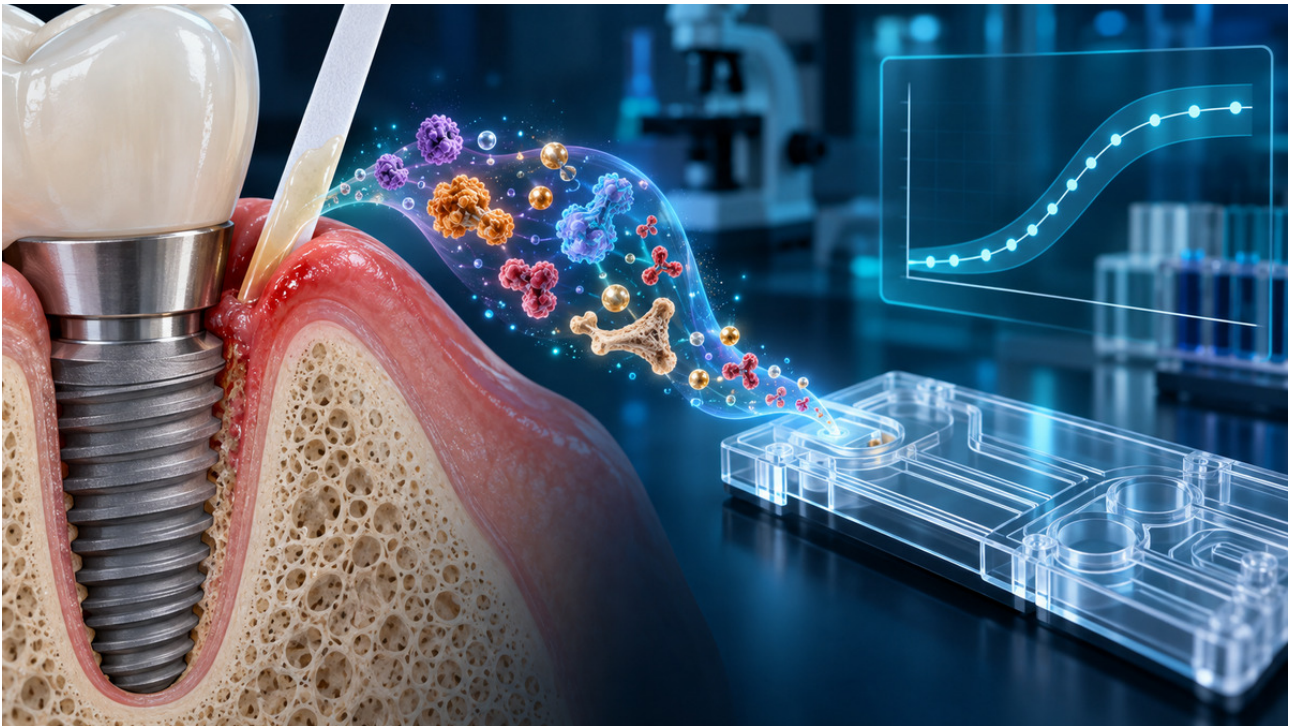


From Sampling to Signal: Biomarkers and Diagnostic Validation in Peri-implant Disease

Local fluids, systemic measures, point-of-care candidates and the evidence required for clinical translation



Core message: a biomarker becomes clinically useful only when the biological question, sampling matrix, analytical method and intended decision are aligned. Statistical separation between groups is not enough.

Educational material for dental students, postgraduate trainees and academic teachers

Clinical question determines biomarker role

Peri-implantitis is currently diagnosed from clinical inflammation and progressive supporting bone loss. Probing, bleeding and/or suppuration, soft-tissue dimensions and comparative radiography remain the diagnostic foundation. Biomarkers are being investigated because these measures often describe established tissue change rather than the molecular activity that precedes or accompanies it.

Different biomarker purposes

Purpose	Question	Required evidence
Diagnostic	Is disease present now?	Accuracy against a defined reference standard in the intended population.
Prognostic	Will the site progress or recur?	Longitudinal sampling before a prespecified future outcome.
Predictive	Who is more likely to benefit from a specific intervention?	Demonstration of treatment-by-marker interaction.
Monitoring	Has biological activity changed over time?	Repeatability, biological variation and responsiveness to meaningful clinical change.
Mechanistic	Which pathway is active?	Biological plausibility supported by tissue, fluid or experimental evidence.

The intended use must be defined before the assay is evaluated

A marker that distinguishes established peri-implantitis from health in a cross-sectional study may still fail to predict future bone loss. A marker that changes after treatment may reflect reduced inflammation without improving patient outcomes. Each claim requires a matching design and endpoint.

Diagnostic, prognostic, predictive and monitoring claims are not interchangeable. Performance in one role does not validate another.

Sampling matrix defines the biological window

The same analyte can have a different meaning depending on where it is measured. A local sample is closer to the peri-implant lesion, while a systemic sample integrates signals from many organs and exposures.

Matrix	Biological scope	Advantages	Main limitations
Peri-implant crevicular fluid (PICF/PISF)	Site-specific fluid next to the implant sulcus.	Minimally invasive; repeatable; close to local tissue activity.	Small volume; plaque, saliva or blood contamination; technique-sensitive.
Saliva	Whole-mouth mixture from glands, mucosa, teeth, implants and microbial communities.	Easy collection; suitable for screening and repeated sampling.	Dilution and poor site localization; influenced by oral and systemic conditions.
Serum or plasma	Systemic inflammatory and metabolic environment.	Standardized laboratory platforms; broad medical context.	Cannot identify the anatomical source of inflammation.
Complete blood count	Circulating immune-cell and platelet populations.	Accessible, inexpensive and suitable for composite indices.	Highly nonspecific; sensitive to infection, medication and systemic disease.
Peri-implant tissue	Cellular and spatial lesion biology.	Direct histological, transcriptomic and proteomic information.	Invasive and unsuitable for routine repeated monitoring.

PICF as a local compartment

PICF contains cytokines, chemokines, proteolytic enzymes and mediators of bone turnover. Its proximity to the site makes it attractive for local questions, but concentration can change with fluid volume and sampling duration. Reporting total amount and concentration may lead to different interpretations.

Local and systemic matrices answer different questions. A high serum marker cannot be assigned to an implant without supporting causal evidence.

Biomarker domains in peri-implant disease

Domain	Examples	Biological interpretation	Clinical limitation
Inflammatory cytokines	IL-1 beta, IL-6, TNF-alpha, IL-17	Immune activation and amplification of inflammatory signalling.	Concentrations overlap between health, mucositis and peri-implantitis.
Proteolytic enzymes	Active MMP-8, other MMPs, cathepsin K	Collagen and extracellular-matrix degradation.	Activity may occur in other oral inflammatory sites.
Bone-turnover mediators	RANKL, OPG, RANKL/OPG ratio, bone fragments	Osteoclast differentiation and balance of bone resorption.	Assays, thresholds and sampling protocols vary.
Vascular and stress markers	VEGF, cortisol	Vascular response and stress-related signalling.	Affected by systemic physiology and time of collection.
Microbial features	Species, virulence genes, community functions	Biofilm composition and functional potential.	Disease reflects dysbiosis; no single organism is diagnostic.
Systemic markers	CRP, IL-6, WBC, NLR, PLR, MLR, SII, AISI/PIV	Whole-body inflammatory context.	Not site-specific and vulnerable to confounding.
Omics signatures	Transcriptomic, proteomic and metagenomic panels	Multidimensional lesion or microbial phenotype.	Often high-dimensional, exploratory and not externally validated.

Group difference is not diagnostic performance

A statistically significant difference in mean concentration does not show that individual patients can be classified accurately. Distributions may overlap substantially. Diagnostic evaluation requires prespecified thresholds, blinded reference assessment, sensitivity, specificity, predictive values and validation in a clinically relevant spectrum of disease.

Biological relevance and clinical usefulness are separate tests. A mediator can be central to pathogenesis yet perform poorly as a stand-alone diagnostic marker.

Collection and analytical control

Preamerical variation can be as important as the biological signal. A robust biomarker study records the sampled surface, isolation method, strip position, collection time, visible contamination, fluid volume, storage temperature, freeze-thaw history and assay platform.

Source of variation	Potential effect	Control strategy
Sampling site	Different surfaces of one implant may have different activity.	Define the exact site and avoid unplanned pooling.
Collection duration and depth	Changes recovered volume and tissue contact.	Use a standardized time and insertion method.
Bleeding or saliva contamination	Introduces proteins and cells from another compartment.	Isolate the field and document exclusion criteria.
Fluid volume	Can dilute or concentrate the measured analyte.	Report volume and specify concentration versus total amount.
Storage and handling	Protein degradation and repeated freezing alter results.	Use a predefined processing and storage protocol.
Assay platform	Detection limits and antibody specificity differ.	Report manufacturer, calibration and analytical range.
Case definition	Changes which sites are labelled diseased.	Use consensus definitions and disclose thresholds.

Repeatability and biological variation

A clinically interpretable change must exceed expected analytical error and short-term biological fluctuation. Without repeatability data, a difference between two visits may reflect sampling noise rather than true change in lesion activity.

Normalization and pooling

Pooling several implant sites may improve sample volume but can dilute a focal active lesion. Normalizing to total protein or fluid volume may reduce some variation while introducing other assumptions. The chosen strategy should match the clinical question and be declared before analysis.

A precise assay cannot rescue an inconsistent sample. Standardization begins at the implant surface, not in the statistical software.

Point-of-care candidates and bone-related markers

Active matrix metalloproteinase-8

Active MMP-8 reflects collagenolytic activity and can be measured using chairside lateral-flow or quantitative point-of-care formats. Recent studies and systematic reviews report promising discrimination of peri-implantitis, but published thresholds and performance estimates depend on the population, clinical reference definition, fluid matrix and assay. aMMP-8 should therefore be treated as an adjunct rather than an independent replacement for probing and radiography.

RANKL, OPG and bone remodelling

RANKL promotes osteoclast differentiation, whereas osteoprotegerin acts as a decoy receptor that limits RANKL signalling. The RANKL/OPG relationship is biologically close to bone resorption and has shown differences between healthy and diseased peri-implant sites in meta-analytic work. Evidence remains limited by heterogeneous sampling and analytical protocols.

Candidate	Potential strength	Reason for caution
aMMP-8	Activity-related collagen degradation; chairside testing is feasible.	Not unique to peri-implantitis; threshold and external validation remain important.
IL-1 beta / IL-6	Frequently studied inflammatory mediators.	Substantial overlap among clinical states and method-dependent values.
RANKL / OPG	Direct relationship with osteoclast biology and bone remodelling.	Assay heterogeneity and inconsistent reporting of concentration or ratio.
Multiplex panels	Can represent several biological domains simultaneously.	Higher risk of overfitting; requires independent validation and calibration.

Point-of-care does not mean point-of-diagnosis

A rapid result is valuable only when it leads to a defined action that improves care. The test must have acceptable accuracy, reproducibility, usability and cost-effectiveness in the intended clinical setting.

Promising is not synonymous with validated. Chairside availability does not remove the need for a reference standard, threshold validation and clinical-impact testing.

Single markers, combinations and systemic adjuncts

Single-analyte limitations

Peri-implantitis involves microbial challenge, immune signalling, connective-tissue degradation and bone resorption. A single analyte rarely captures all phases. Its concentration may be high for several biological reasons, and a low value may reflect timing, sampling or pathway redundancy.

Multimarker panels

Combining pro-inflammatory, regulatory, proteolytic and bone-related markers can improve discrimination within a development dataset. Every additional variable also increases the risk of overfitting. Model complexity should be justified by improvement beyond clinical measures and confirmed in an independent cohort.

Blood-based inflammatory indices

CRP, IL-6, leukocyte counts and CBC-derived indices such as NLR, PLR, MLR, SII and AISI/PIV describe systemic inflammatory context. They do not localize the source. No universally accepted peri-implantitis-specific threshold has been validated for these indices, and acute infection, medication, autoimmune disease, cancer, obesity, diabetes and smoking can dominate the signal.

Approach	Useful question	Minimum evidence
Single local marker	Is one pathway more active at this site?	Repeatable assay and accuracy against a defined clinical reference.
Local multimarker panel	Does a biological signature improve classification?	Penalized model development, calibration and external validation.
Systemic marker or CBC index	Is systemic inflammatory background different?	Adjustment for systemic and behavioural confounders.
Integrated model	Does biomarker information add to clinical and radiographic data?	Demonstrated incremental value and clinical-impact analysis.

The relevant comparison is not biomarker versus nothing. It is biomarker plus standard assessment versus standard assessment alone.

Reading diagnostic-accuracy results

Sensitivity

Proportion of diseased cases correctly classified as positive.

How often does the test detect disease when disease is present?

Specificity

Proportion of non-diseased cases correctly classified as negative.

How often does the test remain negative when disease is absent?

Positive predictive value

Probability of disease after a positive result.

How convincing is a positive result in this tested population?

Negative predictive value

Probability of no disease after a negative result.

How reassuring is a negative result in this tested population?

ROC AUC

Overall discrimination across possible thresholds.

How well are diseased and non-diseased observations ranked within the study?

Predictive values depend on prevalence

Even when sensitivity and specificity remain stable, positive and negative predictive values change with disease prevalence. A test evaluated in a specialist peri-implantitis clinic may produce different predictive values when used for screening a low-risk maintenance population.

AUC is not enough

A high AUC does not guarantee good calibration, a clinically useful threshold or benefit to patients. Confidence intervals, spectrum of disease, missing data, threshold selection and the independence of the validation sample all affect interpretation.

Ask what decision the metric supports. Discrimination, calibration and clinical utility describe different properties of a test.

From discovery to clinical translation

Stage	Core requirement	Frequent failure
Analytical validation	Accuracy, precision, detection limits and stability of the assay.	Assuming a commercial platform performs identically in a new matrix.
Clinical validation	Performance in the intended patients against a defined reference standard.	Case-control sampling with unrealistically clear disease extremes.
External validation	Testing in a different centre, population and operator setting.	Reporting only internal cross-validation.
Incremental value	Improvement beyond probing, bleeding and radiography.	Comparing the biomarker with no clinical model.
Clinical utility	Evidence that using the result improves decisions or outcomes.	Equating statistical accuracy with patient benefit.

Prediction and treatment response

Prediction requires that biomarker sampling precedes future progression. Treatment-response research requires a marker that changes reproducibly when inflammation is controlled and whose change relates to meaningful clinical outcomes. Regression to the mean, natural fluctuation and concurrent systemic changes must be considered.

Precision diagnostics and machine learning

Transcriptomic, metagenomic and multi-omic signatures can identify candidate pathways and patient clusters. Machine-learning performance is easily inflated by small datasets, feature selection outside the validation loop and lack of external testing. Models should report calibration, clinically relevant comparators and transparent handling of missing data.

A practical evidence ladder

- Biological plausibility: the analyte participates in a relevant pathway.
- Analytical reliability: the assay measures the analyte accurately and reproducibly.
- Clinical association: values differ between defined groups.
- Diagnostic or prognostic validity: prespecified performance is confirmed.
- Incremental value: the marker improves assessment beyond existing methods.
- Clinical utility: marker-guided decisions improve care or outcomes.

Translation fails when one rung is skipped. Biological discovery is the beginning of validation, not its endpoint.

Clinical integration and selected references

Clinical and biological synthesis

- Use clinical inflammation and progressive bone loss as the diagnostic foundation.
- Choose the sampling matrix according to whether the question is local or systemic.
- Interpret analyte values in light of collection method, fluid volume and assay platform.
- Treat aMMP-8, bone-related mediators and multimarker panels as adjunctive candidates.
- Require external validation and incremental value before adopting a new diagnostic panel.
- Do not infer prognosis, treatment response or systemic causality from cross-sectional separation.

Selected references

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Educational use and limitations

This material presents current biomarker concepts and validation principles. It does not replace individual clinical assessment, laboratory interpretation or medical consultation.

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