Biologic Markers of Failing Implants

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KEYWORDS

• Peri-implantitis • Microbiological markers • Biological markers

KEY POINTS

- The diagnosis of peri-implantitis benefits from clinical, radiographic, microbiological, and biological information.
- Practitioners and patients can use biomarkers to identify risk of disease, disease activity, disease progression, and response to therapy.
- Peri-implantitis is a biofilm-induced condition. The microbial composition of peri-implantitis lesions is mixed, nonspecific, and less diverse than that of periodontitis but includes *Fusobacterium, Prevotella, Porphyromonas, Streptococcus, Campylobacter*, and *Neisseria* species.
- Failed implants are often associated with enteric bacteria, spirochetes, and opportunistic bacteria (ie, *Staphylococcus aureus*).
- Protein biomarkers detected in peri-implant crevicular fluid provide insight into the underlying biology of the disease and specificity regarding the stage of the disease.

INTRODUCTION

As a result of clinical translational research, biomarkers are becoming increasingly available. They supplement clinical and radiographic information, allowing clinicians to make better decisions. Patients can also use biomarkers to obtain information about their health status and the need for dental care. Although biomarkers are most commonly used to decide whether a patient has a disease, their usefulness is more expansive. As **Fig. 1** shows, biomarkers are important for identifying severity of disease, ongoing activity of disease, disease progression, and response to therapy. With respect to periodontal disease, salivary analytes interleukin 1 beta (IL-1β), matrix metalloprotease 8 (MMP-8), and macrophage inflammatory protein-1 alpha (MIP-1α)

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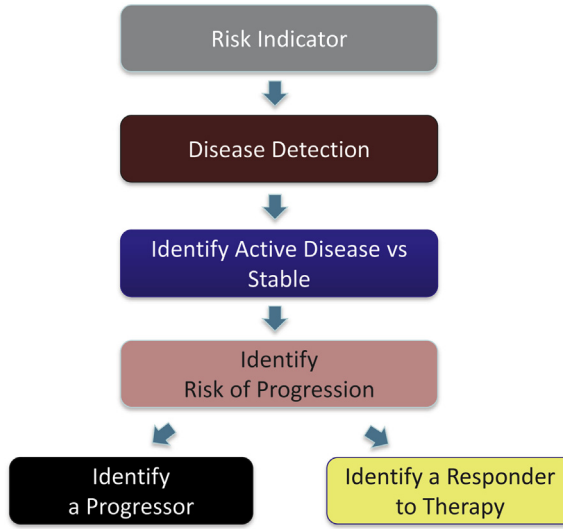

Fig. 1. Potential roles of biomarkers.

have recently been shown to serve in these roles. For example, high salivary concentrations of these analytes are associated with periodontal disease,[1–3] whereas high salivary concentration of MIP-1α is also a predictor of risk, that is, predictive of alveolar bone loss 6 to 9 months before radiographic evidence is apparent.[4] Oral fluid biomarkers can also be used to indicate response to therapy and have recently been shown to be useful in this role.[5] Together, the identification of biomarkers that have clinical utility for risk identification, disease detection, and identification of disease progression and response to therapy is the basis for establishing personalized care in the modern health care age and serve as the context for this article on peri-implantitis and failing dental implants. Specifically, this article discusses the milieu of microbes and proteins, that constitute the underlying biology of implant osseointegration and disease progression that can serve as indicators of implant health or failure.

DEFINITIONS

Peri-implantitis is a potentially progressive condition involving infection, inflammation, connective tissue destruction, and bone resorption.[6] The condition is characterized by microbial infection, deep probing depths, bleeding on probing, suppuration, and radiographic bone loss.[7–9] Risk factors include cigarette smoking, poor oral hygiene, and a previous history of periodontitis.[10] Peri-implantitis does not necessarily mean that the implant will fail. The implant can be salvaged if peri-implantitis is diagnosed early, if risk factors are reduced or eliminated, and if the site is treated appropriately.[11] In contrast, implant failure is defined as the inability of the host tissue to establish or maintain osseointegration, which is clinically diagnosed by mobility of the implant (**Fig. 2**).

Implant failures are classified as early or late, depending on the time of placement and the implant's functionality. Early failure occurs before prosthetic rehabilitation and before the implant is placed into function. Early failures generally result from surgical trauma, overheating of the bone during implant surgery, insufficient bone surrounding the implant, early loading of the implant, or perioperative bacterial

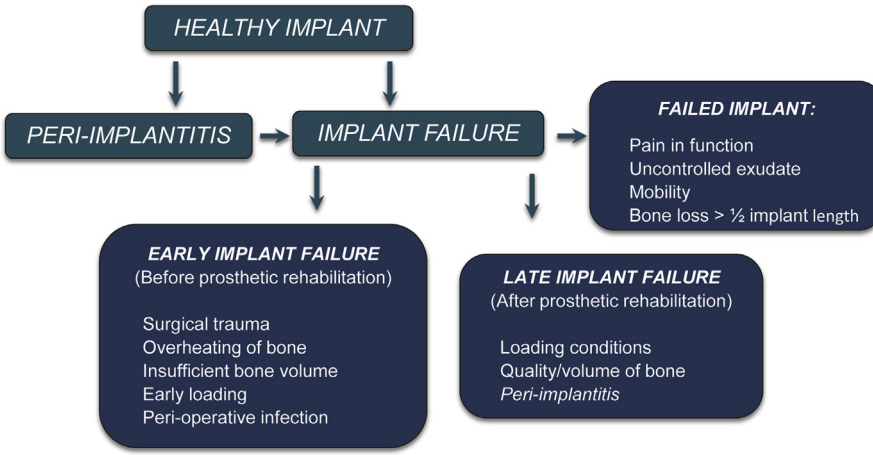

Fig. 2. Implant failure: definitions, etiology, and clinical features.

infection. Late failure occurs after prosthetic rehabilitation and indicates that established osseointegration has not been maintained. Late failures can further be classified as early or delayed depending on whether implant failure is observed before or after the first year of loading. Delayed late failures are generally associated with changes in loading conditions, the quality and volume of bone relations, and peri-implantitis.[12,13]

A failed implant is a clinical diagnosis defined by one or more of the following criteria: pain in function, presence of uncontrolled exudate, mobility, or radiographic alveolar bone loss more than half the length of the implant. Clinicians generally recommend removal of a failed implant[14]; however, because implant failure is a process requiring time, a clinician can experience the dilemma of a failing implant, characterized by progressive loss of alveolar bone support. In this instance, the clinician must decide what action to take and whether clinical, radiographic, or biological information can help in the decision-making process that could ultimately prevent the loss of the implant. Clinicians should be aware that a failing implant is associated with the accumulation of microbial plaque and bacterial infection around the implant. These microbes elicit many biological mediators, and it is conceivable that the milieu of microbial and biological molecules that surround and emanate from the sulcus of the implant can distinguish a healthy implant from one associated with peri-implantitis.

MICROBIOLOGICAL MARKERS OF FAILING DENTAL IMPLANTS

Peri-implantitis accounts for 10% to 50% of implant failures after the first year of loading.[12] Microorganisms play an important role in peri-implantitis; therefore, the identification of peri-implantitis-associated microbiota or bacteria is crucial for an understanding of peri-implantitis pathogenesis and of the bacteria that could serve as microbial biomarkers of this condition. **Table 1** summarizes the results of studies conducted during the last 3 decades that have analyzed the microbiota of peri-implantitis sites. Details regarding these studies are discussed below.

Bacterial Colonization and Microbial Composition Around Healthy Implants

Longitudinal studies of biofilm formation around dental implants have shown that bacterial colonization occurs immediately after implant placement (within 30 minutes).[15]

Table 1
Longitudinal studies of microbial colonization around teeth and implants

Authors	Follow-up	Implants (Patients)	Method	Results
Salvi et al,[19] 2008	12 mo	17 I 17 T (n = 13)	DNA-H	Sum of bacterial counts at T sites was higher than at I sites. *Pm, Lb, Cs,* and *Pi* were most prevalent at I sites at 12 mo (>30 × 10^5 cells). *Sg, Fn* ssp*, polymorphum,* and *vincentii* were most prevalent at T sites at 12 mo (>50 × 10^5 cells). *F* spp, *Strep* spp, *Pm,* and *Sa* were higher at I sites. Few differences in bacterial species between tooth and implant at 12 mo.
Furst et al,[15] 2007	3 mo	17 I 17 T (n = 14)	DNA-H	Bacterial colonization around implants starts at 30 min. *Pg, Td,* and *Tf* were present at implant sites at 12 wk. *Sa* was present at 15% of the implant sites at 12 wk.
Quirynen et al,[17] 2006	18 mo	NR (n = 42)	DNA-H culture	Red (5%) and orange (20%) complex bacteria were present around the implant at 1 wk. Red (8%) and orange (33%) complex bacteria were present around the implant at 3 mo. The subgingival microbiota was similar at I and T sites at 3 mo.
Leonhardt et al,[22] 2002	10 y	57 I 261 T (n = 15)	Culture	*Pg, Pi, Aa, C* ssp, and *Cr* were detected at baseline and at 10-yr follow-up at T and I sites. These bacterial species are members of the normal resident microbiota.
Hultin et al,[21] 2000	10 y	43 I 31 T (n = 15)	DNA probe	No marked differences were found between T and I at 10 yr. *Td, Si,* and *Pm* were the most common bacteria at I and T sites. *Aa, Pg, Td,* and *Tf* were found at implant sites with > 2-mm bone loss.
van Winkelhoff et al,[20] 2000	12 mo	NR (n = 20)	Culture	Prevalence of bacteria was similar at I and T sites at 6 mo. Implant failure was associated with high levels of *Pg.* *Aa* was not detectable around I sites.
Sbordone et al,[18] 1999	3 y	42 I 25 T (n = 25)	Culture	*Pg and C* ssp were the most prevalent bacteria around implants at 1 yr. Significantly fewer motile rods were found at implant sites than at teeth at 1 yr. No statistically significant difference in periodontopathogens at implant and tooth sites.

Abbreviations: Aa, *Aggregatibacter actinomycetemcomitans*; *Cr*, *Campylobacter rectus*; *Cs*, *Campylocytophaga sputigena*; *C* ssp, *Capnocytophaga* subspecies; DNA-H, DNA-DNA hybridization; *Fn*, *Fusobacterium nucleatum*; I, implant; *Lb*, *Leptotrichia buccalis*; NR, not reported; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Pm*, *Peptostreptococcus micros*; *Si*, *Streptococcus intermediate*; *Strep* spp, *Streptococcus* subspecies; T, teeth; *Td*, *Treponema denticola*; *Tf*, *Tannerella forsynthesis*.

The microbiota associated with healthy peri-implant tissues are dominated by gram-positive facultative cocci and rods and by low proportions of gram-negative anaerobic rods.[15,16] Initial colonization of peri-implant sites with periodontitis-associated bacteria (eg, *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythensis*) is detected as early as 2 weeks after placement.[17] The composition of bacteria around healthy teeth and around healthy implant sites is reported to remain similar for as long as 2 years.[17–19] However, the sum of bacterial counts of 40 periodontopathogenic species analyzed with DNA-DNA hybridization was higher around normal teeth than at implant sites both at baseline (immediately after implant placement) and 1 year later.[19] Also, the most predominant microbes around implants 1 year after placement were *Peptostreptococcus micros, Leptotrichia buccalis, Capnocytophaga sputigena*, and *Prevotella intermedia* (>30 $\times$ 10^5 cells), whereas the most predominant microbes at tooth sites were *Streptococcus goordinii* and *Fusobacterium nucleatum* subspecies *polymorphum,* and *vincentii* (>50 $\times$ 10^5 cells).[19] However, the presence of putative periodontal pathogens at peri-implant sites does not dictate loss or failure of an implant attachment, provided proper oral hygiene measures and periodontal supportive therapy are maintained.[18,20] Two follow-up studies of clinical, radiographic, and microbiological parameters of osseointegrated implants in partially edentulous patients treated for periodontal disease did not report marked differences in microbial flora between healthy implants and teeth at baseline and 10 years later.[21,22] In fact, putative bacterial species, including *P gingivalis, P intermedia, Aggregatibacter actinomycetemcomitans*, *Capnocytophaga* spp, and *Campylobacter rectus*, were present at clinically healthy implant sites at a 10-year examination. Therefore, it is suggested that periodontopathogens are present at implant sites as part of the normal resident microbiota and are not the sole factor affecting the long-term outcome of implant treatment.[22]

Microbial Composition Around Implants Associated with Peri-implantitis

Today, it is well accepted that peri-implant mucositis and peri-implantitis are induced by biofilm.[8,23] Although no single candidate bacterium is responsible for the infection of any implant system (**Table 2**), *Staphylococcus aureus* has been suggested to be important for dental implant failure because its specific affinity for titanium surfaces.[24] Recent reports have shown that *S aureus* is an early colonizer of implant surfaces. It is found at implant sites 3 months to 1 year after implant placement.[19,25] Early implant failures are reported to be associated with *S aureus* because of low serum antibody titers, which suggests an impaired host response to this microorganism.[26] A cluster of bacteria, including *S aureus*, has been found to be more prevalent at sites of peri-implantitis (30.2%) than at healthy implant sites (14.1%),[25] and the absence of *S aureus* is suggested to indicate implant health.[19] However, a recent study investigating the presence of opportunistic bacteria at peri-implantitis sites found *Pseudomonas aeruginosa* in 3 of the 31 patients examined and *S aureus* in only one.[27] Therefore, although *S aureus* and other opportunistic bacteria appear to play a role in select cases of peri-implantitis and implant failure, additional investigations are required because of the limited number of studies addressing this problem and the differences in the methods used.

In addition to opportunistic bacteria, specific periodontal pathogens have been identified at healthy implant sites, peri-implant mucositis sites, and peri-implantitis sites.[21,28] Several studies identified increased levels of red complex bacteria (*P gingivalis, T denticola, T forsythensis*) and *P gingivalis* at peri-implantitis sites.[25,27,29–31] Because of the similarity of the microbiota around normal teeth and adjacent implants, it has been suggested that bacterial flora at the adjacent tooth site can act as a

Table 2
Case-control and retrospective human studies on microbial composition in peri-implantitis

Authors	Implants (Patients)	Method	S	PDx	Clinical Measures	Results
Albertini et al,[27] 2014	48 PI 33 T (n = 33)	PCR Culture	Yes	Yes	BOP/SUP Radiograph	*Pg, Pi, Tf, Td* prevalence was similar at Peri-I and T sites. *Pa* (12%), *Ca* (3%), and *Sa* (3%) were detected Peri-I group.
Persson & Renvert,[25] 2013	166 PI 47 HI (n = 213)	DNA-H	Yes	Yes	PPD Radiograph	A cluster of bacteria including *Tf, Pg, Ts, Sa, Si,* and *Hi* were higher at Peri-I sites compared with HI (30.4% vs 14.1%). History of PDx, age, and *Tf* were found associated with Peri-I.
Heuer et al,[33] 2012	9 G 9 PM (n = 9)	DNA-H	NR	No	PD BOP Plaque	Microbial diversity of G sites was more complex than PM sites. *Fusobacterium, Prevotella, Porphyromonas, Streptococcus, Campylobacter,* and *Neisseria* were most prevalent at PM sites.
Dabdoub et al,[35] 2013	33 HT/HI 23 HT/DI 8 DT/HI 17 DT/DT (n = 81)	16S RNA Pyroseq.	NR	Yes	PD BOP/SUP GI Mobility Radiograph	Periodontal pathogens are found in 37% of the DI sites. *Staphylococcus* and *Treponema* are associated with DI compared with T sites. Most abundant species remain different between I and T sites. Geographic proximity is not sufficient to explain peri-implant microbial composition.
Koyanagi et al,[37] 2013	6 PI 6 PDx (n = 6)	16S RNA	No	Yes	PD BOP/SUP Radiograph	Microbial composition of Peri-I sites was more diverse than PDx. Periodontopathogens were detected in lower amounts in Peri-I sites.
Cortelli et al,[36] 2013	53 HI/53 HT 50 PM/50 G 50 PI/50 PDx (n = 306)	16S RNA	No	Yes	PD CAL BOP/SUP Radiograph	Peri-implant mucositis and Peri-I shared similar microbial composition. Composition of bacterial species was different between PI and PDx. Bacterial frequency was higher in teeth compared with implants *Pg* was associated with Peri-I.
Kumar et al,[34] 2012	10 PDx 10 PI 10 HI 10 HT (n = 40)	16S RNA Pyroseq	NR	Yes	PD CAL BOP Plaque	Peri-I and HI biofilms are less diverse than PDx and HT-related biofilms. PI had higher levels of *Actinomyces*, *Peptococcus, Mycoplasma, Eubacterium, Campylobacter, Butyrivibrio, S mutans,* and *Treponema* compared with HI and HT. Peri-I is a microbiologically heterogenous gram-negative infection.

Renvert et al,[28] 2007	31 PI 127 PM 55 HI (n = 231)	DNA-H	NR	Yes	PPD BOP Radiograph	Edentulous and dentate subjects showed similar microbial profiles. Peri-I, PM, and HI sites had similar bacterial profile regardless of implant disease status.
Shibli et al,[30] 2008	22 PI 22 HI (n = 44)	DNA-H	No	No	Radiograph BOP/SUP	HI sites had similar supra/subgingival plaque composition. Peri-I sites had higher levels of red complex bacteria in supra/subgingival plaque. Peri-I sites had different bacterial composition between subra- and subgingival plaque.
Botero et al,[29] 2005	16 PI 15 HI 23 T (n = 19)	Culture	NR	NR	PD BOP/SUP Radiograph	Peri-I sites showed increased levels of gram-enteric rods and *Pg.* A correlation was found between subgingival colonization in PI and neighboring T sites for enteric rods and *Pg.*
Hultin et al,[31] 2002	45 PI 53 HI 133 T (n = 36)	DNA-H	Yes	No	PPD GI Plaque Radiograph	*Pg, Pi, Bf, Aa,* and *Td* were detected in HI and PI sites and around teeth. *Pg, Pi, Bf, Aa,* and *Td* were detected $>10^6$ in PI sites. Peri-I is a site-specific infection rather than a specific host response.
Leonhardt et al,[16] 1999	NR (n = 88)	Culture	Yes	Yes	PD BOP/SUP Radiograph	Edentulous patients have not harbored periodontopathogens. Enterics were found commonly in Peri-I sites compared with HI sites. PI sites carried more periodontopathogens HI sites.
Sbordone et al,[38] 1995	19 FI (n = 13)	Culture	NR	NR	PD PAL BOP/SUP Plaque GI	Fusiform bacteria, spirochetes, and motile curved rods were isolated at failing implant sites.

Abbreviations: Aa, *Aggregatibacter actinomycetemcomitans*; BOP, bleeding on probing; *Ca*, *Candida albicans*; *Cr*, *Campylobacter rectus*; *Cs*, *Capnocytophaga sputigena*; *C* ssp, *Capnocytophaga* subspecies; DNA-C, DNA-DNA checkerboard; DNA-H, DNA-DNA hybridization; *Fn*, *Fusobacterium nucleatum*; G, gingivitis; *Hi*, *Haemophilus intermedia*; HI, healthy implants; HT, healthy teeth; I, Implants; *Lb*, *Leptotrichia buccalis*; NR, not reported; *Pa*, *Pseudomonas aeruginosa*; PDx, periodontal disease; Peri-I, peri-implantitis; *Pg*, *Porphyromonas gingivalis*; *Pi*, *Prevotella intermedia*; *Pm*, *Peptostreptococcus micros*; PM, peri-implant mucositis; PPD, periodontal probing depth; Pyroseq, Pyrosequencing; S, Smoking; *Si*, *Streptococcus intermediate*; *Strep* spp, *Streptococcus* subspecies; SUP, suppuration; *Td*, *Treponema denticola*; *Tf*, *Tannerella forsynthesis*; *Ts*, *Treponema socranskii*.

reservoir for the implant sulcus.[32] However, increasing evidence suggests that the immunology, histology, and microbiology of peri-implant diseases are different from those of periodontal diseases.[22,33–35] In a case-control study, peri-implant, mucositis lesions, characterized by bleeding and inflammation of the peri-implant mucosa, contained fewer diverse microbes (6 microbial genera) than gingivitis lesions (19 microbial genera). The most common genera found at peri-implant mucositis lesions were *Fusobacterium, Prevotella, Porphyromonas, Streptococcus, Campylobacter*, and *Neisseria*.[33] Similarly, the microbial composition of peri-implantitis lesions was less diverse than that of periodontitis lesions, and periodontal pathogens were detected at lower frequencies around peri-implantitis sites than around natural teeth.[34–37] Most of the abundant species in peri-implant lesions are reported to be different from those around natural teeth.[35] For example, peri-implantitis lesions contained higher levels of *Actinomyces*, *Peptococcus, Campylobacter, Butyrivibrio, Streptococcus mutans*, *and nonmutans Streptococcus than* healthy implant sites. Peri-implantitis sites also yielded higher levels of *Peptostreptococcus*, *Mycoplasma, Eubacterium*, *Campylobacter, S mutans, and Treponema* than did periodontitis sites.[34] Of potential importance with respect to biomarkers is the finding that enteric bacteria, spirochetes, and opportunistic bacteria have often been detected as the most abundant members of the microbiota at the sites of failed implants.[16,29,38]

The available information about whether the presence of a single bacterium or a cluster of bacteria can be used as a biomarker for distinguishing peri-implant disease from peri-implant health appears to be limited and requires further evaluation. Identifying good biomarkers of peri-implantitis and implant failure is difficult because investigators have used various methods of sampling and methods for analyzing bacteria (eg, bacterial culture, DNA hybridization, and 16S RNA), and have used small, geographically restricted patient populations. These problems have prevented conclusions regarding the specificity of the peri-implant microbiota. In addition, it is thought that structural and topographic differences between implant surfaces and natural tooth surfaces may influence a unique and unknown bacterial composition that has not yet been identified.[39] Thus, additional studies that recognize these concepts are needed so that we can fully characterize the microbiota related to peri-implant disease. As a result, these studies can lead to better prevention and management strategies for peri-implantitis.

PROTEINS AS BIOLOGICAL MARKERS FOR PERI-IMPLANTITIS

Peri-implantitis is a progressive condition that, if left untreated, involves 3 biological phases: inflammation, connective tissue destruction, and bone resorption. As such, researchers have sought concentrations of biological molecules associated with these 3 biological phases in oral fluids, with the goal of finding early predictors of susceptibility and detecting the molecules associated with the early, intermediate, and late endpoints of this disease. With the identification of specific molecular markers of peri-implantitis, clinicians should be able to monitor disease progression and devise preventive strategies and interventional therapies that can limit the progression of this disease. In addition, biomarkers should help define the interval for periodic recall and the response to therapies. This section provides an overview of protein biomarkers, associated with the host response, that are detected in the oral fluid of patients with peri-implantitis. **Table 3** provides details of studies that have investigated the biomarkers found in the crevicular fluid surrounding healthy and failing implants.

Inflammatory Biomarkers

The immune system responds predictably to infection and injury with inflammation in the affected area. Accordingly, microbial biofilm on peri-implant surfaces induces an inflammatory response in susceptible hosts; this response involves features shared with and distinct from features of periodontitis.[40,41] The classic inflammatory peptides and proteins that respond to bacterial inflammation are cytokines and chemokines produced by T lymphocytes and monocytes/macrophages. In addition, many nonspecific markers of inflammation make up the inflammatory milieu. These proteins appear in peri-implant crevicular fluid (PICF) or gingival crevicular fluid (GCF) samples taken from the area around the implant and could serve as markers of inflammation and early detection.[42] In addition, the volume of PICF is increased during peri-implantitis, and this information can be informative to the clinician.[43]

The proteins myeloperoxidase (MPO) and lactoferrin are found in the granules of neutrophils that are released after tissue injury and inflammation. MPO is a marker of inflammation that also serves as a biomarker of cardiovascular disease,[44] whereas lactoferrin is known to regulate the immune response and protect against bacterial infection.[45] The utility of these 2 proteins as biomarkers of peri-implantitis has been suggested. Liskmann and colleagues[46] collected clinical measures of periodontal health and PICF from 24 adults with 64 oral implants. They found that MPO levels were significantly higher around inflamed implants than around healthy implants and were associated with increased pocket depth, a higher gingival index, and bleeding on probing. Similarly, Hultin and colleagues[31] found that in 17 patients, the concentrations of lactoferrin were higher in PICF from peri-implantitis sites than in PICF from stable implant sites.

Prostaglandins are produced by cyclooxygenases during inflammation and contribute to the clinical features of inflammation.[47] They are found at higher concentrations in fluids emanating from sites of gingivitis and periodontitis,[5] and they were recently detected in PICF. Basegmez and colleagues[48] detected higher levels of prostaglandin E2 around implants as probing depths and time after implantation increased.

IL-1β, a proinflammatory cytokine, is known to be important in the pathogenesis of periodontal disease.[49] It contributes to the activation of osteoclasts, bone resorption, and down-regulation of type 1 collagen expression in bone.[50] IL-1β has been detected at higher levels in GCF and saliva associated with severe periodontitis, periodontal disease progression, and, recently, peri-implantitis.[1,51,52] A study involving 13 patients with 50 implants found that the concentrations of IL-1β were 3 to 9 times higher at peri-implantitis sites than at healthy implant sites.[53] Similarly, a study involving 16 patients with 34 endoseous titatnium implants, found that mean levels of IL-1β in PICF were significantly higher at the 6 peri-implantitis sites than at the 20 healthy implant sites.[43] In contrast, Hultin and colleagues[31] studied 17 patients with 45 implants and found that the concentrations of IL-1β at peri-implantitis sites were not different from those at stable implant sites.

As time progresses and the inflammatory phase matures, peri-implantitis involves the connective tissue destruction phase. This phase is associated with neutrophil- and macrophage-derived enzymes that degrade collagen and extracellular matrices. Thus, the predominant enzymes involved are MMPs. This family of enzymes has been detected in saliva and GCF from patients with periodontal disease[1,54] and recently in PICF from patients with severe peri-implantitis and during periods of ongoing bone loss activity. Arakawa and colleagues[55] used Western blot analysis in a study involving 64 patients. They found that MMP-8 was the main collagenase in PICF from severe peri-implantitis sites and during periods of ongoing bone loss activity. Concentrations

Table 3
Relevant studies involving protein biomarkers of peri-implantitis

Authors	Biomarker	Study Design	Implants (Patients)	Implant Function/ Year	Smoker	Clinical Measures	Outcome
Panagakos et al,[53] 1996	IL-1β	Case control study	17 HI 33 Peri-I (n = 13)	7–30 mo	NR	PI GI BOP PD	Increased levels of IL-1β and pro–IL-1β is detected in Peri-I sites compared with HI.
Hultin et al,[31] 2002	IL-1β	Cross-sectional study	45 implants (n = 17)	2–13 y	NR	Radiographic BL Inflammation Suppuration	Level of IL-1β was found similar in stable and diseased sites.
Murata et al,[43] 2002	Osteocalcin Dpd IL-1β	Cross-sectional case control study	20 HI 6 Peri-I 8 PM (n = 16)	9–112 mo	Nonsmokers	BL PD PI GI	Osteocalcin was significantly higher in PM sites compared with HI. IL-1β was significantly higher in Peri-I sites compared with PM and HI sites. Dpd was not detected in either PICF or GCF.
Liskmann et al,[46] 2004	MPO	Cross-sectional study	31 HI 34 Peri-I (n = 40)	NR	NR	PG GI BOP	MPO activity is increasing with increasing PD and GI. MPO activity is higher when BOP is present.
Arikan et al,[57] 2008	sRANKL, OPG	Cohort study	79 HI 3 Peri-I 4 PM (n = 39)	12–16 mo	Nonsmokers	Radiographic BL BOP PD Suppuration	OPG needs further investigation as a possible biomarker of implant health status.

Arikan et al,[56] 2011	ICTP, sRANKL, OPG, Albumin	Case control study	21 HI 18 Peri-I (n = 28)	2–17 y	Nonsmokers	Radiographic BL CAL PD BOP GI	PI group showed lower amounts and concentrations of OPG and higher amounts and concentrations of ICTP. Local levels of OPG and ICTP reflect alveolar bone resorption.
Arakawa et al,[55] 2012	MMP-1, MMP-8, MMP-13	Case control study	162 implants 4 HI 4 Peri-I (n = 64)	1–7.4 y	NR	Radiographic BL	Incidence of Peri-I was 3.7% of implants. MMP-8 only biomarker detected in PICF from Peri-I sites.
Basegmez et al,[48] 2012	PGE2, MMP-8	Longitudinal study	72 HI (n = 28)	18 mo	Nonsmokers	PI GI PD	MMP-8 levels in PICF showed a significant correlation between PD and GI. MMP-8 seems to be an early marker of connective tissue destruction.
Rakic et al,[58] 2013	sRANKL RANK OPG	Cross-sectional study	25 HI 23 Peri-I 22 CP (n = 70)	≥2 y	Nonsmokers	PD rCAL BOP PI	sRANK/RANK/OPG levels were higher in PI group compared with HI. sRANK/RANK/OPG levels correlated with clinical parameters, BOP, PD, rCAL. RANK levels were associated with PI compared with CP.
Rakic et al,[59] 2014	sRANKL RANK OPG Catepsin-K Sclerostin	Cross-sectional study	58 HI 52 Peri-I 54 PM (n = 164)	≥2 y	Nonsmokers	Radiographic BL rCAL PD BOP PI	Increased sRANKL, OPG and sclerostin levels were associated with Peri-I sites. Increased cathepsin-C levels were associated with PM.

Abbreviations: BL, bone loss; BOP, bleeding on probing; CAL, clinical attachment level; CP, chronic periodontitis; Dpd, Deoxypyridinoline; HI, healthy implant; NR, not reported; PD, probing depth; Peri-I, peri-implantitis; PI, plaque index; PM, peri-mucositis; rCAL, relative clinical attachment level.

of MMP-8 were higher in PICF from 72 implants at 6 and 18 months after implantation, a period coincident with the maturation of the inflammatory phase.[48] The activity of elastase (MMP-12) is also found to be higher at peri-implantitis sites than at healthy control implant sites.[31] In contrast, MMP-1 and MMP-13 have not been detected at peri-implantitis sites.[55]

The advanced stages of peri-implantitis are associated with bone remodeling, radiographic evidence of bone loss, and implant mobility. Osteoclasts, osteoblasts, and bone signaling molecules are active during this phase. A study by Murata and colleagues[43] (2002) was one of the first to detect osteocalcin, a biomarker of bone formation, in PICF from peri-implant mucositis sites. However, these investigators did not detect deoxypyridinoline, a marker of bone resorption, in PICF from peri-implantitis sites. Another marker and by-product of bone resorption is C-telopeptide pyridinoline cross-links of type I collagen (ICTP). Concentrations of ICTP are significantly higher in PICF from patients with peri-implantitis than in PICF from patients with healthy implants[56]; however, little consistent information is available about ICTP concentrations at specific peri-implantitis sites. Osteoprotegerin (OPG), a decoy receptor for the receptor activator of nuclear factor-kappa B ligand (RANKL), has been detected in PICF from implants sites,[57] and concentrations of OPG are reported to be significantly lower in patients with peri-implantitis than in patients with healthy implants.[56] However, findings regarding OPG are conflicting. In a study involving 70 patients, Rakic and colleagues[58] found that concentrations of OPG were significantly higher in PICF from 23 peri-implantitis sites than in PICF from 25 healthy peri-implant sites.

Like OPG, the role of soluble RANKL as a biomarker of the bone resorption phase of peri-implantitis is controversial. Arikan and colleagues[56] found that the concentrations of soluble RANKL were significantly lower in PICF with peri-implantitis sites than in PICF from healthy implant sites. In contrast, Rakic and colleagues[58] detected soluble RANKL at higher concentrations in PICF from peri-implantitis sites than in PICF from healthy implant sites. In a recent study involving 52 patients with peri-implantitis, 54 patients with mucositis, and 58 patients with healthy peri-implant tissue, Rakic and colleagues[59] validated their earlier findings, that is, they found that the concentrations of OPG, RANK, and soluble RANKL were significantly higher in patients with peri-implantitis than in patients with healthy peri-implant tissues. Rakic and colleagues also found that sclerostin concentrations were significantly higher in the PICF of patients with peri-implantitis than in PICF of patients with healthy peri-implant tissues.

Currently, few studies have validated biomarkers of peri-implantitis, and few interventional studies have examined microbes and biomarkers. Thus, at this time, the information about biomarkers of peri-implantitis appears to be preliminary, and additional studies are necessary for confirming the specificity of the biomarkers. Benefits will be gained from prospective studies that involve larger numbers of patients and use robust assay techniques. In addition, it is likely that specificity will be gained from using a panel of biomarkers that target each of the 3 phases of peri-implantitis: inflammation, connective tissue destruction, and bone resorption.

SUMMARY

Microbiology

- Bacterial accumulation at peri-implant sites begins soon after implant placement.
- Similar bacterial species are detected at implant sites and tooth sulcus sites, yet the complexity of the microbiota around teeth is greater than that around implants in both health and disease.

- Increased numbers of red complex bacteria, specifically *P gingivalis*, are detected at peri-implantitis sites.
- Peri-implant disease and periodontal disease are associated with different bacterial compositions, a finding indicating that the microbiology of peri-implant disease is different from that of periodontitis.
- A few studies report that enteric rods, spirochetes, and opportunistic bacteria, including *S aureus* are associated with peri-implantitis and implant failure.
- Future studies involving large study populations that use optimal bacterial sampling and analyses are necessary for identifying specific microbiota and biomarkers of peri-implantitis and failing implants.

Protein Biomarkers

- Inflammatory biomarkers (MPO, lactoferrin, IL-1β, prostaglandins) appear to be early indicators of peri-implantitis.
- MMP-8 seems to be useful for monitoring the connective tissue destruction phase of peri-implant disease, but this finding requires validation.
- The bone remodeling biomarkers OPG, RANK, and soluble RANKL are promising biomarkers that may be associated with bone loss around implants.
- Additional studies involving larger numbers of subjects are necessary for validating biomarkers of peri-implantitis.

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