DNA-based methods, which reveal potential microbial capabilities, metatranscriptomic analysis reflects microbial behaviour in real time, making it an invaluable source of functional diagnostic biomarkers (17,38,43).

One of the main advantages of metatranscriptomics lies in its ability to capture dynamic variations in gene expression in response to clinical interventions. Following non-surgical or surgical treatment of PI, significant changes in the transcriptional activity of key microbial pathways can be observed. For example, successful treatment is often associated with decreased expression of virulence genes, particularly those related to proteolysis, lipopolysaccharide synthesis and the oxidative stress response, as well as increased expression of metabolic pathways involved in biofilm stabilization and nutrient exchange (43,49).

Conversely, persistent or reactivated expression of pathogenic transcripts after treatment may indicate incomplete microbial suppression and serve as a predictive biomarker for disease recurrence (17,48,49).

Monitoring gene expression profiles before and after treatment may therefore provide valuable prognostic information regarding the efficacy of therapeutic strategies. A shift in the metatranscriptomic signature toward profiles resembling those of a healthy peri-implant environment suggests restoration of microbial balance, whereas persistence of a pathogenic transcriptional profile reflects ongoing dysbiosis despite clinical improvement. This real-time molecular assessment enables personalized treatment planning and early detection of relapse risk (35,38,55).

Furthermore, integrating microbial metatranscriptomic biomarkers with host transcriptional responses, such as cytokines, MMPs, or immune regulatory genes, improves the accuracy of treatment outcome predictions. These multi-omics models enable identification of patients most likely to respond to mechanical debridement or adjuvant antimicrobials, as well as those who may require regenerative or laser-assisted therapies. Importantly, these RNA-based biomarkers can be detected non-invasively in PICF or saliva, facilitating their incorporation into chairside diagnostic tests for longitudinal monitoring (38,48–50).

In summary, the metatranscriptome extends beyond simple diagnostics to serve as a platform for functional and prognostic biomarkers. By revealing the expression dynamics of

microbial and host genes in response to treatment, it provides clinicians with molecular indicators of therapeutic success, risk of recurrence and long-term peri-implant stability. This functional understanding represents a crucial step toward precision medicine in implantology, in which therapeutic strategies are guided by real-time biological activity and not solely by clinical signs (17,38,53).

1.8 Integrative omics approaches for precision peri-implant disease management

Integrative omics approaches combine multiple types of molecular data, such as metagenomics, metatranscriptomics, proteomics and metabolomics to provide a comprehensive understanding of the mechanisms underlying peri-implant diseases. This strategy enables the simultaneous assessment of microbial composition, gene expression and host responses, thereby linking dysbiosis, inflammation and tissue destruction at the molecular level (17,38,43).

By combining microbial and host data, integrative omics approaches can reveal causal molecular networks and identify multidimensional biomarkers that more accurately reflect disease activity. For example, combining metatranscriptomic profiles of pathogenic species with the expression of host cytokines or proteases such as IL-1β and MMP-8 improves diagnostic accuracy and enables the early detection of PI (23,48–50). These integrated models surpass single-parameter approaches by incorporating both microbial activity and host immune responses within a unified analytical framework (43,48).

Recent studies have shown that multi-omics analyses can also facilitate patient stratification and treatment monitoring. Individuals exhibiting persistent expression of virulence transcripts or inflammatory mediators after treatment may be at increased risk of relapse, whereas normalization to a eubiotic, health-associated profile indicates treatment success (17,35,38,54). The integration of metabolomics data further refines this approach, as metabolic profiles reflect microbial and host cellular responses to inflammation (42).

Advances in bioinformatics and artificial intelligence now facilitate the integration of large omics datasets, enabling the development of predictive models capable of distinguishing peri-implant health from disease and anticipating treatment outcomes (24,51). In the foreseeable future, these multi-omics platforms could be adapted into chairside diagnostic tools, guiding truly personalized management of peri-implant diseases.

In summary, integrative omics approaches link microbial ecology, gene expression and host immunity to provide a unified molecular view of PI. This concept supports precision

implantology, in which prevention, diagnosis and therapy are tailored to each patient's unique biological profile (17,38,43,48,51).

1.9 Current state of the subject

Recent research has positioned the peri-implant microbiome and metatranscriptome as promising sources of diagnostic biomarkers for PI. Studies integrating 16S rRNA sequencing with metatranscriptomic profiling have identified disease-associated species such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, in conjunction with functional biomarkers including urocanate hydratase and NADH:ubiquinone reductase, achieving high diagnostic accuracy (AUC ≈ 0.85) (50,51). These findings highlight the potential of RNA-based analyses to capture active microbial functions driving inflammation.

However, no universally accepted microbial or transcriptional signature has yet been validated. Systematic reviews emphasize that methodological heterogeneity, including differences in sampling procedures, sequencing platforms and data processing, limit reproducibility and clinical translation (48). Furthermore, many studies remain cross-sectional, offering limited insight into the temporal dynamics of disease progression or response to treatment (20,43).

Overall, current evidence supports the diagnostic potential of metagenomic and metatranscriptomic biomarkers in PI; however, future research should focus on standardized protocols, longitudinal validation and the integration of multi-omics data to enable reliable and clinically applicable diagnostic strategies (17).

1.10 Justification

PI remains a major cause of implant failure, characterized by inflammation and progressive bone loss around osseointegrated implants. Current diagnostic tools rely primarily on clinical and radiographic parameters, which often detect the disease only after irreversible tissue destruction has occurred. This limitation underscores the need to develop molecular biomarkers capable of identifying early biological alterations preceding clinical symptoms (43).

Recent advances in microbiomic and metatranscriptomic profiling have opened new perspectives for elucidating the microbial and functional mechanisms underlying peri-implant diseases. These high-throughput techniques enable the simultaneous analysis of microbial composition and gene expression activity, thereby revealing the functional state of the peri-implant ecosystem and its role in inflammation and tissue destruction (17,38). Unlike DNA-based

approaches, metatranscriptomics captures microbial behaviour in real time, providing a dynamic view of disease progression and treatment response (53).

However, current research on microbiome- and metatranscriptome-based biomarkers remains fragmented and methodologically heterogeneous, with differences in sample collection, sequencing platforms and analytical protocols limiting reproducibility and clinical applicability (48). A systematic review is therefore essential to critically synthesize existing data, identify consistent molecular signatures, and assess their diagnostic accuracy, reproducibility, and predictive potential (17,43,48).

By consolidating and analysing these results, this study aims to clarify the clinical relevance of omics-based biomarkers in PI and to lay the foundations for precision implantology, in which therapeutic strategies are guided by molecular activity rather than delayed clinical manifestations.

2. OBJECTIVE

Following an exhaustive systematic review, the objective of this project is to analyse and synthesize the existing literature to determine whether microbiome and metatranscriptome analyses can identify specific microbial and functional signatures associated with PI in adults with dental implants, compared with clinically healthy peri-implant sites and to assess their potential as diagnostic biomarkers.

3. MATERIAL AND METHODS

This systematic review complies with guidelines of the PRISMA declaration (Preferred Reporting Items for Systematic reviews and Meta-analysis, 2020).

3.1. Identification of the PICO question

Using the PICO method, the research question of this work was defined as: *"In adults with dental implants, do microbiome and metatranscriptome analyses, compared with clinically healthy peri-implants sites, identify specific microbial and functional signatures that can serve as biomarkers for peri-implantitis?"*

This research question is based on:

P for population: Adults with dental implant(s).

I for intervention: Microbiome and/or Metatranscriptome analyses.

C for comparison: Clinically healthy peri-implant site

O for outcomes: Identification of discriminatory microbial and functional signatures (biomarkers, potential ect.,)

3.2. Data sources and extraction

The bibliographic search for this study was obtained from PubMed, Medline Complete, EBSCO and Google Scholar database using MeSH terms and unspecific free text word. The final search terms were defined after iterative processes of trials and improvement. The Boolean operators as AND, OR, NOT were also added to keywords to obtain more precise results.

An example of the search equation used in Medline Complete is as follows:

("microbiome" AND "metatranscriptome") AND ("biomarkers" OR "diagnostic biomarkers" OR "disease markers") AND ("peri-implantitis" OR "peri-implant disease" OR "peri-implant inflammation").

Controlled vocabulary and free-text terms were combined using Boolean operators, and the search syntax was adapted for each database.

Duplicate articles were rejected**.**

Filters were also put, as years of publication: from 2015 to 2025 and articles in English, French and Spanish were potentially selected.

All the criteria of inclusion and exclusion were resumed in the next subsection, in table 2.

3.3. Eligibility criteria

Table 2. Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
English, French, Spanish articles	Studies on microbiome and metatranscriptome for a different aim than peri-implantitis diagnostic relation
Articles less than 10 years from publication	Article from 2015 or before
In vivo studies	Duplicates articles
	In vitro, animal study designs, and ex vivo studies.
	Studies without full-text articles.

4. RESULTS

4.1. Study selection

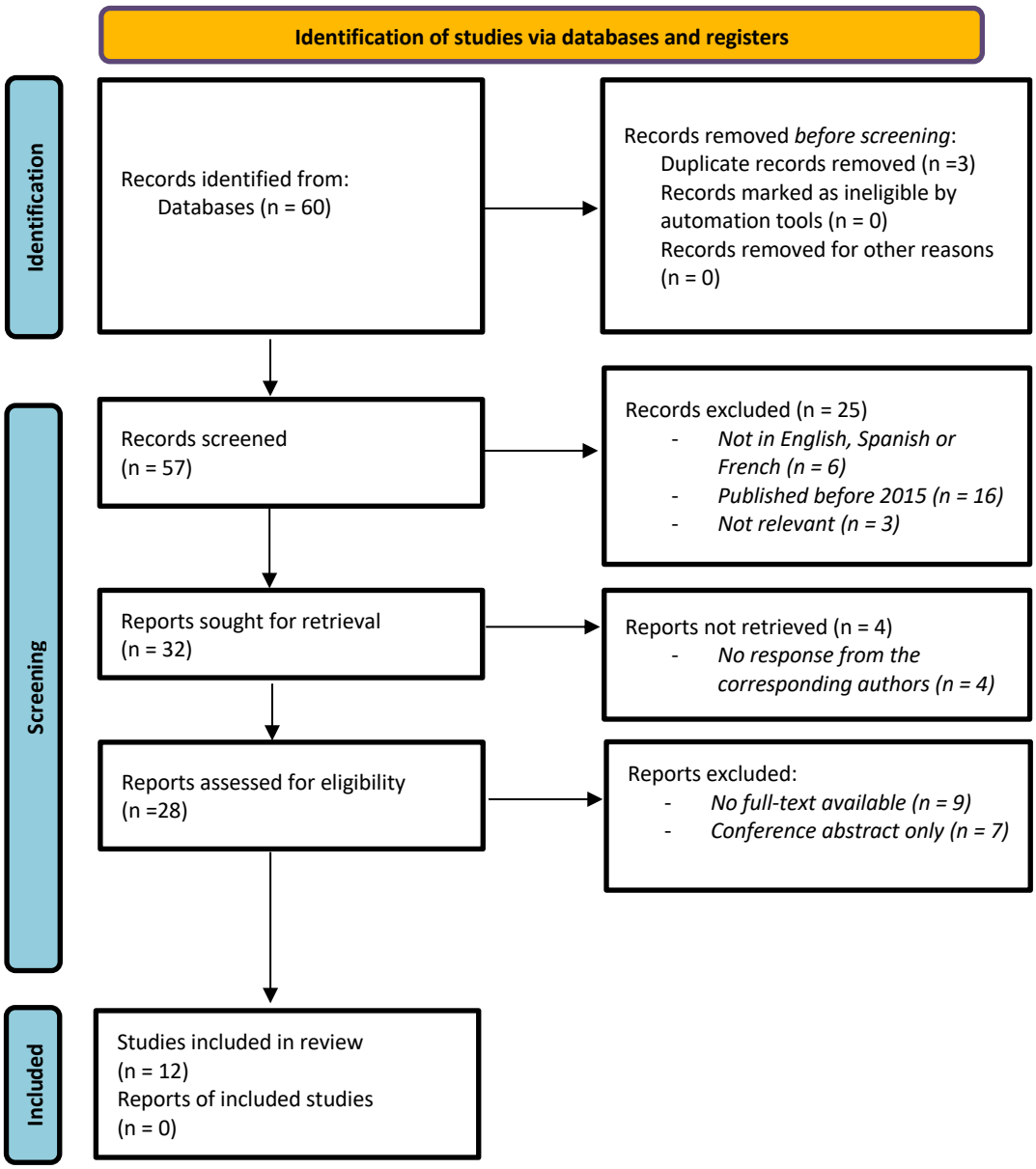


Figure 2. PRISMA 2020 flow diagram.
Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD*, et al.* The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

4.2. Summary of the results

The results are shown in Tables 3, 4, 5 and 6.

Table 3. Characteristics of the included studies

Reference	Study type	Sample size (Healthy sites/ PI sites)	Type of omics	Main findings
Sanz-Martin *et al.* (2017) (52).	Cross-sectional	Healthy (n = 32) / PI (n = 35)	16s rRNA sequencing.	Significant compositional differences between healthy and PI sites, with identification of a distinct disease-associated core microbiome.
Ghensi *et al. (*2020) (56).	Observational cross-sectional cohort	Healthy (n = 35) / PI (n = 41)	Shotgun metagenomics sequencing (microbiome).	Distinct, site-specific PI microbiome with reduced diversity and convergence toward a defined disease-associated profile.
Joshi *et al.* (2025) (17).	Cross-sectional with validation cohort	Healthy (n = 24) / PI (n = 24) Validation cohort: healthy (n = 34) / PI (n = 34)	Full-length 16s + metatranscriptomics (RNAseq; EC/KEGG) + ML.	Integrated full-length 16S and metatranscriptomic analyses revealed distinct taxonomic and functional shifts in PI, with improved disease classification when combining DNA and RNA profiles.

Reference	Study type	Sample size (Healthy sites / PI sites)	Type of omics	Main findings
Komatsu *et al.* (2020) (38).	Cross-sectional	N/A / N/A (21 patients with both PI and periodontitis; no healthy implant controls)	Shotgun metagenomics + metatranscriptomics (public data) + network + RNA/DNA activity comparison.	Taxonomic and functional potentials are largely similar; PI exhibited a distinct and more condensed transcriptional co-occurrence network compared with PT.
Yu *et al.* (2024) (6).	Cross-sectional with a short-term longitudinal component	Healthy (n = 18) / PI (n = 22)	Long read 16S rRNA	Full-length 16S rRNA sequencing revealed distinct core microbiome profiles between PI and PT, with limited short-term microbiome modification following nonsurgical peri-implant therapy.
Song *et al.* (2024) (57).	Cross-sectional	Healthy (n = 10) / PI (n = 10)	Shotgun metagenomics	Shotgun metagenomic analysis revealed distinct taxonomic and functional profiles in PI, with microbial composition influenced by the periodontal environment.

Reference	Study type	Sample size (Healthy sites / PI sites)	Type of omics	Main findings
Bessa *et al.* (2025) (3).	Observational cross-sectional pilot study	Healthy (n = 20) / PI (n = 20)	Shotgun metagenomics.	Shotgun metagenomic profiling revealed distinct taxonomic and functional shifts in PI, with microbiome alterations extending to clinically healthy implants and saliva within the same oral cavity.
Ganesan *et al.* (2022) (18).	Cross-sectional pilot study	Healthy (n = 5) / PI (n = 5)	Dual transcriptomic (host + microbiome).	Dual host–microbial transcriptomic analysis revealed disruption of homeostatic host–bacterial interactions in PI, consistent with a shift toward a chronic, non-healing wound environment.
Bazzani *et al.* (2024) (58).	Longitudinal	Healthy (n = 32) / PI (n = 31) Pre / Post treatment cohort	Shotgun metagenomics.	Longitudinal shotgun metagenomics demonstrated that clinical treatment induces a shift of the PI plaque microbiome toward a health-associated configuration, with site-specific and partially reversible dynamics.

Reference	Study type	Sample size (Healthy sites / PI sites)	Type of omics	Main findings
Ghensi *et al.* (2025) (59).	Observational cross-sectional	Healthy (n = 31) / PI (n = 31)	Shotgun metagenomics + ML.	Expanded shotgun metagenomic confirmed strong, condition-specific microbiome signatures across peri-implant health states, with PI exhibiting a convergent and less variable dysbiotic configuration.
Szafranski *et al.* (2025) (19).	Observational cross-sectional	Healthy (n = 56) / PI (n = 32)	Full-length 16s + Metatranscriptomics.	Integrative multi-omics profiling revealed distinct ecological community types and coordinated microbial–phage–host activity patterns across peri-implant health and PI.
Fedre *et al.* (2025) (60).	Observational cross-sectional	Healthy (n = 10) / PI (n = 10)	Metatranscriptomics.	Cross-sectional metatranscriptomic profiling revealed a functionally reprogrammed and transcriptionally distinct microbiome in PI, characterized by increased microbial diversity and a pathogenic activity shift compared with healthy implants.

Abbreviation: EC = Enzyme classes, KEGG = Kyoto Encyclopedia of Genes and Genomes, ML = machine learning

Table 4. Microbial signatures associated with peri-implantitis.

ID's	Comparison	Increased taxa in PI	Decreased taxa in PI
Sanz-Martin *et al.* (2017) (52).	Healthy vs PI.	*Porphyromonas, Tannerella, Treponema, Filifactor.*	*Rothia, Neisseria.*
Ghensi *et al.* (2020) (56).	Healthy vs PI.	Disease-associated strain-level anaerobic consortium.	Reduced health-associated variability.
Joshi *et al.* (2025) (17).	Healthy vs PI.	*Prevotella, Porphyromonas, Treponema, Fusobacterium.*	*Streptococcus spp.*
Komatsu *et al.* (2020) (38).	PI vs PT.	*Solobacterium moorei, Prevotella denticola (high activity).*	—
Yu *et al.* (2024) (6).	PI vs control implants (CI); also compared with PT and control teeth (CT).	*Treponema denticola* (predominant, especially PD 8–10 mm); novel associations: *Thermovirga lienii*, *Dialister invisus*.	Health-associated species enriched in CI/CT: *Rothia aeria*, *Neisseria spp.*,
Song *et al.* (2024) (57).	Healthy vs. PI ± PT.	Red complex species (*P. gingivalis*, *T. forsythia*, *T. denticola*) and *P. endodontalis*; additional enrichment of *Prevotella spp.*, *F. alocis*, *C.*	Health-associated commensals including *Actinomyces spp.;* ↑ Actinobacteria in healthy sites.
Bessa *et al.* (2025) (3).	Healthy vs PI; intra-subject healthy implants in PI patients also analysed.	Dysbiotic anaerobic taxa; ecosystem-wide shift.	Partial microbiome shift in healthy implants.

ID's	Comparison	Increased taxa in PI	Decreased taxa in PI
Ganesan *et al.* (2022) (18).	Healthy vs PI (paired intra-subject implants).	Greater abundance and transcriptional activity of diverse Gram-negative taxa (including *Proteobacteria, Bacteroidetes, Spirochaetes*); overall ↑ microbial diversity in PI sites.	Health-associated commensal taxa with predominant metabolic activity in healthy sites; lower diversity compared to PI.
Bazzani *et al.* (2024) (58).	Pre / post PI.	Disease-associated taxa decreased after successful therapy; "favorable shifts" in subgingival plaque microbiome.	Increase in health-associated commensals after treatment (directional shift).
Ghensi *et al.* (2025) (59).	Healthy vs PI.	Convergent, low variability dysbiotic signature.	Health-specific variability lost.
Szafranski *et al.* (2025) (19).	Healthy vs PI.	*Dialister, Prevotella, Parvimonas, Mogibacterium.*	*Streptococcus* dominant in health.
Fedre *et al.* (2025) (60).	Healthy vs PI.	Increased diversity; pathogenic transcript-associated taxa.	Health-associated commensals.

Abbreviations: ID's: identification.

Table 5. Functional and metatranscriptomic signatures in peri-implantitis.

ID's	Microbial functional shift in PI	Virulence-associated markers	Host interaction / immune response signatures	Health – associated functional profile
Joshi *et al.* (2025) (17).	↑ Amino acid catabolism (histidine, lysine, tryptophan); ↑ NADH: ubiquinone reductase; ↑ phosphoenolpyruvate carboxykinase.	Urocanate hydratase; tripeptide aminopeptidase.	Coordinated microbial metabolic activation linked with inflammatory environment.	↑ Carbohydrate metabolism; ↑ anabolic pathways.
Komatsu *et al.* (2020) (38).	Altered RNA/DNA activity ratios; condensed transcriptional co-occurrence network.	↑ GAPDH (plasmin receptor); adhesion/invasion-associated enzymes.	Functional activity differentiated PI from PT.	—
Song *et al.* (2024) (57).	↑ Flagellar assembly pathways; ↑ motility genes.	Motility-associated virulence pathways.	—	—
Ganesan *et al.* (2022) (18).	Disruption of microbial homeostasis.	—	↑ Host inflammatory transcripts; dysregulated immune pathways; chronic wound-like profile.	Balanced host–microbial transcriptome.
Bazzani *et al.* (2024) (58).	Post-treatment metabolic shift toward health-associated configuration.	Reduction of dysbiotic functional expression after therapy.	Functional normalization after treatment.	Restoration of health-like pathways.

ID's	Microbial functional shift in PI	Virulence-associated markers	Host interaction / immune response signatures	Health – associated functional profile
Ghensi *et al.* (2025) (59).	Condition-specific metabolic clustering; reduced functional variability in PI.	Dysbiotic metabolic convergence.	—	Higher metabolic variability in health.
Szafranski *et al.* (2025) (19).	↑ Catabolic metabolism; ↑ amino acid degradation; ↑ hypoxia-related pathways; ↑ phage–bacteria interactions.	↑ Virulence-associated anaerobic activity; complex biofilm morphology.	↑ Host inflammatory signaling; ↑ ribosomal components (PIM); ↑ hypoxia response in PI.	Aerotolerant taxa; ↑ anabolic metabolism.
Fedre *et al.* (2025) (60).	Functional reprogramming toward pathogenic metabolism.	↑ Proteolytic and pathogenic transcriptional signatures.	Inflammatory microenvironment-associated expression.	Homeostatic metabolic balance.

Abbreviations: PIM = peri-implant mucositis.

Table 6. Biomarker potential and diagnostic discrimination.

ID's	Biomarkers type	Diagnostic performance / relevance	Validation method
Joshi *et al.* (2025) (17).	Combined taxonomic + enzymatic RNA markers.	AUC ≈ 0.85	Training + independent validation cohort; ML classifier.
Komatsu *et al.* (2020) (38).	RNA/DNA activity ratios.	Functional differentiation PI vs PT.	Integrated network + comparative analysis.
Ganesan *et al.* (2022) (18).	Dual host–microbial transcriptomic pattern.	Distinct inflammatory profile.	Differential expression analysis.

ID's	Biomarkers type	Diagnostic performance / relevance	Validation method
Bazzani *et al.* (2024) (58).	Microbiome shifts after treatment.	Functional normalization.	Longitudinal pre/post comparison.
Ghensi *et al.* (2025) (59).	Shotgun microbiome signature.	High discriminatory power.	Machine learning modelling.
Szafranski *et al.* (2025) (19).	Community type IV + catabolic signature.	Clear separation PI vs health.	Statistical clustering (SIMPROF, PERMANOVA).

5. DISCUSSION

5.1. Integrated microbial and functional reprogramming in peri-implantitis

This systematic review aimed to determine whether microbiome and metatranscriptome analyses can identify discriminatory microbial and functional signatures capable of distinguishing PI from clinically healthy peri-implant sites.

Across the 12 included studies, a consistent pattern emerged: PI is characterized not only by taxonomic dysbiosis but also by coordinated functional reprogramming of the peri-implant biofilm, suggesting that disease involves both ecological disruption and metabolic adaptation within the microbial community.

Taxonomic convergence toward dysbiosis

Multiple metagenomic investigations demonstrated enrichment of anaerobic and proteolytic taxa in I, including *Porphyromonas, Treponema, Prevotella, Fusobacterium, Dialister* and *Parvimonas* species (6,17,19,28,52,59). Early illumina-based analyses identified enrichment of red-complex species together with depletion of health-associated genera such as *Rothia* and *Neisseria* (52), while subsequent strain-level shotgun metagenomics studies confirmed disease-specific microbial configurations (56,59).

Importantly, Ghensi *et al.* (2020, 2025) (56,59) reported that PI microbiomes exhibited reduced inter-individual variability and converged toward a more homogeneous dysbiotic structure. This ecological convergence suggests that PI may represent a stable disease-associated microbial state rather than random compositional perturbation.

However, no single pathogen was universally identified across all studies. The absence of a standardized microbial signature likely reflects the polymicrobial nature of PI, substantial inter-individual microbiome variability and the influence of environmental and host-related modifiers.

Functional metabolic specialization

Beyond compositional shifts, metatranscriptomic studies revealed that PI is marked by increased expression of metabolic and virulence-associated pathways.

Joshi *et al.* (2025) (17) demonstrated upregulation of amino acid catabolism pathways (histidine, lysine, tryptophan), NADH:ubiquinone oxidoreductase, and phosphoenolpyruvate carboxykinase, achieving high predictive accuracy (AUC ≈ 0.85) when taxonomic and enzymatic

markers were combined. Similarly, Szafranski *et al.* (2025) (19) identified enhanced catabolic metabolism, hypoxia-related pathways and virulence-associated anaerobic activity in PI lesions. Komatsu *et al.* (2020) (38) further demonstrated altered RNA/DNA activity ratios and a condensed transcriptional co-occurrence network in PI compared with PT, suggesting tighter functional coordination within dysbiotic peri-implant biofilms.

Collectively, these findings indicate that PI is associated with metabolic specialization favouring proteolysis, hypoxic adaptation and inflammatory metabolite production. Notably, functional signatures appeared more consistent across studies than purely taxonomic profiles, supporting the concept that disease activity may be more reliably characterized at the pathway level than at the species level.

5.2. Host-microbiome interaction and systems-level disease

Several investigations indicated that PI cannot be interpreted solely as a microbial infection but rather as a host–microbiome network disorder characterized by reciprocal interactions between dysbiotic biofilms and host inflammatory responses.

Ganesan *et al.* (2022) (18) performed dual host–microbial transcriptomics analysis and identified disruption of homeostatic host-microbe interactions, with increased host inflammatory gene expression and a chronic wound-like molecular profile in PI. Similarly, Szafranski *et al.* (2025) (19) reported coordinated microbial-phage-host transcriptional activity and increased hypoxia-related host signalling in peri-implant disease.

These findings support a systems biology model in which microbial metabolic reprogramming and host immune activation occur simultaneously and reinforce one another. Within this framework, inflammatory host responses may create a hypoxic and nutrient-rich environment that promotes anaerobic microbial metabolism, while microbial virulence factors further amplifies host-mediated tissue destruction. Consequently, PI may be better conceptualized as a self-perpetuating dysbiotic–inflammatory feedback loop rather than a unidirectional infectious process.

5.3. Diagnostic potential of multi-omics biomarkers

A central objective of this review was to assess whether metagenomic and metatranscriptomic signatures demonstrate potential as diagnostic biomarkers. Several studies provided encouraging evidence supporting this hypothesis.

Joshi *et al.* (2025) (17) reported strong discriminatory capacity (AUC ≈ 0.85) using machine-learning models integrating DNA- and RNA-derived markers, while Ghensi *et al.* (2025)

(59) demonstrate improved biomarkers performance through shotgun metagenomics coupled with predictive modeling. Longitudinal data further support the biological relevance of these findings. Bazzani *et al.* (2024) (58) showed that successful peri-implant therapy was associated with a shift toward a health-associated microbial configuration, indicating that microbiome signatures are dynamic and responsive to treatment.

Importantly, functional biomarkers such as urocanate hydratase, tripeptide aminopeptidase and virulence-associated enzyme appear to provide greater biological specificity than compositional markers alone (17,19,38). This suggests that RNA-based profiling may offer superior diagnostic resolution by identifying metabolically active disease processes rather than simply microbial presence.

However, these promising diagnostic performance metrics should be interpreted with caution. Most predictive models were developed using relatively small study populations and have not undergone extensive external validation, which raises concerns about potential overfitting and limited generalizability. Consequently, the diagnostic utility of these biomarkers in routine clinical practice remains preliminary and requires confirmation in larger, independent multicenter cohorts.

5.4. Comparison with conventional inflammatory biomarkers

Traditional peri-implant diagnostic markers, including IL-1β and MMP-8, primarily reflect host inflammatory activity and have demonstrated clinical utility in identifying peri-implant disease (48,50). However, these markers predominantly detect established inflammatory burden and provide limited insight into microbial drivers or metabolic states.
In contrast, microbiome and metatranscriptome analyses can identify:

- Ecological dysbiosis
- Functional virulence activation
- Community-level metabolic specialization
- Coordinated host-microbial transcriptional responses

Accordingly, omics-based biomarkers may complement rather than replace conventional inflammatory markers. An integrated multi marker panel combining host inflammatory mediators with microbial functional signature could theoretically improve both biological specificity and early diagnostic sensitivity.

Nevertheless, despite their superior mechanistic resolution, omics-based approaches currently remain substantially more expensive, technically demanding and less accessible than

conventional biomarker assays. Their clinical translation will therefore depend on simplification into targeted, cost-effective diagnostic platforms suitable for chairside implementation.

5.5. Limitations of the current evidence

Despite promising findings, several important methodological limitations must be acknowledged.

First, most included studies were cross-sectional (6,17,19,28,38,52,56,59,60), limiting causal interference. Observed microbial and transcriptional signatures demonstrate association with disease but do not establish whether these changes precede clinical PI or arise secondarily to inflammatory tissue breakdown.

Second, sample sizes varied considerably and several transcriptomic investigations included relatively small cohort (18,60), thereby limiting statistical power and generalizability.

Third, substantial methodological heterogeneity exists across studies. Differences are present in sequencing technologies, including short-read and long-read 16S sequencing, shotgun metagenomics, and RNA-seq, as well as in bioinformatic pipelines and diagnostic definitions of PI. This variability complicates direct comparison between studies and likely contributes to the absence of a universally reproducible microbial signature.

Additionally, potentially important confounding variables including smoking status, history of periodontitis, implant surface characteristics, prosthetic design and maintenance protocols were inconsistently controlled across studies and may independently influence microbiome composition.

Finally, external validation cohorts remain limited. Only a limited subset of studies incorporated machine-learning validation strategies (17,59).

5.6. Future perspectives: toward precision implantology

The cumulative evidence suggests that PI represents a convergent dysbiotic and functionally microbial state characterized by ecological collapse and coordinated metabolic adaptation.

Future research should prioritize:

- Large scale longitudinal validation studies
- Standardization of sequencing and bioinformatic workflows
- Integration of metagenomics, metatranscriptomics, proteomics and metabolomics
- Reduction of complex omics signatures into clinically deployable targeted biomarkers panels

Although metatranscriptomics is not yet feasible as a routine chairside use. Its value in biomarker discovery is significant. Translation into simplified RT-qPCR based enzyme panels or targeted molecular analysis could represent a realistic pathway toward precision implantology.

5.7. Overall interpretation

This review demonstrates that microbiome and metatranscriptome analyses consistently identify disease associated ecological and functional signatures in PI. Functional reprogramming, particularly increased amino acid catabolism, hypoxia adaptation and virulence associated transcription, appears central to disease activity and may represent a more reproducible disease defining feature than taxonomic composition alone.

Although current evidence remains predominantly associative and methodologically heterogenous, integration of DNA- and RNA-derived data provides biologically meaningful discrimination between peri-implant health and disease.

Taken together, these findings support the emerging potential of multi-omics biomarkers to improve early diagnosis, therapeutic monitoring and personalized management of PI. However, substantial methodological, translational and validation challenges remain before omics-based diagnostics can be integrated into routine clinical implant practice. Nonetheless, these advances represent an important step toward precision implant dentistry.

6. CONCLUSIONS

This systematic review demonstrates that microbiome and metatranscriptome analyses can identify distinct microbial and functional signatures that differentiate peri-implantitis from clinically healthy peri-implant sites. Peri-implantitis is consistently associated with enrichment of anaerobic dysbiotic taxa and functional metabolic reprogramming characterized by increased virulence, amino acid catabolism, hypoxia-related activity, and coordinated host–microbial inflammatory responses.

These findings support the potential of microbiome- and metatranscriptome-derived signatures as emerging diagnostic biomarkers for peri-implantitis and suggest that they may offer greater biological specificity than conventional inflammatory markers by reflecting active disease-related microbial function. However, their clinical translation remains limited by methodological heterogeneity, insufficient external validation, and the predominance of cross-sectional evidence.

Overall, multi-omics biomarkers represent a promising step toward precision diagnostics in peri-implantitis and may contribute to more personalized diagnostic and therapeutic strategies

in implant dentistry, provided that future longitudinal and standardized validation studies confirm their clinical utility.

7. SUSTAINABILITY

The development of microbiome and metatranscriptome-based biomarkers for peri-implantitis contributes to a more sustainable and responsible model of oral healthcare. From an economic perspective, these molecular diagnostic tools promote early detection and personalized treatment, reducing the need for invasive or repetitive procedures, thereby lowering healthcare costs and optimizing resource use (SDG 3: Good Health and Well-being). On an environmental level, precision diagnostics help minimize the consumption of antibiotics and clinical materials by enabling targeted therapies, which supports a reduction in medical waste and antimicrobial resistance, an essential aspect of sustainable medical practice (SDG 12: Responsible Consumption and Production). In terms of social sustainability, this research encourages equitable access to innovative healthcare, improving patient's quality of life and long-term oral health outcomes. Moreover, integrating sustainability principles into biomedical research promotes ethical awareness, responsible data use, and interdisciplinary collaboration. Overall, this project aligns with the global agenda for sustainable development by advancing efficient, ethical, and environmentally conscious dental healthcare.

8. BIBLIOGRAPHY

1. Jung RE, Pjetursson BE, Glauser R, Zembic A, Zwahlen M, Lang NP. A systematic review of the 5-year survival and complication rates of implant-supported single crowns. Clin Oral Implants Res. 2008;19(2):119–30.

2. Diaz P, Gonzalo E, Villagra LJG, Miegimolle B, Suarez MJ. What is the prevalence of peri-implantitis? A systematic review and meta-analysis. BMC Oral Health. 2022;22(1):449.

3. Bessa LJ, Egas C, Pires C, Proença L, Mascarenhas P, Pais RJ, et al. Linking peri-implantitis to microbiome changes in affected implants, healthy implants, and saliva: a cross-sectional pilot study. Front Cell Infect Microbiol. 2025;15:1543100.

4. Li Q, Lu H, Zhang M, Ye Y, Chen Q, Sun P. Epigenetic factors associated with peri-implantitis: a review. J Zhejiang Univ-Sci B. 2025;26(7):657–74.

5. Anuntakarun S, Thaweesapphithak S, Krasaesin A, Prommanee S, Arunyanak S, Kungsadalpipob K, et al. Microbiome shifts in peri-implantitis: Longitudinal characterization of dysbiosis and resolution. Int Dent J. 2025;75(5):100951.

6. Yu P, Tu C, Wara-aswapati N, Wang C, Tu Y, Hou H, et al. Microbiome of periodontitis and peri-implantitis before and after therapy: Long-read 16S rRNA gene amplicon sequencing. J Periodontal Res. 2024;59(4):657–68.

7. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Periodontol. 2018;89 (Suppl 1):S1-8. Available from: https://aap.onlinelibrary.wiley.com/doi/10.1002/JPER.18-0157

8. Hong I, Koo KT, Oh SY, Park HW, Sanz-Martín I, Cha JK. Comprehensive treatment protocol for peri-implantitis: an up-to date narrative review of the literature. J Periodontal Implant Sci. 2024;54(5):295.

9. Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM, et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol [Internet]. 2018 [cited 2025 Oct 29];45(S20). Available from: https://onlinelibrary.wiley.com/doi/10.1111/jcpe.12957

10. Heitz-Mayfield LJA, Salvi GE. Peri-implant mucositis. J Clin Periodontol [Internet]. 2018 [cited 2025 Oct 28];45(S20). Available from: https://onlinelibrary.wiley.com/doi/10.1111/jcpe.12953

11. Heitz-Mayfield LJA. Peri-implant mucositis and peri-implantitis: key features and differences. Br Dent J. 2024;236(10):791–4.

12. Benedek C, Kerekes-Máthé B, Bereșescu L, Buka IZ, Bardocz-Veres Z, Geréb I, et al. Influencing factors regarding the severity of peri-implantitis and peri-Implant mucositis. Diagnostics. 2024;14(14):1573.

13. Astolfi V, Ríos-Carrasco B, Gil-Mur FJ, Ríos-Santos JV, Bullón B, Herrero-Climent M, et al. Incidence of peri-implantitis and relationship with different conditions: A retrospective study. Int J Environ Res Public Health. 2022;19(7):4147.

14. Ríos-Osorio N, Ladino LG, Guerrero-Torres M. Structure, biology, and function of peri-implant soft tissues in health and disease: a comprehensive review of the literature. J Periodontal Implant Sci. 2025;55:e10.

15. Chan MH, Kang J. Diagnosis and treatment of periimplant mucositis and peri-implantitis. Dent Clin North Am. 2024;68(1):167–202.

16. Dreyer H, Grischke J, Tiede C, Eberhard J, Schweitzer A, Toikkanen SE, et al. Epidemiology and risk factors of peri-implantitis: A systematic review. J Periodontal Res. 2018;53(5):657–81.

17. Joshi AA, Szafrański SP, Steglich M, Yang I, Qu T, Schaefer-Dreyer P, et al. Integrative microbiome- and metatranscriptome-based analyses reveal diagnostic biomarkers for peri-implantitis. Npj Biofilms Microbiomes. 2025;11(1):175.

18. Ganesan SM, Dabdoub SM, Nagaraja HN, Mariotti AJ, Ludden CW, Kumar PS. Biome-microbiome interactions in peri-implantitis: A pilot investigation. J Periodontol. 2022;93(6):814–23.

19. Szafranski SPD. High-resolution taxonomic profiling and metatranscriptomics identify microbial, biochemical, host and ecological factors in peri-implant disease. 2025;

20. Salvi GE, Stähli A, Imber J, Sculean A, Roccuzzo A. Physiopathology of peri-implant diseases. Clin Implant Dent Relat Res. 2023;25(4):629–39.

21. Becker ST, Beck-Broichsitter BE, Graetz C, Dörfer CE, Wiltfang J, Häsler R. Peri-Implantitis versus periodontitis: functional differences indicated by transcriptome profiling. Clin Implant Dent Relat Res. 2014;16(3):401–11.

22. Kensara A, Saito H, Mongodin EF, Masri R. Microbiological profile of peri-implantitis: Analyses of peri-implant microbiome. J Prosthodont. 2024;33(4):330–9.

23. La Monaca G, Pranno N, Patini R, Polimeni A, Cordaro M, Cristalli MP. Biomarkers in peri-implant crevicular fluid of healthy implants and those with peri-implant diseases: A systematic review and meta-analysis. J Oral Pathol Med. 2025;54(5):267–82.

24. Zhang X, Wang Z, Hu L, Shen X, Liu C. Identification of Potential Genetic Biomarkers and Target Genes of Peri-Implantitis Using Bioinformatics Tools. De Molon RS, editor. BioMed Res Int. 2021;2021(1):1759214.

25. Santostasi N. Relationship between interleukin 1 (IL-1) and peri-implantitis: systematic literature review and meta-analysis. 2022 [cited 2025 Oct 28]; Available from: https://osf.io/chupt/

26. Li Y, Li X, Guo D, Meng L, Feng X, Zhang Y, et al. Immune dysregulation and macrophage polarization in peri-implantitis. Front Bioeng Biotechnol. 2024;12:1291880.

27. Corrêa MG, Pimentel SP, Ribeiro FV, Cirano FR, Casati MZ. Host response and peri-implantitis. Braz Oral Res. 2019;33(suppl 1):e066.

28. Song Z, Cheng Y, Chen M, Xie X. Macrophage polarization in bone implant repair: A review. Tissue Cell. 2023;82:102112.

29. Yu YM, Lu YP, Zhang T, Zheng YF, Liu YS, Xia DD. Biomaterials science and surface engineering strategies for dental peri-implantitis management. Mil Med Res. 2024;11(1):29.

30. Shiels S, Mangum L, Wenke J. Revisiting the "race for the surface" in a pre-clinical model of implant infection. Eur Cell Mater. 2020;39:77–95.

31. Alexander R, Liu X. Soft tissue integration around dental implants: A pressing priority. Biomaterials. 2025;324:123491.

32. Monje A, Barootchi S, Rosen PS, Wang H. Surgical- and implant-related factors and onset/progression of peri-implant diseases: An AO/AAP systematic review. J Periodontol. 2025;96(6):542–61.

33. Araujo MG, Lindhe J. Peri-implant health. J Periodontol [Internet]. 2018 [cited 2025 Oct 31];89(S1). Available from: https://aap.onlinelibrary.wiley.com/doi/10.1002/JPER.16-0424

34. Tallarico M, Canullo L, Caneva M, Özcan M. Microbial colonization at the implant-abutment interface and its possible influence on peri-implantitis: A systematic review and meta-analysis. J Prosthodont Res. 2017;61(3):233–41.

35. Di Spirito F, Giordano F, Di Palo MP, D'Ambrosio F, Scognamiglio B, Sangiovanni G, et al. Microbiota of Peri-Implant Healthy Tissues, Peri-Implant Mucositis, and Peri-Implantitis: A Comprehensive Review. Microorganisms. 2024;12(6):1137.

36. Gazil V, Bandiaky ON, Renard E, Idiri K, Struillou X, Soueidan A. Current Data on Oral Peri-Implant and Periodontal Microbiota and Its Pathological Changes: A Systematic Review. Microorganisms. 2022;10(12):2466.

37. Renvert S, Persson GR, Pirih FQ, Camargo PM. Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Periodontol [Internet]. 2018 [cited 2025 Oct 31];89(S1). Available from: https://aap.onlinelibrary.wiley.com/doi/10.1002/JPER.17-0588

38. Komatsu K, Shiba T, Takeuchi Y, Watanabe T, Koyanagi T, Nemoto T, et al. Discriminating Microbial Community Structure Between Peri-Implantitis and Periodontitis With Integrated Metagenomic, Metatranscriptomic, and Network Analysis. Front Cell Infect Microbiol. 2020;10:596490.

39. Caselli E, Fabbri C, D'Accolti M, Soffritti I, Bassi C, Mazzacane S, et al. Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. BMC Microbiol. 2020;20(1):120.

40. Deo P, Deshmukh R. Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23(1):122.

41. Aguiar-Pulido V, Huang W, Suarez-Ulloa V, Cickovski T, Mathee K, Narasimhan G. Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis: Supplementary Issue: Bioinformatics Methods and Applications for Big Metagenomics Data. Evol Bioinforma. 2016;12s1:EBO.S36436.

42. Condor AM, Kui A, Condor DC, Negucioiu M, Buduru SD, Lucaciu PO. Metabolomics Applications for Diagnosing Peri-Implantitis: A Systematic Review of In Vivo Studies. Diagnostics. 2025;15(8):990.

43. Ardila CM, Pineda-Vélez E, Vivares-Builes AM. Transcriptomic and Metagenomic Biomarkers in Peri-Implantitis: A Systematic Review, Diagnostic Meta-Analysis, and Functional Meta-Synthesis. Med Sci. 2025;13(3):187.

44. Califf RM. Biomarker definitions and their applications. Exp Biol Med. 2018;243(3):213–21.

45. Strimbu K, Tavel JA. What are biomarkers?: Curr Opin HIV AIDS. 2010;5(6):463–6.

46. Ahmad A, Imran M, Ahsan H. Biomarkers as Biomedical Bioindicators: Approaches and Techniques for the Detection, Analysis, and Validation of Novel Biomarkers of Diseases. Pharmaceutics. 2023;15(6):1630.

47. Shah A, Grimberg DC, Inman BA. Classification of Molecular Biomarkers. Société Int D'Urologie J. 2020;1(1):8–15.

48. Lumbikananda S, Srithanyarat SS, Mattheos N, Osathanon T. Oral Fluid Biomarkers for Peri-Implantitis: A Scoping Review. Int Dent J. 2024;74(3):387–402.

49. Radu CM, Radu CC, Zaha DC. Salivary and Microbiome Biomarkers in Periodontitis: Advances in Diagnosis and Therapy—A Narrative Review. Medicina (Mex). 2025;61(10):1818.

50. Sachelarie L, Stefanescu CL, Murineanu RM, Grigorian M, Zaharia A, Scrobota I, et al. Role of Salivary Biomarkers IL-1β and MMP-8 in Early Detection and Staging of Periodontal Disease. Medicina (Mex). 2025;61(4):760.

51. Ardila CM, Vivares-Builes AM, Yadalam PK. Artificial Intelligence Models for Diagnosis of Periodontitis Using Non-Invasive Biological Markers: A Systematic Review and Meta-Analysis of Patient-Based Studies. Med Sci. 2025;13(3):159.

52. Sanz-Martin I, Doolittle-Hall J, Teles RP, Patel M, Belibasakis GN, Hämmerle CHF, et al. Exploring the microbiome of healthy and diseased peri-implant sites using Illumina sequencing. J Clin Periodontol. 2017;44(12):1274–84.

53. Duran-Pinedo A, Solbiati JO, Teles F, Yanping Z, Frias-Lopez J. Longitudinal host-microbiome dynamics of metatranscription identify hallmarks of progression in periodontitis. Microbiome. 2025;13(1):119.

54. Saxena A, Rastogi PK, Lal N, Verma UP, Singhal R, Ahmad MdK. Evaluating the role of inflammatory biomarkers as a diagnostic tool in peri-implantitis. J Oral Biol Craniofacial Res. 2025;15(2):228–33.

55. Moaven H, Giacaman A, Beltrán V, Sam YH, Betancur D, Mainas G, et al. Biomarker Expression of Peri-Implantitis Lesions before and after Treatment: A Systematic Review. Int J Environ Res Public Health. 2022;19(21):14085.

56. Ghensi P, Manghi P, Zolfo M, Armanini F, Pasolli E, Bolzan M, et al. Strong oral plaque microbiome signatures for dental implant diseases identified by strain-resolution metagenomics. Npj Biofilms Microbiomes. 2020;6(1):47.

57. Song L, Feng Z, Zhou Q, Wu X, Zhang L, Sun Y, et al. Metagenomic analysis of healthy and diseased peri-implant microbiome under different periodontal conditions: a cross-sectional study. BMC Oral Health. 2024;24(1):105.

58. Bazzani D, Heidrich V, Manghi P, Blanco-Miguez A, Asnicar F, Armanini F, et al. Favorable subgingival plaque microbiome shifts are associated with clinical treatment for peri-implant diseases. Npj Biofilms Microbiomes. 2024;10(1):12.

59. Ghensi P, Heidrich V, Bazzani D, Asnicar F, Armanini F, Bertelle A, et al. Shotgun Metagenomics Identifies in a Cross-Sectional Setting Improved Plaque Microbiome Biomarkers for Peri-Implant Diseases. J Clin Periodontol. 2025;52(7):999–1010.

60. Fedre RW, Ramakhrisnand R, Firman H, Mahmood A. Metatranscriptomic Profiling of the Subgingival Microbiome in Peri-implantitis versus Healthy Implants: Identifying Key Dysbiotic Pathways. Crown J Dent Health Res. 2025;3(1):38–51.