Diagnostic Biomarkers for Oral and Periodontal Diseases

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Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth. It is widely accepted that the initiation and the progression of periodontitis are dependent on the presence of virulent microorganisms capable of causing disease. Although the bacteria are initiating agents in periodontitis, the host response to the pathogenic infection is critical to disease progression [1–3]. After its initiation, the disease progresses with the loss of collagen fibers and attachment to the cemental surface, apical migration of the junctional epithelium, formation of deepened periodontal pockets, and resorption of alveolar bone [4]. If left untreated, the disease continues with progressive bone destruction, leading to tooth mobility and subsequent tooth loss. Periodontal disease afflicts over 50% of the adult population in the United States, with approximately 10% displaying severe disease concomitant with early tooth loss [5].

A goal of periodontal diagnostic procedures is to provide useful information to the clinician regarding the present periodontal disease type, location, and severity. These findings serve as a basis for treatment planning

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and provide essential data during periodontal maintenance and disease-monitoring phases of treatment.

Traditional periodontal diagnostic parameters used clinically include probing depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs assessing alveolar bone level [6]. The strengths of these traditional tools are their ease of use, their cost-effectiveness, and that they are relatively noninvasive. Traditional diagnostic procedures are inherently limited, in that only disease history, not current disease status, can be assessed. Clinical attachment loss readings by the periodontal probe and radiographic evaluations of alveolar bone loss measure damage from past episodes of destruction and require a 2- to 3-mm threshold change before a site can be identified as having experienced a significant anatomic event [7]. Advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers (Table 1).

There are several key questions regarding current clinical decision making: How can clinicians assess risk for periodontal disease? What are the useful laboratory and clinical methods for periodontal risk assessment? and What can be achieved by controlling periodontal disease using a risk profile? [8–11]. Risk factors are considered modifiers of disease activity. In association with host susceptibility and a variety of local and systemic conditions, they influence the initiation and progression of periodontitis and successive changes on biomarkers [12–14]. Biomarkers of disease in succession play an important role in life sciences and have begun to assume a greater role in diagnosis, monitoring of therapy outcomes, and drug discovery. The challenge for biomarkers is to allow earlier detection of disease evolution and more robust therapy efficacy measurements. For biomarkers to assume their rightful role in routine practice, it is essential that their relation to the mechanism of disease progression and therapeutic intervention be more fully understood (Table 2) [13].

Table 1
Diagnostic tools to measure periodontal disease at the molecular, cellular, tissue, and clinical levels

Level	Example of process	Example of diagnostic tools
Molecular	Activation of receptors for endotoxin: CD-14; Toll-like receptors	Polymerase chain reaction; DNA-DNA hybridization; laser-capture microdissection
Cellular	Inflammatory cell activation such as neutrophils; osteoclast activation	ELISA; immunohistochemistry
Tissue	Downgrowth of junctional epithelium; bone and connective tissue loss	Histomorphometry; immunohistochemistry
Clinical	Attachment loss	Periodontal probing
	Bone loss	Radiographs

Table 2
Predictors for periodontal diseases

Term	Definition
Risk marker	An attribute or event that is associated with increased probability of disease but is not necessarily a causal factor
Risk indicator	An event that is associated with an outcome only in cross-sectional studies
Risk factor	An action or event that is related statistically in some way to an outcome and is truly causal
Risk determinant	An attribute or event that increases the probability of occurrence of disease
Biomarker	A substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

Adapted from Refs. [128,154,155].

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 [15]. This article highlights recent advances in the use of biomarker-based disease diagnostics that focus on the identification of active periodontal disease from plaque biofilms [16], gingival crevicular fluid (GCF) [17], and saliva [18]. Mediators that are released into GCF and saliva as biomarkers of disease are shown in Fig. 1. The authors also present an overview of well-studied mediators associated with microbial identification, host response factors, and bone resorptive mediators.

Microbial factors for the diagnosis of periodontal diseases

Of the more than 600 bacterial species that have been identified from subgingival plaque, only a small number have been suggested to play a causal role in the pathogenesis of destructive periodontal diseases in the susceptible host [16]. Furthermore, technologic advances in methodologies such as analysis of 16S ribosomal RNA bacterial genes indicate that as many as several hundred additional species of not-yet-identified bacteria may exist [19]. The presence of bacteria adjacent to the gingival crevice and the intimate contact of bacterial lipopolysaccharide with the host cells trigger monocytes, polymorphonuclear leukocytes (neutrophils), macrophages, and other cells to release inflammatory mediators such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, and prostaglandin E_2 [3]. The role of host response factors derived from GCF and saliva is discussed later.
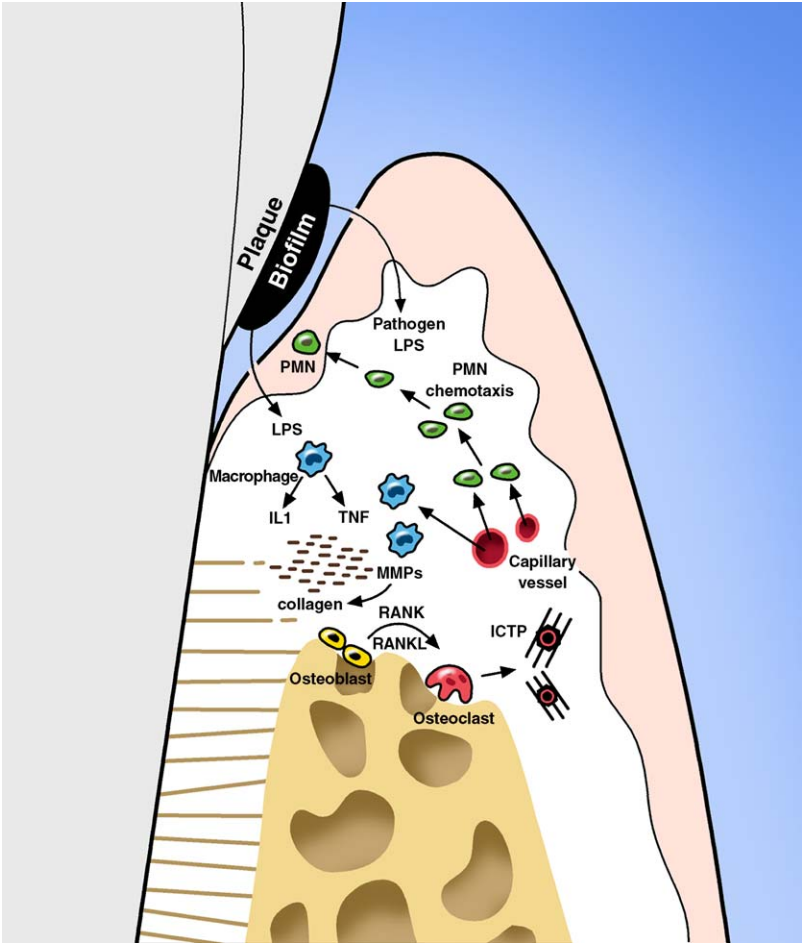
Fig. 1. Schematic representation of the initial events triggered by lipopolysaccharide (LPS) from plaque biofilms on periodontal tissues. Pathogens present in plaque biofilm activate chemotaxis of polymorphonuclear leucocytes (PMN) as a first line of defense against infection. Monocytes and activated macrophages respond to endotoxin by releasing cytokines (tumor necrosis factor [TNF] and interleukin 1 [IL1]) that are mediators for bone resorption. Matrix metalloproteinases (MMPs) released by fibroblast and PMNs are potent collagen destruction enzymes. TNF, IL-1, and receptor activator of NF-κB ligand (RANKL) are elevated in disease sites and play an important role in osteoclastogenesis and bone resorption. Tissue degradation molecules such as pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) that are specific to bone resorption are released into the GCF and can be used as biomarkers for periodontal disease and the previously mentioned cytokines and enzymes. RANK, receptor activator of NF-κB.

A number of specific periodontal pathogens have been implicated in periodontal diseases, including *Tanerella forsythensis*, *Porphyromonas gingivalis*, and *Treponema denticola*. These three organisms are members of the "red complex" of bacteria (and exhibit benzoyl-DL-arginine-naphthyla-mide, or BANA, activity) [20,21] that are highly implicated in the progression of periodontal diseases [22]. *Actinobacillus actinomycetemcomi-tans* has been linked with early-onset forms of periodontal disease and aggressive periodontitis, whereas red complex bacteria are associated with chronic periodontitis [23]. The rationale for the use of microbial analysis for periodontitis monitoring is to target pathogens implicated in disease to (1) identify specific periodontal diseases, (2) identify antibiotic susceptibility of infecting organisms colonizing diseased sites, and (3) predict disease activity. Thus, the goal of microbiologic monitoring is twofold (disease monitoring and disease treatment guidance); however, microbial tests (eg, BANA test, DNA probe analysis, or culturing) have failed to predict future disease progression [24]. These findings can be explained by the fact that the presence of specific periodontal pathogens is necessary to initiate periodontal disease but not sufficient to cause disease in the nonsusceptible host [25].

A recent systematic review of the topic asked the focused question, "In patients with periodontal diseases, does microbial identification influence patient management compared with treatment prescribed without this information?" [24]. The answer to this question is central for the clinical utility of microbial-based diagnostic tests for oral and periodontal diseases. If tests do not influence clinical decision making, then in essence, there is no clinical value for the test. Of the 24 studies (a total of 835 subjects) noted in the review, 13 reported microbial identification as an aid in treatment planning [26–38]. The investigators concluded that the literature lacks studies with a high evidence rating; the most-pertinent studies were case reports or case series without controls [24]. Furthermore, although some practitioners consider microbial identification a valuable adjunct to the management of patients with periodontal diseases, there is a lack of strong evidence to support this practice. It is also possible that as-yet-unidentified, uncultivable microbial species are essential to disease initiation and progression. If so, microbial-based tests for these species are obviously unavailable. Future studies are needed in this area to justify the use of microbial testing to predict progression of periodontal diseases [24]. New strategies that combine microbial identification with host response or tissue breakdown factors using discriminant analysis may better improve the ability of microbial analysis to predict future periodontal disease around teeth and dental implants [39,40].

Host response and inflammatory mediators as potential biomarkers

Periodontal inflammation occurs in the gingival tissue in response to plaque bacteria biofilms [3,41]. Gingivitis is characterized by an initial

increase in blood flow, enhanced vascular permeability, and the influx of cells (neutrophils and monocyte-macrophages) from the peripheral blood to the gingival crevice [42]. Subsequently, T cells and B cells appear at the infection site. After they appear at the lesion, these cells produce a myriad of cytokines such as IL-1β, IL-6, TNF-α, and immunoglubulins as an antigen-specific response [4]. Initially, tissue degradation is limited to epithelial cells and collagen fibers from the connective tissue. Later on, the inflammatory process may reach periodontal supportive tissue, leading to bone resorption (see Fig. 1) [43].

GCF has been extensively investigated for the release of host response factors. It includes a mixture of molecules from blood, host tissue, and plaque biofilms, such as electrolytes, small molecules, proteins, cytokines, antibodies, bacterial antigens, and enzymes [44–60]. Host cell–derived enzymes such as matrix metalloproteinases (MMPs) are an important group of neutral proteinases implicated in the destructive process of periodontal disease that can be measured in GCF [44,61–67]. The neutrophils are the major cells responsible for MMP release at the infected site, specifically MMP-8 (collagenase-2) and MMP-9 (gelatinase-B) [65]. Although MMP-8 is able to potently degrade interstitial collagens, MMP-9 degrades several extracellular matrix proteins [68–71]. Kinane et al [64] and Mantyla et al [65] presented the use of a rapid chairside test based on the immunologic detection of elevated MMP-8 in GCF to diagnose and monitor the course and treatment of periodontitis. With a threshold of 1 mg/L MMP-8 activity, the test provided a sensitivity of 0.83 and specificity of 0.96, demonstrating value as a potential tool to differentiate periodontitis from gingivitis and healthy sites and to monitor treatment of periodontitis.

Macrophages and polymorphonuclear leukocytes, in response to the chemoattractant effect of bacterial lipopolysaccharide [72], are activated to produce important inflammatory mediators—notably, TNF-α, IL-1, IL-6, and other cytokines [73] related to the host response and tissue destruction [72]. Holmlund et al [74] investigated bone resorption activity, IL-1α, IL-1β, and IL-1 receptor antagonist levels in GCF in sites having no signs of periodontal disease and in sites having horizontal or angular periodontal bone loss. The amounts of IL-1α, IL-1β, and IL-1 receptor antagonist from GCF were quantified by ELISA. It was observed that levels of bone resorption activity, IL-1α, IL-1β, and IL-1 receptor antagonist were significantly higher in GCF from diseased sites compared with healthy sites but did not relate to defect morphology [74].

The severity of periodontitis is associated with local (GCF or tissue) increases in IL-1β, TNF-α, prostaglandins such as prostaglandin E_2 [4], and MMPs [61,67,71], whereas inhibition of these substances produces substantial reductions in periodontal disease. Specifically for IL-1 and TNF, local protein blockade in a monkey model of periodontitis produced significant reductions in bone loss [75,76], highlighting the important role of these mediators in periodontal disease.

Advanced stages of periodontal lesions are populated by a large proportion of B lymphocytes and plasma cells [77–79] and increased levels of immunoglobulins in GCF [54,80–82]. Plombas et al [83] investigated GCF and whole saliva from periodontitis patients and periodontally healthy adults for the presence of IgA and IgG antibodies to *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*. Compared with healthy patients, the GCF of periodontitis patients contained significantly higher levels of IgA and IgG antibodies to the four microorganisms tested.

Porphyromonas gingivalis and *Actinobacillus actinomycetemcomitans* are examples of well-studied gram-negative pathogens implicated in the immune and inflammatory host response in periodontal disease [84]. *Porphyromonas gingivalis* produces by far the greatest proteolytic activity through peptidases, elastases, trypsinlike proteases, and collagenases [85] that can be monitored by GCF analysis [86,87]. Figueredo et al [62] compared elastase and collagenase activities in GCF before and after nonsurgical periodontal treatment. Improvement in clinical parameters after therapy was accompanied by a significant reduction in the values of total elastase activity, free elastase, MMP-8, and collagenolytic activity in gingivitis and periodontitis sites.

Aspartate aminotransferase, a tissue destruction biomarker released from necrotic cells in GCF, is associated with periodontitis severity [88,89]. Aspartate aminotransferase–positive sites are positively correlated with higher prevalence of *Porphyromonas gingivalis*, *Streptococcus intermedius*, *Peptostreptococcus micros*, *Campylobacter concisus*, *Bacteroides forsythus*, *Camplobacter gracilis*, *Campylobacter rectus*, and *Selenomonas sputigena* [90]. Moreover, to evaluate the relationship between aspartate aminotransferase and periodontal disease, periodontitis subjects were monitored for 12 months using a chairside assay. After nonsurgical therapy, the percentage of sites exhibiting higher levels of aspartate aminotransferase and bleeding on probing was significantly lower at 6 and 12 months compared with baseline. Elevated levels of aspartate aminotransferase, however, were present at sites that did not subsequently exhibit disease progression [89]. Therefore, the biomarker does not discriminate between progressive sites and sites that are stable but inflamed.

In summary, GCF carries multiple molecular factors derived from the host response and is considered a significant protective mechanism in periodontal infection (Table 3) [91]. These host response factors represent important mediators that can aid in the development of periodontal diagnostics.

Bone-specific markers of tissue destruction for periodontal diagnosis

Of the 50 or more different components in GCF and saliva evaluated to date for periodontal diagnosis, most lack specificity to alveolar bone

Table 3
Examples of biomarkers of periodontal disease identified from plaque biofilm, gingival
crevicular fluid, or saliva

Category mediator	Examples
Microbial factors	DNA probes or culturing of putative periodontal pathogens (eg, *Porphyromonas gingivalis, Tanerella forsythensis, Treponema denticola*)
Host response factors	IL-1β; TNF-α; aspartate aminotransferase; elastase
Connective tissue breakdown products	Collagen telopeptides; osteocalcin; proteoglycans; fibronection fragments

destruction and essentially constitute soft tissue inflammatory events [92]. When examining the destruction of alveolar bone that is preceded by a microbial infection and inflammatory response, the measurement of connective tissue–derived molecules may lead to a more accurate assessment of tissue breakdown due to the tremendous variability of the host response among individuals [92].

Advances in bone cell biology over the past decade have resulted in several new biochemical markers for the measurement of bone homeostasis. With mounting evidence for a relationship between osteoporosis and oral bone loss, investigators have sought to develop better biologic markers to determine and predict oral bone loss [93]. Two of the more well studied mediators (bone collagen fragments and osteocalcin) are presented in the following text.

Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen

Type I collagen composes 90% of the organic matrix of bone and is the most abundant collagen in osseous tissue [94]. Collagen degradation products have emerged as valuable markers of bone turnover in a multitude of bone resorptive and metabolic diseases [95]. Pyridinoline cross-links represent a class of collagen degradative molecules that include pyridinoline, deoxypyridinoline, N-telopeptides, and C-telopeptides [96]. Pyridinoline and deoxypyridinoline are mature intermolecular cross-links of collagen. Subsequent to osteoclastic bone resorption and collagen matrix degradation, pyridinoline, deoxypyridinoline, and amino- and carboxyterminal cross-linked telopeptides of type I collagen are released into the circulation. Because the cross-linked telopeptides result from post-translational modification of collagen molecules, they cannot be reused during collagen synthesis and are therefore considered specific biomarkers for bone resorption [97]. In addition, the value of pyridinoline cross-links as potential markers of bone turnover relates to their specificity for bone. In skin and other soft tissues, histidine cross-links are the predominant form and no pyridinoline-like structures exist. Recently, a degradation fragment originating from the

helical part of type I collagen and consisting of the 620–633 sequence of the α1 chain has been identified to correlate highly with amino- and carboxyterminal telopeptides associated with bone resorption [98].

The pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a 12- to 20-kd fragment of bone type I collagen released by digestion with trypsin or bacterial collagenase [99]. Elevated serum ICTP and other pyridinoline cross-linked components have been shown to be correlated with the bone resorptive rate in several bone metabolic diseases including osteoporosis [100], rheumatoid arthritis [101], and Paget's disease [102]. Furthermore, pyridinoline cross-links demonstrated significant decreases in postmenopausal osteoporotic subjects after bisphosphonate [103] or estrogen [104] therapy.

Given their specificity for bone resorption, pyridinoline cross-links represent a potentially valuable diagnostic aid in periodontics because biochemical markers specific for bone degradation may be useful in differentiating between the presence of gingival inflammation and active periodontal or peri-implant bone destruction [105]. Several investigations have explored the ability of pyridinoline cross-links to detect bone resorption in periodontitis lesions [39,63,106–108], in peri-implantitis [40], and in response to periodontal therapy [40,61,66,107,109–111].

Palys et al [39] related ICTP levels to the subgingival microflora of various disease states on GCF. Subjects were divided into groups representing health, gingivitis, and chronic periodontitis, and GCF and plaque samples were collected from each subject. The samples were analyzed for ICTP levels and the presence of 40 subgingival species using checkerboard DNA-DNA hybridization techniques. ICTP levels differed significantly between health, gingivitis, and periodontitis subjects, and related modestly to several clinical disease parameters. ICTP levels were also strongly correlated with whole-subject levels of several periodontal pathogens including *Tanerella forsythensis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Treponema denticola*. Oringer et al [40] examined the relationship between ICTP levels and subgingival species around implants and teeth in 20 partially edentulous and 2 fully edentulous patients. No significant differences were found among ICTP levels and subgingival plaque composition between implants and teeth. Strong correlations were found between elevated ICTP levels at implant sites and colonization with organisms associated with failing implants such as *Prevotella intermedia*, *Fusobacterim nucleatum* subsp *vincentii*, and *Streptococcus gordonii* [40].

Diagnostic tools have also been applied to evaluate the response to active periodontal therapy. Golub et al [63] found that treatment of chronic periodontitis patients with scaling and root planing (SRP) and an MMP inhibitor (subantimicrobial doxycycline hyclate) resulted in a 70% reduction in GCF ICTP levels after 1 month, concomitant with a 30% reduction in collagenase levels. An investigation of periodontitis patients treated with

SRP also demonstrated significant correlations between GCF ICTP levels and clinical periodontal disease parameters, including attachment loss, pocket depth, and bleeding on probing [93]. In addition, elevated GCF ICTP levels at baseline, especially at shallow sites, were found to be predictive for future attachment loss as early as 1 month after sampling. Furthermore, treatment of a group of periodontitis subjects by SRP and locally delivered minocycline led to rapid reductions in GCF ICTP levels [63].

Studies assessing the role of GCF ICTP levels as a diagnostic marker of periodontal disease activity have produced promising results to date. ICTP has been shown to be a good predictor of future alveolar bone and attachment loss, was strongly correlated with clinical parameters and putative periodontal pathogens, and demonstrated significant reductions after periodontal therapy [92]. Controlled human longitudinal trials are needed to fully establish the role of ICTP as a predictor of periodontal tissue destruction, disease activity, and response to therapy in periodontal patients.

Osteocalcin

Osteocalcin is a calcium-binding protein of bone and is the most abundant noncollagenous protein in mineralized tissues [112]. Osteocalcin is synthesized predominantly by osteoblasts [113] and has an important role in bone formation and turnover [114,115]. Osteocalcin exhibits chemoattractive activity for osteoclast progenitor cells and monocytes [116–118], and its synthesis in vitro is stimulated by 1,25-dihydroxyvitamin D_3. It has also been shown to promote bone resorption, and stimulate differentiation of osteoclast progenitor cells [112,119]. Elevated serum osteocalcin levels have been shown during periods of rapid bone turnover (eg, osteoporosis, multiple myeloma, and fracture repair) [120,121]. Serum osteocalcin is presently a valid marker of bone turnover when resorption and formation are coupled and is a specific marker of bone formation when formation and resorption are uncoupled [115,120,122,123].

Several studies have investigated the relationship between GCF osteocalcin levels and periodontal disease [49,63,106,124–126]. Kunimatsu et al [124] reported a positive correlation between GCF osteocalcin aminoterminal peptide levels and clinical parameters in a cross-sectional study of periodontitis and gingivitis patients. The investigators also reported that osteocalcin could not be detected in patients with gingivitis. In contrast, Nakashima et al [126] reported significant GCF osteocalcin levels from periodontitis and gingivitis patients. Osteocalcin levels were also significantly correlated with pocket depth, gingival index scores, and GCF levels of alkaline phosphatase and prostaglandin E_2. In a longitudinal study of untreated periodontitis patients with ≥ 1.5 mm attachment loss during the monitoring period, GCF osteocalcin levels alone were unable to distinguish

between active and inactive sites [49]. When a combination of the biochemical markers osteocalcin, collagenase, prostaglandin E_2, α_2-macroglobulin, elastase, and alkaline phosphatase was evaluated, however, increased diagnostic sensitivity and specificity values of 80% and 91%, respectively, were reported [49].

A longitudinal study using an experimental periodontitis model in beagle dogs reported a strong correlation between GCF osteocalcin levels and active bone turnover as assessed by bone-seeking radio pharmaceutical uptake [106]. Osteocalcin, however, was shown to possess only modest predictive value for future bone loss measured by computer-assisted digitizing radiography. Moreover, treatment of chronic periodontitis patients with subantimicrobial doxycycline failed to reduce GCF osteocalcin levels [63], and a cross-sectional study of periodontitis patients reported no differences in GCF osteocalcin levels between deep and shallow sites in the same patients [125]. In addition, osteocalcin levels in the GCF during orthodontic tooth movement were highly variable between subjects and lacked a consistent pattern related to the stages of tooth movement [127]. Taken together, the results of these studies show a potential role for intact osteocalcin as a bone-specific marker of bone turnover but not as a predictive indicator for periodontal disease. Greater promise appears to be in the detection of aminoterminal osteocalcin fragments for periodontal disease detection. Additional longitudinal studies may be warranted to more fully elucidate the utility of osteocalcin as a periodontal disease activity diagnostic aid.

Role of oral fluid biomarkers in periodontal diagnosis

A biomarker or biologic marker, according to the most recent definition [128], is a substance that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Because saliva and GCF are fluids easily collected and contain locally and systemically derived markers of periodontal disease, they may offer the basis for a patient-specific biomarker assessment for periodontitis and other systemic diseases [18,129].

Due to the noninvasive and simple nature of their collection, analysis of saliva and GCF may be especially beneficial in the determination of current periodontal status and a means of monitoring response to treatment [130,131]. Many studies have shown that the determination of inflammatory mediator levels in biologic fluids is a good indicator of inflammatory activity. Therefore, studies related to the pathogenesis of periodontal diseases usually examine whether biochemical and immunologic markers in saliva or GCF might reflect the extent of periodontal destruction and possibly predict future disease progression [18,129]. Oral fluid biomarkers that have been studied for periodontal diagnosis include proteins of host

origin (ie, enzymes and immunoglobulins), phenotypic markers, host cells, hormones, bacteria and bacterial products, ions, and volatile compounds [18,132–135]. Table 3 lists a sample of compounds obtained by diagnostic screening of saliva or GCF.

Future directions

There is a plethora of possibilities for the future use of oral fluids in biotechnology and health care applications, especially in the field of diagnostics. A tremendous amount of research activity is currently under way to explore the role of oral fluids as a possible medium in a variety of applications.

Recent advances in HIV diagnosis have been made using oral fluids. A commercially available kit (OraSure, OraSure Technologies, Bethlehem, Pennsylvania) has an oral specimen collection device that is placed between the buccal mucosa and buccal gingiva for 2 to 5 minutes to collect HIV-1 antibodies (not the virus) from the tissues of the cheek and gingiva. OraSure HIV-1 does not collect saliva but rather a sample called oral mucosal transudate. For different fluids (oral fluid, finger-stick or venipuncture whole blood or plasma specimens), the alternative test OraQuick (OraSure Technologies) provides accurate results for HIV-1 and HIV-2 in 20 minutes. The collector pad is placed in a vial with preservative and sent to a clinical laboratory for testing with an initial "screening" assay (ELISA). If necessary, a supplementary test (Western blot assay) is performed to verify the results of the screening assay. This process is referred to as the OraSure testing algorithm [136].

Several researchers have focused on genetic single nucleotide polymorphisms in the study of periodontitis. There is a genetic susceptibility test currently available for severe chronic periodontitis (Interleukin Genetics, Waltham, Massachusetts). This system works by detection of two types of IL-1 genetic alleles, IL-1α +4845 and IL-1β +3954 [137]. Individuals identified as "genotype positive," or found to have both of these alleles, are more likely to have the phenotype of overexpression of this gene. The increased GCF and salivary IL-1 predisposes the patient to the severe form of chronic periodontitis by way of a hyperinflammatory response to bacterial challenge. In this way, genomics has been found to be applicable in the prediction of predisposition to periodontitis in certain patient populations [138]. Socransky et al [139] took a different approach in researching IL-1 gene polymorphisms in periodontitis patients. These investigators linked previous findings regarding the association of IL-1 polymorphisms and severity of adult periodontitis with microbial species found in IL-1 genotype-negative versus IL-1 genotype-positive patients. These researchers concluded that those who were IL-1 genotype positive tended to have higher levels of the more damaging microbial species (red

and orange complex organisms) associated with periodontal inflammation [139].

Li et al [140] investigated the potential use of genomics in the development of salivary diagnostics. They performed microarray testing of cell-free saliva for RNA profiling. RNA was isolated from unstimulated saliva that was collected from healthy subjects. After analysis by microarray and quantitative polymerase chain reaction, they found that it was possible to profile messenger RNAs, of which there were thousands present in the saliva. More recently, the group demonstrated the potential of salivary IL-8 levels to predict patients afflicted with squamous cell carcinoma [141].

Salivary immunocomponents have also been studied at length in oral health, including immunoglobulin subclass, immunoglobulin isotypes, and antibody levels [142–148]. Other salivary constituents that have been investigated for diagnostic uses include epithelial keratins [149], occult blood [150], salivary ions such as calcium and phosphates [151,152], and serum markers such as cortisol [153–155].
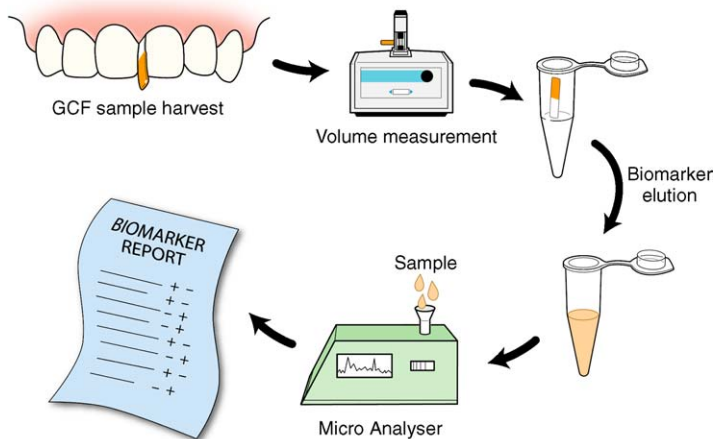

Fig. 2. Futuristic chairside diagnostic test based on GCF sampling. Considering the GCF fluid as a potential analyte for the screening of multiple biomarkers, a rapid, chairside diagnostic tool (represented in the figure as a Micro Analyser) or a "mini-lab" could be used by clinicians for risk assessment and decision making on treatment planning. The advantages of such a tool would be enhanced predictability of clinical outcomes and well-informed patients regarding personalized treatment needs. As shown, a simple clinical procedure for GCF collection could be used, followed by extraction of analytes from the test strip. The fluid present on the test strip would be subjected to volumetric quantification. After an elution procedure to "wash" and retrieve the compounds from the fluid, the sample would be analyzed. An immediate comprehensive risk report profile and biomarkers screening would enable evidence-based decision making.

Summary

Researchers in the biotechnology and medical realm are currently investigating the use of oral fluids for the diagnosis of oral and systemic diseases and for drug development. In the pharmaceutical industry, the use of biomarkers is avidly being developed for use in tailored dosing and drug metabolism studies. Professionals in seemingly unrelated arenas such as the insurance industry, the Environment Protection Agency, and Homeland Security are interested in the possible use of oral fluids to monitor biomarkers. Under investigation are possible uses of GCF and saliva in the preliminary screening for biological/chemical warfare agent exposure, environmental toxin detection, and screening for metabolites of drugs of abuse.

In the field of oral disease diagnosis, there has been a steady growing trend during the last 2 decades to develop tools to monitor periodontitis. From physical measurements such as periodontal probing to sophisticated genetic susceptibility analysis and molecular assays for the detection of biomarkers on the different stages of the disease, substantial improvements have been made on the understanding of the mediators implicated on the initiation and progression of periodontitis. At the same time, this evolutionary process has promoted the discovery of new biomarkers and the development of new therapeutic approaches mainly using host modulation. Moreover, new diagnostic technologies such as nucleic acid and protein microarrays and microfluidics are under development for risk assessment and comprehensive screening of biomarkers. These recent advances are leading to the development of more powerful diagnostic tools for practitioners to optimize their treatment predictability (Fig. 2).

References

[1] Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. J Periodontol 1992;63(4 Suppl):322–31.

[2] Genco RJ. Host responses in periodontal diseases: current concepts. J Periodontol 1992; 63(Suppl 4):338–55.

[3] Kirkwood KL, Taba M Jr, Rossa C, et al. Molecular biology of the host-microbe interaction in periodontal diseases. Selected topics: molecular signaling aspects of pathogen-mediated bone destruction in periodontal diseases. In: Newman M, Takei H, editors. Carranza's periodontology 10th edition. St. Louis (MO): Elsevier; in press.

[4] Offenbacher S. Periodontal diseases: pathogenesis. Ann Periodontol 1996;1(1):821–78.

[5] Albandar JM. Periodontal diseases in North America. Periodontol 2000 2002;29:31–69.

[6] Armitage GC. The complete periodontal examination. Periodontol 2000 2004;34:22–33.

[7] Goodson JM. Conduct of multicenter trials to test agents for treatment of periodontitis. J Periodontol 1992;63(Suppl 12):1058–63.

[8] Page RC, Martin J, Krall EA, et al. Longitudinal validation of a risk calculator for periodontal disease. J Clin Periodontol 2003;30(9):819–27.

[9] Genco RJ. Assessment of risk of periodontal disease. Compend Suppl 1994;18:678–83.

[10] Lamster IB. Current concepts and future trends for periodontal disease and periodontal therapy, part 2: classification, diagnosis, and nonsurgical and surgical therapy. Dent Today 2001;20(3):86–91.

[11] Lang NP, Tonetti MS. Periodontal diagnosis in treated periodontitis. Why, when and how to use clinical parameters. J Clin Periodontol 1996;23(3 Pt 2):240–50.

[12] Genco RJ. Current view of risk factors for periodontal diseases. J Periodontol 1996; 67(Suppl 10):1041–9.

[13] Colburn WA. Biomarkers in drug discovery and development: from target identification through drug marketing. J Clin Pharmacol 2003;43(4):329–41.

[14] Kamma JJ, Giannopoulou C, Vasdekis VG, et al. Cytokine profile in gingival crevicular fluid of aggressive periodontitis: influence of smoking and stress. J Clin Periodontol 2004; 31(10):894–902.

[15] Souza SL, Taba M Jr. Cross-sectional evaluation of clinical parameters to select high prevalence populations for periodontal disease: the site comparative severity methodology. Braz Dent J 2004;15(1):46–53.

[16] Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontol 2000 2002;28:12–55.

[17] Uitto VJ. Gingival crevice fluid—an introduction. Periodontol 2000 2003;31:9–11.

[18] Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis—a review. J Clin Periodontol 2000;27(7):453–65.

[19] Paster BJ, Boches SK, Galvin JL, et al. Bacterial diversity in human subgingival plaque. J Bacteriol 2001;183(12):3770–83.

[20] Loesche WJ, Lopatin DE, Giordano J, et al. Comparison of the benzoyl-DL-arginine-naphthylamide (BANA) test, DNA probes, and immunological reagents for ability to detect anaerobic periodontal infections due to *Porphyromonas gingivalis, Treponema denticola*, and *Bacteroides forsythus*. J Clin Microbiol 1992;30(2):427–33.

[21] Loesche WJ, Kazor CE, Taylor GW. The optimization of the BANA test as a screening instrument for gingivitis among subjects seeking dental treatment. J Clin Periodontol 1997; 24(10):718–26.

[22] Socransky SS, Haffajee AD, Cugini MA, et al. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25(2):134–44.

[23] Zambon JJ. Periodontal diseases: microbial factors. Ann Periodontol 1996;1(1):879–925.

[24] Listgarten MA, Loomer PM. Microbial identification in the management of periodontal diseases. A systematic review. Ann Periodontol 2003;8(1):182–92.

[25] Offenbacher S, Jared HL, O'Reilly PG, et al. Potential pathogenic mechanisms of periodontitis associated pregnancy complications. Ann Periodontol 1998;3(1):233–50.

[26] Muller HP, Streletz E, Muller RF, et al. Microbiologic diagnosis and treatment of periodontally involved, "hopeless" teeth. Int J Periodontics Restorative Dent 1991;11(5): 376–86.

[27] Levy D, Csima A, Birek P, et al. Impact of microbiological consultation on clinical decision making: a case-control study of clinical management of recurrent periodontitis. J Periodontol 1993;64(11):1029–39.

[28] Rosenberg ES, Torosian JP, Hammond BF, et al. Routine anaerobic bacterial culture and systemic antibiotic usage in the treatment of adult periodontitis: a 6-year longitudinal study. Int J Periodontics Restorative Dent 1993;13(3):213–43.

[29] Fine DH. Microbial identification and antibiotic sensitivity testing, an aid for patients refractory to periodontal therapy. A report of 3 cases. J Clin Periodontol 1994;21(2):98–106.

[30] Ishikawa I, Umeda M, Laosrisin N. Clinical, bacteriological, and immunological examinations and the treatment process of two Papillon-Lefevre syndrome patients. J Periodontol 1994;65(4):364–71.

[31] Ishikawa I, Kawashima Y, Oda S, et al. Three case reports of aggressive periodontitis associated with *Porphyromonas gingivalis* in younger patients. J Periodontal Res 2002; 37(5):324–32.

[32] Renvert S, Dahlen G, Wikstrom M. Treatment of periodontal disease based on microbiological diagnosis. Relation between microbiological and clinical parameters during 5 years. J Periodontol 1996;67(6):562–71.

[33] Worch KP, Listgarten MA. Treatment considerations in rapidly progressive periodontitis: a case report. Compend Contin Educ Dent 1998;19(12):1203–6.

[34] Worch KP, Listgarten MA, Korostoff JM. A multidisciplinary approach to the diagnosis and treatment of early-onset periodontitis: a case report. J Periodontol 2001;72(1): 96–106.

[35] Kamma JJ, Lygidakis NA, Nakou M. Subgingival microflora and treatment in prepubertal periodontitis associated with chronic idiopathic neutropenia. J Clin Periodontol 1998; 25(9):759–65.

[36] De Vree H, Steenackers K, De Boever JA. Periodontal treatment of rapid progressive periodontitis in 2 siblings with Papillon-Lefevre syndrome: 15-year follow-up. J Clin Periodontol 2000;27(5):354–60.

[37] Eickholz P, Kugel B, Pohl S, et al. Combined mechanical and antibiotic periodontal therapy in a case of Papillon-Lefevre syndrome. J Periodontol 2001;72(4):542–9.

[38] Pacheco JJ, Coelho C, Salazar F, et al. Treatment of Papillon-Lefevre syndrome periodontitis. J Clin Periodontol 2002;29(4):370–4.

[39] Palys MD, Haffajee AD, Socransky SS, et al. Relationship between C-telopeptide pyridinoline cross-links (ICTP) and putative periodontal pathogens in periodontitis. J Clin Periodontol 1998;25(11 Pt 1):865–71.

[40] Oringer RJ, Palys MD, Iranmanesh A, et al. C-telopeptide pyridinoline cross-links (ICTP) and periodontal pathogens associated with endosseous oral implants. Clin Oral Implants Res 1998;9(6):365–73.

[41] Novaes Junior AB, Souza SL, Taba M Jr, et al. Control of gingival inflammation in a teenager population using ultrasonic prophylaxis. Braz Dent J 2004;15(1):41–5.

[42] Madianos PN, Papapanou PN, Sandros J. Porphyromonas gingivalis infection of oral epithelium inhibits neutrophil transepithelial migration. Infect Immun 1997;65(10): 3983–90.

[43] Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. Lab Invest 1976;34(3):235–49.

[44] Soder B, Jin LJ, Wickholm S. Granulocyte elastase, matrix metalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers with persistent periodontitis. J Clin Periodontol 2002;29(5):384–91.

[45] Golub LM, Kleinberg I. Gingival crevicular fluid: a new diagnostic aid in managing the periodontal patient. Oral Sci Rev 1976;8:49–61.

[46] Golub LM, McNamara TF, Ryan ME, et al. Adjunctive treatment with subantimicrobial doses of doxycycline: effects on gingival fluid collagenase activity and attachment loss in adult periodontitis. J Clin Periodontol 2001;28(2):146–56.

[47] Cimasoni G. Crevicular fluid updated. Monogr Oral Sci 1983;12(III-VII):1–152.

[48] Estreicher A, Broggiato A, Duroux P, et al. Low molecular-weight proteins in human gingival crevicular fluid. Arch Oral Biol 1996;41(8–9):733–8.

[49] Nakashima K, Giannopoulou C, Andersen E, et al. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. J Clin Periodontol 1996;23(9):832–8.

[50] Kojima T, Andersen E, Sanchez JC, et al. Human gingival crevicular fluid contains MRP8 (S100A8) and MRP14 (S100A9), two calcium-binding proteins of the S100 family. J Dent Res 2000;79(2):740–7.

[51] Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, et al. Gingival crevicular fluid levels of interleukin-1beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. J Periodontol 2004;75(9):1203–8.

[52] Lamster IB, Kaufman E, Grbic JT, et al. Beta-glucuronidase activity in saliva: relationship to clinical periodontal parameters. J Periodontol 2003;74(3):353–9.

[53] Engebretson SP, Grbic JT, Singer R, et al. GCF IL-1beta profiles in periodontal disease. J Clin Periodontol 2002;29(1):48–53.

[54] Grbic JT, Lamster IB, Fine JB, et al. Changes in gingival crevicular fluid levels of immunoglobulin A following therapy: association with attachment loss. J Periodontol 1999;70(10):1221–7.

[55] Grbic JT, Lamster IB, Mitchell-Lewis D. Inflammatory and immune mediators in crevicular fluid from HIV-infected injecting drug users. J Periodontol 1997;68(3): 249–55.

[56] Gustafsson A, Asman B, Bergstrom K. Elastase and lactoferrin in gingival crevicular fluid: possible indicators of a granulocyte-associated specific host response. J Periodontal Res 1994;29(4):276–82.

[57] Lerner UH, Modeer T, Krekmanova L, et al. Gingival crevicular fluid from patients with periodontitis contains bone resorbing activity. Eur J Oral Sci 1998;106(3):778–87.

[58] Rasmussen L, Hanstrom L, Lerner UH. Characterization of bone resorbing activity in gingival crevicular fluid from patients with periodontitis. J Clin Periodontol 2000;27(1): 41–52.

[59] Shapiro L, Goldman H, Bloom A. Sulcular exudate flow in gingival inflammation. J Periodontol 1979;50(6):301–4.

[60] Novaes AB Jr, Shapiro L, Fillios LC, et al. Gingival fluid fucose to protein ratios as indicators of the severity of periodontal disease. J Periodontol 1980;51(2):88–94.

[61] Gapski R, Barr JL, Sarment DP, et al. Effect of systemic matrix metalloproteinase inhibition on periodontal wound repair: a proof of concept trial. J Periodontol 2004;75(3): 441–52.

[62] Figueredo CM, Areas A, Miranda LA, et al. The short-term effectiveness of non-surgical treatment in reducing protease activity in gingival crevicular fluid from chronic periodontitis patients. J Clin Periodontol 2004;31(8):615–9.

[63] Golub LM, Lee HM, Greenwald RA, et al. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. Inflamm Res 1997;46(8):310–9.

[64] Kinane DF, Darby IB, Said S, et al. Changes in gingival crevicular fluid matrix metalloproteinase-8 levels during periodontal treatment and maintenance. J Periodontal Res 2003;38(4):400–4.

[65] Mantyla P, Stenman M, Kinane DF, et al. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. J Periodontal Res 2003;38(4):436–9.

[66] Oringer RJ, Al-Shammari KF, Aldredge WA, et al. Effect of locally delivered minocycline microspheres on markers of bone resorption. J Periodontol 2002;73(8):835–42.

[67] Ryan ME, Ramamurthy S, Golub LM. Matrix metalloproteinases and their inhibition in periodontal treatment. Curr Opin Periodontol 1996;3:85–96.

[68] Ingman T, Tervahartiala T, Ding Y, et al. Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. J Clin Periodontol 1996;23(12): 1127–32.

[69] Mellanen L, Ingman T, Lahdevirta J, et al. Matrix metalloproteinases-1, -3 and 8 and myeloperoxidase in saliva of patients with human immunodeficiency virus infection. Oral Dis 1996;2(4):263–71.

[70] Teronen O, Konttinen YT, Lindqvist C, et al. Inhibition of matrix metalloproteinase-1 by dichloromethylene bisphosphonate (clodronate). Calcif Tissue Int 1997;61(1):59–61.

[71] Makela M, Salo T, Uitto VJ, et al. Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. J Dent Res 1994;73(8): 1397–406.

[72] Amar S, Oyaisu K, Li L, et al. Moesin: a potential LPS receptor on human monocytes. J Endotoxin Res 2001;7(4):281–6.

[73] Mogi M, Otogoto J, Ota N, et al. Interleukin 1 beta, interleukin 6, beta 2-microglobulin, and transforming growth factor-alpha in gingival crevicular fluid from human periodontal disease. Arch Oral Biol 1999;44(6):535–9.

[74] Holmlund A, Hanstrom L, Lerner UH. Bone resorbing activity and cytokine levels in gingival crevicular fluid before and after treatment of periodontal disease. J Clin Periodontol 2004;31(6):475–82.

[75] Assuma R, Oates T, Cochran D, et al. IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. J Immunol 1998;160(1):403–9.

[76] Graves DT, Delima AJ, Assuma R, et al. Interleukin-1 and tumor necrosis factor antagonists inhibit the progression of inflammatory cell infiltration toward alveolar bone in experimental periodontitis. J Periodontol 1998;69(12):1419–25.

[77] Ranney RR. Immunologic mechanisms of pathogenesis in periodontal diseases: an assessment. J Periodontal Res 1991;26(3 Pt 2):243–54.

[78] Seymour GJ, Cole KL, Powell RN. Analysis of lymphocyte populations extracted from chronically inflamed human periodontal tissues. I. Identification. J Periodontal Res 1985; 20(1):47–57.

[79] Seymour GJ, Cole KL, Powell RN. Analysis of lymphocyte populations extracted from chronically inflamed human periodontal tissues. II. Blastogenic response. J Periodontal Res 1985;20(6):571–9.

[80] Condorelli F, Scalia G, Cali G, et al. Isolation of *Porphyromonas gingivalis* and detection of immunoglobulin A specific to fimbrial antigen in gingival crevicular fluid. J Clin Microbiol 1998;36(8):2322–5.

[81] Kinane DF, Takahashi K, Mooney J. Crevicular fluid and serum IgG subclasses and corresponding mRNA expressing plasma cells in periodontitis lesions. J Periodontal Res 1997;32(1 Pt 2):176–8.

[82] Dibart S, Eftimiadi C, Socransky S, et al. Rapid evaluation of serum and gingival crevicular fluid immunoglobulin G subclass antibody levels in patients with early-onset periodontitis using checkerboard immunoblotting. Oral Microbiol Immunol 1998;13(3):166–72.

[83] Plombas M, Gobert B, De March AK, et al. Isotypic antibody response to plaque anaerobes in periodontal disease. J Periodontol 2002;73(12):1507–11.

[84] Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 1994;5:78–111.

[85] Courant PR, Bader H. Bacteroides melaninogenicus and its products in the gingiva of man. Periodontics 1966;4(3):131–6.

[86] Eley BM, Cox SW. Bacterial proteases in gingival crevicular fluid before and after periodontal treatment. Br Dent J 1995;178(4):133–9.

[87] Eley BM, Cox SW. Cathepsin B/L-, elastase-, tryptase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid: correlation with clinical parameters in untreated chronic periodontitis patients. J Periodontal Res 1992;27(1):62–9.

[88] Lamster IB. Evaluation of components of gingival crevicular fluid as diagnostic tests. Ann Periodontol 1997;2(1):123–37.

[89] Oringer RJ, Howell TH, Nevins ML, et al. Relationship between crevicular aspartate aminotransferase levels and periodontal disease progression. J Periodontol 2001;72(1): 17–24.

[90] Kamma JJ, Nakou M, Persson RG. Association of early onset periodontitis microbiota with aspartate aminotransferase activity in gingival crevicular fluid. J Clin Periodontol 2001;28(12):1096–105.

[91] Takahashi K, Mooney J, Frandsen EV, et al. IgG and IgA subclass mRNA-bearing plasma cells in periodontitis gingival tissue and immunoglobulin levels in the gingival crevicular fluid. Clin Exp Immunol 1997;107(1):158–65.

[92] Giannobile WV. C-telopeptide pyridinoline cross-links. Sensitive indicators of periodontal tissue destruction. Ann N Y Acad Sci 1999;878:404–12.

[93] Giannobile WV, Al-Shammari KF, Sarment DP. Matrix molecules and growth factors as indicators of periodontal disease activity. Periodontol 2000 2003;31:125–34.

[94] Narayanan AS, Page RC. Connective tissues of the periodontium: a summary of current work. Coll Relat Res 1983;3(1):33–64.

[95] Johnell O, Oden A, De Laet C, et al. Biochemical indices of bone turnover and the assessment of fracture probability. Osteoporos Int 2002;13(7):523–6.

[96] Calvo MS, Eyre DR, Gundberg CM. Molecular basis and clinical application of biological markers of bone turnover. Endocr Rev 1996;17(4):333–68.

[97] Eriksen EF, Charles P, Melsen F, et al. Serum markers of type I collagen formation and degradation in metabolic bone disease: correlation with bone histomorphometry. J Bone Miner Res 1993;8(2):127–32.

[98] Garnero P, Delmas PD. An immunoassay for type I collagen alpha 1 helicoidal peptide 620–633, a new marker of bone resorption in osteoporosis. Bone 2003;32(1):20–6.

[99] Risteli J, Elomaa I, Niemi S, et al. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. Clin Chem 1993;39(4):635–40.

[100] Colwell A, Russell RG, Eastell R. Factors affecting the assay of urinary 3-hydroxy pyridinium crosslinks of collagen as markers of bone resorption. Eur J Clin Invest 1993; 23(6):341–9.

[101] Black D, Marabani M, Sturrock RD, et al. Urinary excretion of the hydroxypyridinium cross links of collagen in patients with rheumatoid arthritis. Ann Rheum Dis 1989;48(8): 641–4.

[102] Uebelhart D, Gineyts E, Chapuy MC, et al. Urinary excretion of pyridinium crosslinks: a new marker of bone resorption in metabolic bone disease. Bone Miner 1990;8(1): 87–96.

[103] Garnero P, Gineyts E, Riou JP, et al. Assessment of bone resorption with a new marker of collagen degradation in patients with metabolic bone disease. J Clin Endocrinol Metab 1994;79(3):780–5.

[104] Yasumizu T, Hoshi K, Iijima S, et al. Serum concentration of the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a useful indicator of decline and recovery of bone mineral density in lumbar spine: analysis in Japanese postmenopausal women with or without hormone replacement. Endocr J 1998;45(1):45–51.

[105] Giannobile WV. Crevicular fluid biomarkers of oral bone loss. Curr Opin Periodontol 1997;4:11–20.

[106] Giannobile WV, Lynch SE, Denmark RG, et al. Crevicular fluid osteocalcin and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) as markers of rapid bone turnover in periodontitis. A pilot study in beagle dogs. J Clin Periodontol 1995;22(12):903–10.

[107] Shibutani T, Murahashi Y, Tsukada E, et al. Experimentally induced periodontitis in beagle dogs causes rapid increases in osteoclastic resorption of alveolar bone. J Periodontol 1997;68(4):385–91.

[108] Talonpoika JT, Hamalainen MM. Type I collagen carboxyterminal telopeptide in human gingival crevicular fluid in different clinical conditions and after periodontal treatment. J Clin Periodontol 1994;21(5):320–6.

[109] Perez LA, Al-Shammari KF, Giannobile WV, et al. Treatment of periodontal disease in a patient with Ehlers-Danlos syndrome. A case report and literature review. J Periodontol 2002;73(5):564–70.

[110] Williams RC, Paquette DW, Offenbacher S, et al. Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. J Periodontol 2001;72(11): 1535–44.

[111] Al-Shammari KF, Giannobile WV, Aldredge WA, et al. Effect of non-surgical periodontal therapy on C-telopeptide pyridinoline cross-links (ICTP) and interleukin-1 levels. J Periodontol 2001;72(8):1045–51.

[112] Lian JB, Gundberg CM. Osteocalcin. Biochemical considerations and clinical applications. Clin Orthop 1988;226:267–91.

[113] Bronckers AL, Gay S, Dimuzio MT, et al. Immunolocalization of gamma-carboxyglutamic acid containing proteins in developing rat bones. Coll Relat Res 1985;5(3):273–81.

[114] Ducy P, Geoffroy V, Karsenty G. Study of osteoblast-specific expression of one mouse osteocalcin gene: characterization of the factor binding to OSE2. Connect Tissue Res 1996; 35(1–4):7–14.

[115] Garnero P, Delmas PD. Biochemical markers of bone turnover. Applications for osteoporosis. Endocrinol Metab Clin N Am 1998;27(2):303–23.

[116] Chenu C, Colucci S, Grano M, et al. Osteocalcin induces chemotaxis, secretion of matrix proteins, and calcium-mediated intracellular signaling in human osteoclast-like cells. J Cell Biol 1994;127(4):1149–58.

[117] Glowacki J, Lian JB. Impaired recruitment and differentiation of osteoclast progenitors by osteocalcin-deplete bone implants. Cell Differ 1987;21(4):247–54.

[118] Mundy GR, Poser JW. Chemotactic activity of the gamma-carboxyglutamic acid containing protein in bone. Calcif Tissue Int 1983;35(2):164–8.

[119] Canalis E. Effect of growth factors on bone cell replication and differentiation. Clin Orthop 1985;193:246–63.

[120] Bataille R, Delmas P, Sany J. Serum bone gla-protein in multiple myeloma. Cancer 1987; 59(2):329–34.

[121] Slovik DM, Gundberg CM, Neer RM, et al. Clinical evaluation of bone turnover by serum osteocalcin measurements in a hospital setting. J Clin Endocrinol Metab 1984;59(2): 228–30.

[122] Brown JP, Delmas PD, Malaval L, et al. Serum bone Gla-protein: a specific marker for bone formation in postmenopausal osteoporosis. Lancet 1984;1(8386):1091–3.

[123] Delmas PD, Chatelain P, Malaval L, et al. Serum bone GLA-protein in growth hormone deficient children. J Bone Miner Res 1986;1(4):333–8.

[124] Kunimatsu K, Mataki S, Tanaka H, et al. A cross-sectional study on osteocalcin levels in gingival crevicular fluid from periodontal patients. J Periodontol 1993;64(9):865–9.

[125] Lee AJ, Walsh TF, Hodges SJ, et al. Gingival crevicular fluid osteocalcin in adult periodontitis. J Clin Periodontol 1999;26(4):252–6.

[126] Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandin E2 and alkaline phosphatase in gingival crevicular fluid: their relations to periodontal status. J Clin Periodontol 1994;21(5):327–33.

[127] Griffiths GS, Moulson AM, Petrie A, et al. Evaluation of osteocalcin and pyridinium crosslinks of bone collagen as markers of bone turnover in gingival crevicular fluid during different stages of orthodontic treatment. J Clin Periodontol 1998;25(6):492–8.

[128] Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69(3):89–95.

[129] Ozmeric N. Advances in periodontal disease markers. Clin Chim Acta 2004;343(1–2):1–16.

[130] Zambon JJ, Nakamura M, Slots J. Effect of periodontal therapy on salivary enzymatic activity. J Periodontal Res 1985;20(6):652–9.

[131] Hayakawa H, Yamashita K, Ohwaki K, et al. Collagenase activity and tissue inhibitor of metalloproteinases-1 (TIMP-1) content in human whole saliva from clinically healthy and periodontally diseased subjects. J Periodontal Res 1994;29(5):305–8.

[132] Ferguson DB. Current diagnostic uses of saliva. J Dent Res 1987;66(2):420–4.

[133] Lamster IB, Grbic JT. Diagnosis of periodontal disease based on analysis of the host response. Periodontol 2000 1995;7:83–99.

[134] Mandel ID. The diagnostic uses of saliva. J Oral Pathol Med 1990;19(3):119–25.

[135] Nakamura M, Slots J. Salivary enzymes. Origin and relationship to periodontal disease. J Periodontal Res 1983;18(6):559–69.

[136] Reynolds SJ, Muwonga J. OraQuick ADVANCE Rapid HIV-1/2 antibody test. Expert Rev Mol Diagn 2004;4(5):587–91.

[137] Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997;24(1):72–7.

[138] Greenstein G, Hart TC. Clinical utility of a genetic susceptibility test for severe chronic periodontitis: a critical evaluation. J Am Dent Assoc 2002;133(4):452–9 [quiz: 492–3].

[139] Socransky SS, Haffajee AD, Smith C, et al. Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. J Clin Periodontol 2000;27(11):810–8.

[140] Li Y, Zhou X, St John MA, et al. RNA profiling of cell-free saliva using microarray technology. J Dent Res 2004;83(3):199–203.

[141] St John MA, Li Y, Zhou X, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004;130(8):929–35.

[142] Aufricht C, Tenner W, Salzer HR, et al. Salivary IgA concentration is influenced by the saliva collection method. Eur J Clin Chem Clin Biochem 1992;30(2):81–3.

[143] Wilton JM, Curtis MA, Gillett IR, et al. Detection of high-risk groups and individuals for periodontal diseases: laboratory markers from analysis of saliva. J Clin Periodontol 1989; 16(8):475–83.

[144] Bokor M. Immunoglobulin A levels in the saliva in patients with periodontal disease. Med Pregl 1997;50(1–2):9–11.

[145] Anil S, Remani P, Beena VT, et al. Immunoglobulins in the saliva of diabetic patients with periodontitis. Ann Dent 1995;54(1–2):30–3.

[146] Schenck K, Poppelsdorf D, Denis C, et al. Levels of salivary IgA antibodies reactive with bacteria from dental plaque are associated with susceptibility to experimental gingivitis. J Clin Periodontol 1993;20(6):411–7.

[147] Sandholm L, Tolo K, Olsen I. Salivary IgG, a parameter of periodontal disease activity? High responders to *Actinobacillus actinomycetemcomitans* Y4 in juvenile and adult periodontitis. J Clin Periodontol 1987;14(5):289–94.

[148] Nieminen A, Kari K, Saxen L. Specific antibodies against *Actinobacillus actinomycetemcomitans* in serum and saliva of patients with advanced periodontitis. Scand J Dent Res 1993; 101(4):196–201.

[149] McLaughlin WS, Kirkham J, Kowolik MJ, et al. Human gingival crevicular fluid keratin at healthy, chronic gingivitis and chronic adult periodontitis sites. J Clin Periodontol 1996; 23(4):331–5.

[150] Kopczyk RA, Graham R, Abrams H, et al. The feasibility and reliability of using a home screening test to detect gingival inflammation. J Periodontol 1995;66(1):52–4.

[151] Sewon L, Makela M. A study of the possible correlation of high salivary calcium levels with periodontal and dental conditions in young adults. Arch Oral Biol 1990;35(Suppl):211–2.

[152] Sewon LA, Karjalainen SM, Sainio M, et al. Calcium and other salivary factors in periodontitis-affected subjects prior to treatment. J Clin Periodontol 1995;22(4):267–70.

[153] Genco RJ, Ho AW, Kopman J, et al. Models to evaluate the role of stress in periodontal disease. Ann Periodontol 1998;3(1):288–302.

[154] Beck JD. Risk revisited. Community Dent Oral Epidemiol 1998;26(4):220–5.

[155] Burt BA. Risk factors, risk markers, and risk indicators. Community Dent Oral Epidemiol 1998;26(4):219.