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Review Article

Biomarkers: Carving a Niche in Personalized Periodontics

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Abstract

Periodontal disease is a chronic microbial infection that triggers inflammation-mediated loss of the periodontal ligament and alveolar bone that supports the teeth. Because of the increasing prevalence and associated comorbidities, there is a need for the development of new diagnostic tests that can detect the presence of active disease, predict future disease progression, and evaluate the response to periodontal therapy, thereby improving the clinical management of periodontal patients. The diagnosis of active phases of periodontal disease and the identification of patients at risk for active disease represent challenges for clinical investigators and practitioners. Advances in diagnostic research are moving toward methods whereby the periodontal risk can be identified and quantified by objective measures using biomarkers. Patients with periodontitis may have elevated circulating levels of specific inflammatory markers that can be correlated to the severity of the disease. Advances in the use of oral fluids as possible biological samples for objective measures of the current disease state, treatment monitoring, and prognostic indicators have boosted saliva- and other oral-based fluids to the forefront of technology. This article highlights recent Research methodology and advances in the use of biomarker-based disease diagnostics that focus on the identification of active periodontal disease from plaque biofilms, GCF, and saliva so as to move towards precision periodontics.

Keywords: *Periodontitis; Biomarkers; Diagnostic Aid; Precision; Research*

Introduction

Clinical parameters are key principles of periodontal care, which include regular disruption and reduction of the gingival and subgingival microbiota. Current evidence suggests, that some individuals are more susceptible to develop periodontitis and less responsive to standard bacterial control principles for preventing and treating periodontitis. Therefore, it is unclear whether current clinical parameters are sufficient to monitor disease development and treatment responses in such patients. Biomarkers may contribute to improve diagnostic accuracy in the early detection of periodontitis and are likely to provide decisive contributions to a better assessment of the grade of periodontitis. They may assist both in staging and grading of periodontitis. The proposed framework in 2017 classification allows introduction of validated biomarkers in the case definition system to highlight the potential of systemic impact of the disease in the specific case.

Study planning

From *in-vitro* to *in vivo* technically planned study designs can be done. Specifically randomised controlled trials can be executed with precise evaluation of changes in target biomarker. Improvements in the quality of RCTs are needed, which facilitate application of the

principles of personalized medicine. Larger sample size is preferable to show a significant clinical relevance of the study. Biomedical data from both healthy and sick people should be collected. Potentially, these data will assist in identifying potential risk factors, unknown links with other diseases and permit evaluation of treatment or intervention outcomes. This new field uses contemporary technology to analyse and systematically extract information from these massive data sets and is an emerging field in medicine.

Based on the mechanism which we want to alter or modify, we can select the specific biomarker. It can be either an Inflammatory mediator, Tissue destructive mediators or Bone remodelling mediators (Figure 1).

Type of Biomarker		Major effector functions	References
Inflammation	IL-1β	Potent proinflammatory stimulator Potent effects on cell proliferation, differentiation and function of many innate and specific immunocompetent cells Strong correlation with periodontal disease progression	(Kornman et al., 1997; Akdis et al., 2011)
	IL-6	Regulator of T- and B-cell growth Directs leukocyte trafficking Induces production of acute-phase protein Increase levels of periodontal disease	(Akdis et al., 2011; Lee et al., 2012)
	IL-10	Restriction of excessive inflammatory responses Upregulation of innate immunity and promotion of tissue repair mechanisms	(Ouyang and O'Garra, 2019)
	IL-8	Recruitment and activation of neutrophils Attracts NK cells, T cells and basophils	(Lee et al., 2012)
	TNF-α	Macrophage activation Inducing apoptosis of epithelial cells in the mucosa Regulates MHC class I and II protein and antigen presentation expression Stimulates gingival fibroblasts to produce collagenase	(Erdemir et al., 2004; Akdis et al., 2011)
	CRP	Increases rapidly in response to trauma, inflammation and infection Activates the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, production of cytokines	(Freitas et al., 2012)
	IFN-γ	Key cytokine in bridging innate and adaptive immune system Regulate MHC I and II class protein expression Inhibition of cells growth primarily by increasing levels of cyclin-dependent kinase inhibitors Proapoptotic affects	(Akdis et al., 2011)
	PGE2	Lipid mediator that regulates activation, maturation and cytokine secretion of several immune cells Induced during bacterial pathogenesis	(Agard et al., 2013)
Tissue destruction	MMP-8	Degradation of interstitial collagens Prevalent host proteinase in periodontal disease	(Kinney et al., 2007)
	MMP-9	Proteolytic degradation of extracellular matrix proteins Mediator of tissue destruction and immune responses in periodontal disease	(Cui et al., 2017)
	MMP-13	Expressed by epithelial cells during prolonged inflammation Efficiently degrading type II collagen	(Offenbacher et al., 2010)
	TIMP	Naturally occurring MMP inhibitor that bind MMPs in a 1:1 stoichiometry Decreased levels after periodontal treatment	(Pietruska et al., 2009; Cui et al., 2017)
	Cathepsin-B	Degrades extracellular components, type IV collagen, laminin and fibronectin	(Buck et al., 1992)
Bone remodeling	OPG	Decoy receptor for RANKL Inhibits osteoclast formation	(Bostanci et al., 2007)
	RANKL	Stimulates RANK on the surface of stem cells to form osteoclasts Regulation of bone destruction	(Bostanci et al., 2007)
	ICTP	Pyridinoline cross-links with high specificity for bone (compared to histidine cross-links for soft tissue and skin) Osteoclastic bone resorption initiates the release of cross-linked immunoreactive telopeptides	(Giannobile, 1999; Al-Shammari et al., 2001)
	Calprotectin	Antimicrobial and antifungal activities (improving resistance to *P. gingivalis*) Inhibits immunoglobulin production Neutrophil recruitment and production	(Kinney et al., 2007)
	Osteonectin	Affinity to collagen and hydroxyapatite leading to tissue mineralization Key role in remodeling and repair	(McCauley and Nohutcu, 2002)
	Osteocalcin	High concentration during bone turnover	(Giannobile et al., 1995)
	Osteopontin	Highly concentrated at sites where osteoclasts are attached to the underlying mineral surface Holds a dual function in bone maturation and mineralization as well as bone resorption Highly glycosylated extracellular matrix protein with levels in active sites of bone metabolism	(McCauley and Nohutcu, 2002; Kinney et al., 2007)

IL, Interleukin; NK cells, Natural killer cells; TNF, Tumor necrosis factor; CRP, C-reactive protein; MHC, Major histocompatibility complex; IFN, Interferon; PGE, Prostaglandin E; MMP, matrix metalloproteinases; TIMP, Tissue inhibitor of metalloproteinases; OPG, Osteoprotegerin; RANKL, Receptor activator of nuclear factor kappa-B ligand; ICTP, C-telopeptide pyridinoline cross-links.

Figure 1: *Types of biomarkers and it's functions.*

Study execution

The unmet diagnostic needs in periodontal diagnostics and related requests for a precision approach are as follows:

- Predictive markers measured in healthy individuals in the disease prevention stage.
- Diagnostic markers of disease onset; to estimate patient compliance with the administered treatment, stability of the therapy results, and disease activity in the maintenance phase.
- Prognostic markers for the assessment of disease progression, stage and grade in the treatment planning phase.

Data collection can be done using either static or dynamic biomarkers. Under static biomarkers we have genetic and histopathological biomarkers. Under dynamics we have biochemical and microbiological biomarkers (Figure 2). Recently molecular biology techniques are preferred due to its specificity. This technique includes polymerase chain reaction (PCR), DNA probes, checkerboards DNA hybridization, fluorescent *in-situ* hybridization (FISH), terminal restriction fragment length polymorphism (T-RFLP), 454 pyrosequencing, supported oligonucleotide ligation and detection (SOLiD).

Biomarker Classification	Sampling From	Product Name	Detecting Target	Detecting Principle	Analyzing in
Biochemical assay	GCF	Periocheck	Neutral proteases	Enzymatic digestion reaction (Colorimetric assays)	Chairside
	GCF	PocketWatch	AST	Enzymatic catalysis reaction (Colorimetric assays)	
	GCF	PerioGard	AST	Enzymatic catalysis reaction (Colorimetric assays)	
	Oral rinse	PerioSafe	aMMP-8	Lateral flow test with digital reader (OraLyzer®)	
	GCF	ImplantSafe			
	Oral rinse	SillHa ST-4910	Blood, leukocytes, and protein	Lateral flow test with dual-wavelength reflectometry	
Microbiological assay	Subgingival plaque	Evalusite	*Aa, Pg, Pi*	Sandwich enzyme immunoassay (Colorimetric assays)	Chairside
	Subgingival plaque	BANA-Enzymatic test kit	*Pg, Td, Tf*	BANA hydrolysis reaction (Colorimetric assays)	
	Gums and plaque	OMNIgene ORAL/ OMR-110	Characterization of virus species of all genome type including *Aa, Pg, Pi, Fn, Td, Ec*	DNA hybridization	Company or research laboratory
	Saliva	OMNIgene ORAL/ OM-501, 505			
	Subgingival plaque	Carpegen® Perio Diagnostik	*Aa, Pg, Tf, Td, Fn, Pi*	Real-time qPCR	
	Oral rinse	MyPerioPath®	*Aa, Pg, Td, Tf, En, Fn, Pi, Cr, Pm, Ec, Cs*	DNA hybridization	
	Microbiological samples/subgingival plaque	iai Pado Test	*Aa, Pg, Pi, Td, Tf, Fa*	DNA hybridization	
	Subgingival plaque	micro-IDent®plus11	*Aa, Pg, Pi, Tf, Td, Pm, Fn, Cr, En, Ec, Cs*	DNA hybridization	
Genetic assay	Cheek swab	PerioPredict™	genes for IL-1	DNA hybridization	Company laboratory
	Oral rinse	MyPerioID® IL-6 or IL-1	genes for IL-6 or IL-1	Genetic polymorphisms detection	

GCF: Gingival crevicular fluid, AST: Aspartate aminotransferase, aMMP: active Matrix metalloproteinase, Aa: Aggregatibacter actinomycetemcomitans, Pg: Porphyromonas gingivalis, Pi: Prevotella intermedia, Td: Treponema denticola, Tf: Tannerella forsythia, Fn: Fusobacterium nucleatum, Ec: Eikenella corrodens, En: Eubacterium nodatum, Fn: Fusobacterium nucleatum/periodonticum, Cr: Campylobacter rectus, Pm: Peptostreptococcus (Micromonas) micros, Cs: Capnocytophaga species (gingivalis, ochracea, sputigena), Fa: Filifactor alocis, IL: Interleukin, qPCR: quantitative polymerase chain reaction.

Figure 2: *Classification of biomarkers, it's source, product name, detecting target and it's principle.*

Machine learning algorithms have the capacity to cross-analyze unlimited numbers of clinical and biological parameters while identifying a panel of critical determinants within highly specific patterns, which are further integrated into accurate and interpretable diagnostic information. Therefore, we want to endorse specific, measurable, achievable, realistic and time-bound (SMART) periodontal diagnosis to broaden the horizon of diagnostic criteria, also to shift the conventional paradigm from subjective to objective methods of assessment. Subject segmentation is necessary for improved clinical trial design and precise treatment approaches. It can also serve as a basis for analysis of dynamic responses to treatment strategies and the identification of molecular mechanisms underlying different phenotypes. Bioinformatic analyses using artificial intelligence (AI) on comprehensive 'omics data sets to interpret network dynamics across omics layers help develop precise and personalized treatment schemes for oral and associated systemic conditions. In recent times COVID-19 POINT-OF-CARE testing were used in oral health care settings. In this SARS-CoV-2 is detected in saliva of infected individuals [Wyllie., *et al*. 2020; Azzi., *et al*. 2020; Iwasaki., *et al*. 2020; Sabino-Silva., *et al*. 2020; To., *et al*. 2020a; To., *et al*. 2020b]. Both saliva and posterior oropharyngeal swab sampling are less invasive, more acceptable to patients, and reduce exposure risks for healthcare workers compared to nasopharyngeal swab sampling (Figure 3).

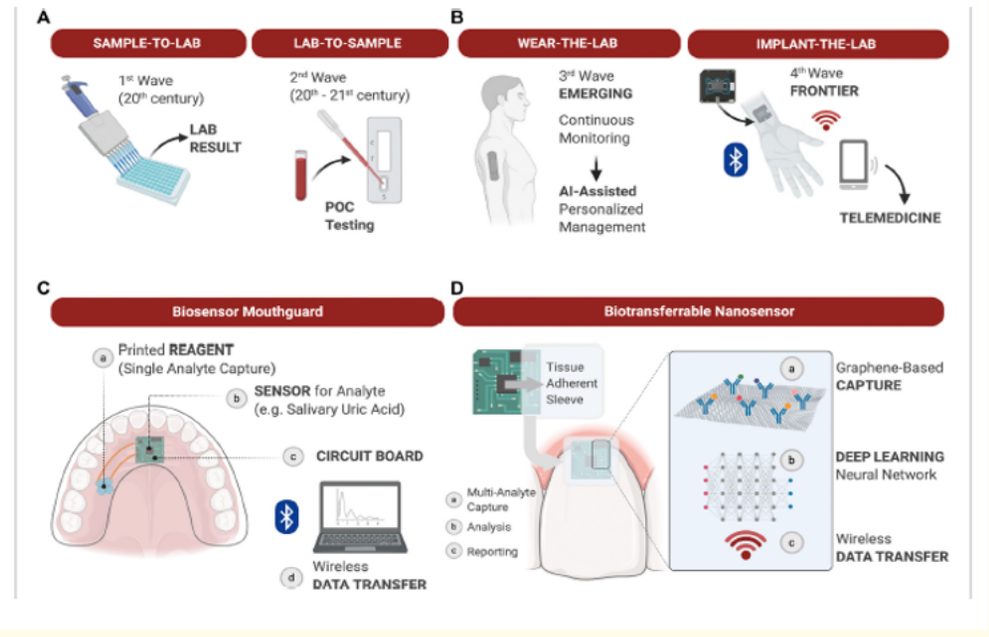

Figure 3: *The evolution of diagnostic devices and wearable lab-on-a-chip's (LOC) for precision medicine applications.*

(A): The most common diagnostic approaches to measuring soluble biomarkers are "sample-to-lab" and "lab-to-sample," i.e. samples are either collected from patients, transferred to the lab and analyzed, or tests are delivered directly at the point-of-care for rapid actionable results in the clinic. Technological advancements of the 21st century allow for the development of LOC analyzers to gather diagnostic information chairside in real time.

(B): The emerging integration of wearable LOC's in health care allows for continuous monitoring of physiological and pathological processes, and provide dense individual-level data for Artificial Intelligence (AI)-assisted personalized management. The next Frontier in LOC development may be the fabrication of biocompatible implantable sensors for continuous measurement of soluble biomarkers difficult to measure through the skin. Such advancements will expand diagnostic capabilities, at-home care and telemedicine.

(C): Example of a wearable biosensor integrated into a mouthguard to capture a single analyte in saliva over time and transduce the signal via Wi-Fi for analysis.

(D): Example of a graphene-based nanosensor adhered to the tooth surface and marginal gingiva to capture and quantify multiple analytes over time. Data is processed onboard and deep learning algorithms applied to establish personal physiological thresholds and out of personal norm trends. Wirelessly transferred output data supports clinical decisions during in-office or teledentistry appointments.

Study reporting

In microbiological markers - Assessment of a panel of key-stone periopathogens, together with opportunists. Specific diagnostic information on shifts in microflora, recognized as a crucial diagnostic indicator in distinguishing health from disease, disease progression, and responsiveness to the performed treatment. Metagenomic and metatranscriptomic methods, together with culturomics, will contribute to the identification of highly specific microbiological markers for accurate diagnosis and prognosis of periodontal disease. In the context of assessing quantitative changes, quantitative RT-PCR is the method of choice. In Biochemical markers- proteomics is an analytical method that can contribute to the identification of highly specific cytokine panels, and different multiplexing methods enable the assessment of a great range of protein profiles. Bone turnover markers are fast-response markers that reflect the nature and volume of the ongoing bone processes, practically enabling the clinician to visualize the bone status at the molecular level, far before clinical/radiological manifestations. The majority of commercially available diagnostic assays are intended for Biological Fluids available in larger volumes, which complicates the analysis of GCF, requiring standardization and adjustment of analytical protocols. The Histopathological studies will contribute to biological definitions of the grading criteria of periodontitis. Finally, the entire process of biomarker validation directly depends on the quality of the clinical aspect of diagnostic studies (Figure 4). A common reason for the gap between biomarker research and implementation in clinical practice is also the lack of appropriate tools for interpreting comprehensive panels.

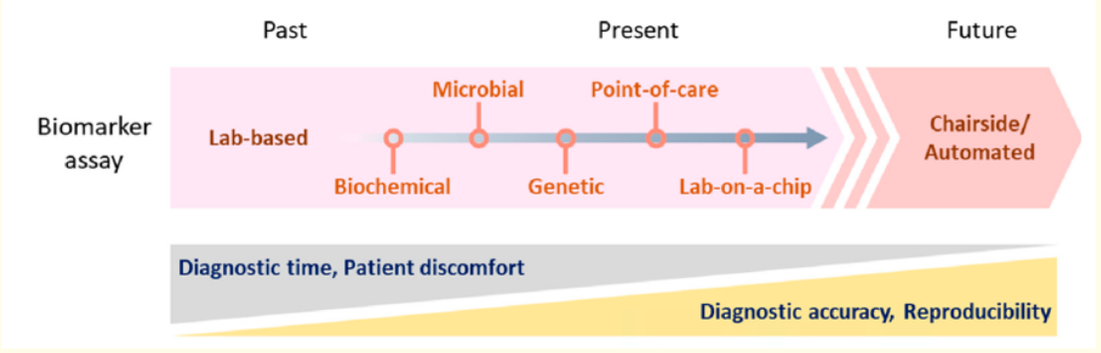

Figure 4: *A roadmap towards precision periodontics.*

Recent evidence based research on biomarkers

- P4 Medicine is based on a proactive approach for clinical patient care incorporating the four "pillars" of prediction, prevention, personalization, and participation for patient management [1].
- Salivary MMP-8 and IL-1β discriminate periodontitis in T2DM [2].
- H NMR-based metabolomic analysis identified and validated salivary biomarkers for periodontitis with large cohorts. The combined use of multiple statistical methods provided more comprehensive information for biomarker selection. Ethanol, taurine, isovalerate, butyrate, and glucose were discovered as salivary biomarkers with good capability to distinguish periodontitis [3].
- In oral rinse, the combination of MMP-8 and chitinase could assign best a subject to the control or periodontitis group. Future studies could focus on the exploration of the combination of MMP-8 and chitinase in oral rinse, which could potentially lead to developing a chair-side test to support periodontal screening and periodontal diagnostics [4].
- Implants diagnosed with peri-implantitis have higher levels of IL-1β and MMP-8 in peri-implant crevicular fluid (PICF) compared to healthy implants. Non-surgical therapy did not influence the inflammatory immune response. sRANKL and Interferon-γ (INF-y) appeared under level of detection using a customized Luminex™ assay [5].
- The aMMP-8 point-of- care test has better specificity than sensitivity to detect periodontitis across its spectrum of severity. The levels of aMMP-8 adjusted by the number of teeth present yielded moderate-to- high accuracy in identifying advanced cases. Moreover, a combined model including the test results with age and smoking performed better than the test alone. The aMMP-8 test may be helpful in conjunction with risk factors/indicators of periodontitis or other screening tools [6].

- Periodontal inflammation represents an occult source of oxidative stress in patients with CKD. Further clinical studies are needed to confirm whether periodontal therapy, as a non-pharmacological approach to reducing systemic inflammatory/ oxidative stress burden, can improve outcomes in chronic kidney disease CKD [7].
- Higher C-reactive protein (CRP) plasma levels were associated with higher recurrence of periodontitis and worse clinical periodontal parameters among irregular compliers (IC) when compared to regular compliers (RC) (Fernando., *et al*. in 2016).
- At 24 hr after surgery, significant variations in mRNA expression of key genes: RAC1, SERPINE1 and TIMP1, involved in scar formation; CDH1, ITGA4 and ITGB5, contributing to myofibroblast differentiation; and IL6 and CXCL1, involved in inflammation [8-10].

Conclusion

Precision periodontics undoubtedly represents the future of high-quality periodontal care, so it is of paramount importance that future research studies strictly adhere to the recommendations for the validation of biomarkers in order to accelerate the process of their implementation in routine clinical practice. Moreover, future studies should focus on the development of biomarker assessment protocols applicable in everyday practice, such as point-of-care testing (POCT), lab-on-chip's (LOC), which is still in the developmental stage in periodontology. Additionally, the development of patient self-testing methods should be seriously considered as well.

Conflict of Interest

Nil.

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