J Clin Periodontol 2011; 38 (Suppl. 11): 85–105 doi: 10.1111/j.1600-051X.2010.01670.x

Journal of Clinical Periodontology

Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis

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Buduneli N, Kinane DF: Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. J Clin Periodontol 2011; 38 (Suppl. 11): 85–105. doi: 10.1111/j.1600-051X.2010.01670.x.

Abstract

Background: A major challenge in clinical periodontics is to find a reliable molecular marker of periodontal tissue destruction with high sensitivity, specificity and utility.

Objectives: The aim of this systematic review is to evaluate available literature on 'the utility of molecular markers of soft and hard periodontal tissue destruction'.

Materials and Methods: Based on the focused question, 'What is the utility of molecular markers of soft and hard periodontal tissue destruction', an electronic and manual search was conducted for human studies presenting clinical data for the potential of molecular markers of tissue destruction in biofluids; gingival crevicular fluid (GCF), saliva, and serum.

Results: Papers fulfilling the inclusion criteria were selected. All relevant data from the selected papers were extracted and recorded in separate tables for molecules in GCF, saliva, and serum.

Conclusion: Within the defined limits of the Problem/Population, Intervention, Comparison, Outcome, the present analysis reveals that (a) no single or combination of markers exists that can disclose periodontal tissue destruction adequately; (b) while the most fruitful source of biomarkers for periodontal destruction appears to be in molecules tightly related to bone and soft tissue destruction, this remains to be objectively demonstrated. Currently, clinical measurements are still the most reliable.

Key words: biomarkers; diagnosis; gingival crevicular fluid; periodontal disease; saliva; serum

Accepted for publication 7 November 2010

Periodontitis is one of the most common oral diseases and is characterized by gingival inflammation and alveolar bone resorption (Savage et al. 2009). More than 500 different bacterial species are able to colonize the oral biofilm and up to 150 different species of bacteria are possible in any individual's subgingival plaque. According to a report by the World Health Organisation, severe periodontitis leading to tooth loss was found in 5–15% of most populations worldwide (Armitage 2004). Hence, it can be considered among the prevalent and important global health problems in terms of quality of life.

A diagnostic tool should provide pertinent information to aid differential diagnosis, screening, presence, location, severity or staging and prognosis of a disease. At present, periodontitis is diagnosed almost entirely on the basis of an array of clinical measurements including probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), plaque index (PI) recordings and radiographical findings. However, PD and CAL measurements by periodontal probes and radiographic bone levels, provide information about past periodontal tissue destruction and do not elucidate the current state of the disease activity nor predict the future. Therefore, one of the major challenges in the field of periodontology is to discover a method of predicting the future of periodontal disease or at least to declare the current state of disease activity. Thus, objective and ideal diagnostic methods

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

This work was supported in part by Ege University (NB) and by the United States Public Health Service National Institute of Health, NIDCR Grant DE017384 to D. F. K. This supplement was supported by an unrestricted grant from Colgate.

for periodontal diagnosis are still being sought. The ideal periodontal diagnostic method should be able firstly to screen susceptible subjects in the general population, secondly to differentiate active and inactive sites, thirdly to predict future tissue destruction in particular individuals and sites, and finally to monitor the response to periodontal therapy.

Periodontitis is described as a multifactorial irreversible and cumulative condition, initiated and propagated by bacteria and host factors (Kinane 2001). Given the complex nature of periodontitis, it is unlikely that one single clinical or laboratory examination can address all issues concerning diagnosis, classification, and prognosis (Van der Velden 2005, Xiang et al. 2010). Moreover, molecular markers of bone resorption have advantages as well as disadvantages as they relate to specificity for bone, ease of detection, pre-analytic stability, and availability of sensitive and specific assays for detection (Koka et al. 2006). Proteins derived from inflamed host tissue and pathogenic bacteria have the potential of being used as markers of periodontitis (Özmeriç 2004).

Numerous molecules in the oral fluids namely; gingival crevicular fluid (GCF) and saliva, as well as molecules in the blood circulation; serum or plasma have been investigated so far in an attempt to provide a sensitive and specific marker for periodontal tissue destruction. GCF and saliva are particularly promising as they can easily be obtained non-invasively and with minimal discomfort to the patient and consist of both locally synthesized and systemically derived molecules. Thus, evaluation of various biologically specific proteins or markers in oral fluids by using immunologic or biochemical methods may provide information on the events going on in the periodontal microenvironment (Kinney et al. 2007).

The immune and inflammatory responses are critical to understanding the pathogenesis of periodontal diseases and they are orchestrated by a number of host-related factors, either intrinsic or induced (Taubman et al. 2005). Under normal physiologic conditions, there is a balance between bone formation and bone resorption. Bone homoeostasis is maintained as long as this balance is preserved. When the structural integrity and/or calcium metabolism is altered, this balance is lost towards either increased bone formation or towards bone resorption. Inflammatory conditions like periodontal diseases and rheumatoid arthritis, and metabolic conditions like osteoporosis are examples of an altered balance between bone formation and bone resorption.

The threshold for periodontitis progression is based upon extensively documented evidence within the periodontal literature. Many epidemiological studies apply a threshold for loss of attachment of changes of 3 mm as the definition of disease progression. The threshold is set at the level of two teeth to minimize the risk of including cases of progression arising because of reasons other than periodontitis and/or measurement error. Presence of $\geqslant 2$ teeth demonstrating a longitudinal loss of proximal attachment of $\geqslant 3$ mm has been proposed as the criteria for case definition of periodontitis progression (Tonetti & Claffey 2005). In situations where serial proximal attachment level measurements are not available, longitudinal radiographic bone loss of $\geqslant 2$ mm at $\geqslant 2$ teeth may be used as a substitute.

The criteria for definition of progressing sites are as stated by the working group in the Fifth European Workshop in Periodontology, in 2005as follows:

- The exposure of interest, along with relevant covariates including age, is used in a multi-factorial model, using the ''case'' as the dependent variable. Adjusted odds ratios and/or relative risk estimates as well as 95% confidence limits should be reported.
- The impact of the putative risk factor on the extent of periodontitis progression (percentage of teeth affected by disease progression) is examined to investigate a dose–response effect, using a new multivariate model.
- Additional models may be developed as needed to test specific hypotheses.

Clinical examination and the clinical periodontal measurements can provide information on the disease activity, only if these measurements are repeated at two time-points. However, knowing the disease activity state might be critical to clinical decision making at one single time point and clinical evaluation cannot fulfil this requirement. This deficiency has spawned dozens of studies with as yet no definitive answer.

There are also well-accepted risk factors such as diabetes and smoking to be considered when determining the diagnosis as well as prognosis of a particular case. Cigarette smoking represents a risk factor for progression of periodontitis, the effect of which may be dose related. Indeed, apart from the plaque biofilm, it is the major environmental risk factor. Heavy smokers should be considered as high-risk individuals for disease progression. The clinical implications for this are that smokers should be identified during patient examination and efforts should be made to modify this behavioural risk factor. Furthermore, smoking or molecules related to smoking such as blood cotinine induced by smoking should be considered as important risk markers of periodontal disease that are relevant to the assessment of prognosis (Calsina et al. 2002, Tang et al. 2009).

The aim of the present review was to provide a systematic review of the state of evidence on whether current periodontal tissue destruction can be disclosed accurately utilizing chemical analysis of various molecules in the biological fluids.

Materials and Methods

Search strategy

A literature search of the last thirty years was performed using the ISI and PubMed database from 1980 to 15 June 2010, with the following search strategy: (''periodontitis'' OR ''periodontal disease'') AND (''progression'' OR ''activity'') AND (''saliva'' OR ''gingival crevicular fluid'' OR ''serum'' OR ''plasma''). The search was limited to the English language. In vitro studies on cell cultures, experimental studies on animal models, polymorphism studies, studies particularly investigating possible role of various therapeutic agents such as subantimicrobial dose doxycycline, anti-inflammatory agents, or dietary supplements, as well as studies comprising only patients with particular systemic diseases such as diabetes or rheumatoid arthritis and studies focused only on smoking were excluded from the present review. Titles and abstracts were screened and full text of publications was obtained for the selected articles. All levels of evidence were included. In addition, the reference lists of review papers were hand searched. To be eligible for inclusion in this systematic review, studies had to meet the following criteria: (1) original

investigations; (2) studies conducted within a human population; (3) studies having a systemically healthy chronic periodontitis group; (4) studies correlating the biochemical findings in biofluids with clinical periodontal parameters: PD, CAL, and BOP; (5) preferentially follow-up and/or intervention studies, but cross-sectional studies were also included in the review.

Outcome variables

The primary outcome variables investigated were PD, CAL, and incidence of BOP.

Results

Clinical methods for periodontal diagnosis

Measuring PD and CAL by a calibrated periodontal probe and assessing gingival inflammation by gingival index (GI) (Löe & Silness 1963) and/or a bleeding index such as BOP within a certain time after probing or papilla bleeding index (PBI) (Saxer & Mühlemann 1975) are still the most reliable clinical periodontal parameters for periodontal diagnosis. Furthermore, PI (Silness & Löe 1964) provides information on the major local aetiological factor; microbial dental plaque. Presence of BOP is still the most reliable clinical finding indicating periodontal disease activity. However, the absence of bleeding is much more valuable in terms of being a highly specific negative predictor of periodontal disease activity (Lang et al. 1986, 1990). This helps us exclude healthy patients but of course does not help us truly focus on the really at risk subjects as if we were to address all patients with BOP we would be overwhelmed with false positives as this approach would be far too sensitive to be useful as a screening technique. Sensitivity is the ability of a diagnostic test to identify the target molecule when this is truly present. Specificity is the probability of a diagnostic test being negative when the target molecule is truly absent. Misleading test results occur when the test is positive and the disease is absent (false positive) or when the test is negative and the disease is present (false negative). An ideal diagnostic test should have sensitivity and specificity values approaching 100%. But unfortunately this is never the case.

Source for samples of the different diagnostic tests

GCFcomponents

GCF is a transudate originating from the gingival plexus of blood vessels in the gingival connective tissue, close to the epithelium lining of the dentogingival space. It also collects resident host cells and microorganisms in the microbial dental plaque as well as their cellular products. GCF provides an accurate representation of tissue and serum concentrations of inflammatory mediators (Giannobile 1997, Champagne et al. 2003, Armitage 2004). Up to now, at least 90 different components in GCF have been evaluated as possible biomarkers for diagnosis of periodontal disease (Loos & Tjoa 2005). These markers can be divided into three major groups: host-derived enzymes and their inhibitors, inflammatory mediators and host-response modifiers, and byproducts of tissue breakdown (Lamster & Ahlo 2007).

A substantial number of studies throughout the 1980s and the 1990s explored the predictive ability of GCF components for identification of progressive periodontal lesions (Heitz-Mayfield 2005). While individual GCF components produced positive predictive values that were superior to individual clinical measures (Chapple et al. 1999), these studies focused largely on the prediction of periodontitis at the site level rather than the identification of high-risk groups and individuals. Data from the selected studies investigating potential biomarkers in GCF in relation to periodontal disease are outlined in Table 1. An early multi-centre study by Lamster et al. (1995) did examine the predictive value of β-glucuronidase (βG) at the patient level, in a population of predominantly recall patients, and demonstrated that subjects with persistently elevated levels of GCF βG at baseline, 2-week and 3-month recalls had between 7 and 14 times (dependent upon the algorithm used) increased risk ratio for periodontitis progression.

Salivary components

Saliva is a mirror of the body that can be used to monitor the systemic as well as the oral health status. Whole saliva contains constituents from exocrine glands secreting into the oral cavity, GCF and dietary and oral plaque components. Saliva is readily available and easily collected without specialized equipment or personnel. Several mediators of chronic inflammation and tissue destruction have been detected in whole saliva of periodontitis patients as described in relatively recent reviews (Kaufman & Lamster 2000, Kinane & Chestnutt 2000, Lamster et al. 2003). In addition, as whole saliva represents a pooled sample with contributions from all periodontal sites, analysis of biomarkers in saliva may provide an overall assessment of disease status as opposed to site-specific GCF analysis (Miller et al. 2006).

Recently, the use of whole saliva as a means of evaluating host-derived products (e.g. salivary gland product, gingival crevice fluid, host enzymes) as well as exogenous components (e.g. oral microorganisms and microbial products) has been suggested as a potential diagnostic marker for disease susceptibility (Şahingür & Cohen 2004). The source of saliva, types of saliva samples, as well as saliva collection methods has been described in detail by Şahingür & Cohen (2004). Development of tests based on the detection of neutrophil defects, genetic markers or the detection and measurement of antibodies specific for periodontal pathogens may be useful in the future. However, there is currently insufficient evidence available for the predictive value of diagnostic tests assessing the host's susceptibility to future periodontitis progression.

Salivary enzymes originate from three major sources; the actual salivary secretions, the host cells found in GCF, and finally disposed bacterial cells from dental plaque and mucosal surfaces. Data from the selected studies evaluating the potential utility of salivary components as diagnostic markers for periodontal tissue destruction are presented in Table 2.

Blood components

Various studies have evaluated the molecular markers of tissue destruction in serum or plasma: these manifestations of periodontal diseases are mainly sought to clarify the possible interactions between periodontitis and various systemic diseases and/conditions such as cardiovascular diseases (CVDs), pregnancy complications, diabetes mellitus, and rheumatoid arthritis. Serum or plasma provides information about the inflammatory stimulus and/or response generated in circulation towards the

Table 1. Studies evaluating possible biomarkers in gingival crevicular fluid (GCF) samples

Reference	Study design	Study groups	Follow-up period	Clinical parameter	Biological sample	Biological parameter	Results
Akalın et al. (2005)	Cross-sectional	26 CP, 18 controls		Routine clinical parameters	GCF, biopsy	SOD	Increased in gingiva, no difference in GCF
Akalın et al. (2007)	Cross-sectional	36 CP, 28 healthy			Serum, saliva, GCF	Lipid peroxidation, malondialdehyde, total antioxidant status	LPO and TOS may play an important role in periodontitis
Baltacıoğlu et al. (2008)	Cross-sectional	33 CP, 24 healthy			Serum, GCF	Total protein, protein carbonylation	Elevated protein carbonylation may be a sign of oxidative stress in CP
Buduneli et al. (2005)	Cross-sectional	20 gingivitis, 20 CP, 20 healthy controls.			GCF, serum	t-PA, u-PA, PAI-1, PAI-2	GCF PAI-2 concentrations were higher in CP, gingivitis than healthy controls. Periodontal disease rather than smoking seems to affect PA system
Chapple et al. (2007)	Prospective	18 CP	3 months after SRP		GCF, plasma	TAOC	GCF TAOC was lower in CP, increased after SRP
Chen et al. (2009)	Cross-sectional	25 CP, 24 healthy			GCF, serum	PAF	Higher PAF in GCF, serum in CP
Dezerega et al. (2010)		15 progression, 18 CP, 10 healthy			GCF	MCP-3	Higher in CP, higher in active sites than inactive sites, may be a diagnostic marker
Dutzan et al. (2009)	Cross-sectional	106 moderate to advanced CP			GCF	IFN-γ	Higher in active sites, may be an indicator for progression
Emingil et al. (2006b)	Cross-sectional	18 GAgP, 29 CP, 20 gingivitis, 20 healthy controls.		PD, CAL, BOP, PI	GCF	Laminin-5 gamma2-chain	CP had elevated laminin-5 gamma2-chain levels
Fitzsimmons et al. (2010)	Cross-sectional, population based	430 periodontitis, 509 healthy controls			GCF	IL-1β, CRP	Higher levels in periodontitis, GCF levels of IL-1β, CRP may indicate higher susceptibility
Gapski et al. (2009)	Multi-centre, prospective, controlled trial	CP SDD+surgery Placebo+surgery	12 months	1° outcome: CAL 2° outcome: PD, BOP, ICTP	GCF	ICTP	SDD decreased ICTP effectively Provided better clinical outcomes
Garg et al. (2009)	Intervention	20 healthy 20 gingivitis 20 CP (also after SRP)	6–8 weeks after SRP	GI, PD, CAL	GCF	Cathepsin K	Cathepsin K increased in CP, decreased with SRP
Golub et al. (2008)	Prospective, controlled	CP, Postmenopausal women SDD: 64 Placebo: 64	2 years		GCF	ICTP, MMP-8, MMP-1, MMP-13, IL-1β	SDD decreased collagenase activity, ICTP
Grant et al. (2010)	Intervention	20 healthy, 20 CP	Before and 3 months after SRP		GCF	Reduced glutathione, oxidized glutathione	GSH, GSSG lower in CP GSH: GSSG ratio increased after SRP
Holzhausen et al. (2010)	Cross-sectional, and intervention	40 moderate CP 40 severe CP 40 healthy controls	At baseline and also 1 month after SRP		GCF	Trypsin-like activity, TNF-α, IL-1α, IL-6, IL-8, PAR_2	PAR_2 was higher in CP than controls. IL-1α, -6, -8 TNF-α, total proteolytic activity were all higher in CP
Ikezawa-Suzuki et al. (2008)	Intervention	35 CP	At baseline and after SRP		GCF	TNF-α, TNF receptor types 1 and 2	Ratio of TNF R2: R1 increased after treatment, may be related to clinical periodontal data

Jepsen et al. (2003)	Cross-sectional	54 teeth with CP, 11 experimental gingivitis			GCF	lysylpyridinoline, hydroxylysylpyridinoline	Increased hydroxylysylpyridinoline, Lysylpyridinoline may be indicators of ongoing destruction
Kardeşler et al. (2008)	Cross-sectional	17 DM+periodontal disease, 17 healthy+periodontal disease, 17 healthy controls			GCF	PGE_2, IL-1β, t-PA, PAI-2	PGE_2, t-PA were higher in DM, and periodontal disease groups than in healthy controls
Kurtiş et al. (1999)		24 CP, 24 healthy		PD, PI, GI, BOP, CAL	GCF	IL-6	Higher in CP, no correlation with clinical data
Lamster et al. (1994)					GCF, saliva, serum	β-glucuronidase	GCF levels correlated with CAL
Lin et al. (2005)	Cross-sectional	14 patients (62 sites divided into 4 groups by PD, BOP).		PD, BOP, CAL	GCF	Oncostatin M, IL-6	Total amounts but not concentrations correlated with disease severity.
Mogi & Otogoto 2007)	Cross-sectional	66 CP; 20 mild, 24 moderate, 22 severe 19 healthy controls			GCF	Cathepsin K	Increased in periodontitis *versus* control subjects, (+) correlation between cathepsin K and RANKL suggesting that both contribute to osteoclastic bone destruction
Mogi et al. (2004)	Cross-sectional				GCF	Cathepsin K, RANKL	Both increased in CP
Nakamura-Minami et al. (2003)	Intervention	21 CP	4 weeks after SRP	Routine clinical parameters	GCF	α-1 protease inhibitor, secretory leukocyte protease inhibitor	Both seem to be related with healing after SRP
Oringer et al. (2002)	Intervention	48 CP; SRP+placebo, SRP+minocycline	Before, 1,3,6 months after SRP		GCF	ICTP, IL-1	ICTP, IL-1 correlated with clinical data, may be disease markers
Palys et al. 1998	Cross-sectional	7 healthy and 8 gingivitis 21 CP		BOP, PD, CAL, PI	GCF subgingival plaque	ICTP, 40 subgingival taxa	Positive relation between ICTP and putative pathogens
Perinetti et al. (2008)	Intervention	16 CP	15, 60 days after SRP	PD, PI, CAL, BOP	GCF	Alkaline phosphatase	Decreased on day 15, increased on day 60 after SRP
Pradeep et al. (2009a, b)	Intervention	20 healthy, 20 gingivitis, 20 CP	CP patients also 6–8 weeks after SRP	PD, CAL, GI	GCF	MCP-1	Increased gradually with severity of periodontal disease, decreased after SRP, correlated with clinical periodontal data; may be a biomarker
Pradeep et al. (2009a, b)	Intervention	20 healthy, 20 gingivitis, 20 CP	CP patients also 6–8 weeks after SRP		GCF	IL-18, MCP-1	Higher levels in CP, +correlation between IL-18 and MCP-1; IL-18 may induce MCP-1, promote inflammation
Rescala et al. (2010)	Cross-sectional	20 GCP, 17 GAgP, 10 gingivitis			GCF	IL-1β, IL-2, IL-4, IL-8, elastase, IFN-γ	IL-1β, elastase were higher in deep sites than shallow sites in periodontitis groups. No significant difference between CP and AgP in GCF levels of biomarkers
Rosin et al. (1999)	Cross-sectional	140 subjects		PI, SBI, GCF flow	Saliva, GCF	Peroxidise, lysozyme	No significant correlation between clinical periodontal indices and salivary data
Srinath et al. (2010)	Cross-sectional	15 healthy, 15 gingivitis, 15 CP		GI, Russell periodontal index	GCF, saliva	Melatonin	Lowest in CP, may have a protective role in periodontal disease
Surna et al. (2009)	Cross-sectional	Healthy Gingivitis CP		PD	GCF, saliva	Lysozyme activity	Lysozyme activity correlated with PD
Teles et al. (2010)	Cross-sectional	20 healthy, 20 CP				IL-1β, IL-8, MMP-8, 40 bacterial taxa	+ correlation between clinical indices, GCF cytokines, orange and red complexes

Table 1. (Contd.)

Reference	Study design	Study groups	Follow-up period	Clinical parameter	Biological sample	Biological parameter	Results
					GCF; subgingival plaque		Healthy sites of CP had higher cytokine levels than healthy group
Thorat Manojkumar et al. (2010)	Intervention	20 healthy, 20 gingivitis, 20 CP	CP patients also 8 weeks after SRP	PD, CAL	GCF	Oncostatin M	Highest in CP, +correlation with PD, CAL; can be a biomarker in periodontitis
Toker et al. (2008)	Intervention	15 healthy, 15 generalized AgP	6 weeks after SRP	PD, CAL, PI, BOP, GI	GCF	IL-1β, IL-1ra, IL-10	IL-1β is high and decreased after SRP
Tsalikis et al. (2001)	Intervention	12 advanced periodontitis	4 weeks after SRP	PD, CAL, BOP	GCF	Aspartate amino transferase	AST decreased after SRP
Türkoğlu et al. (2009)	Cross-sectional			PD, CAL, PI, BOP, PBI	GCF	IL-18, cathelicidin LL-37	Cathelicidin LL-37 increased in CP, may play a role in destruction
Tüter et al. (2007)	Cross-sectional	20 CP, 17 healthy			Serum, GCF	hsCRP	No difference between groups
Yetkin Ay et al. (2009)	Cross-sectional	40 CP, 20 healthy		GI, PD, CAL, BOP, PI	GCF	IL-11, IL-17	Healthy group had highest IL-11: IL-17 ratio
Yin et al. (2000)	Intervention	Periodontitis, gingivitis, healthy	14 days after SRP		GCF	t-PA, PAI-2	Decreased after SRP, may be diagnostic markers
Yoshinari et al. (2004)	Intervention	7 CP	Before and after SRP	Routine clinical parameters	GCF, gingiva	IL-1α, IL-1β, IL-1ra	IL-1 is effective for evaluating the gingival inflammation
Zheng et al. (2006)	Cross-sectional	21 CP, 19 gingivitis, 20 healthy controls			GCF, serum	PAF	PAF levels were higher in CP both in GCF and serum. Serum and GCF levels correlated also with clinical parameters. PAF may play a role in pathogenesis

CP, chronic periodontitis; GCF, gingival crevicular fluid; SOD, superoxide dismutase; LPO, lipid peroxidation; TOS, total oxidant status; t-PA, the tissue/blood vessel type plasminogen activator; u-PA, urokinase type plasminogen activator; PAI, plasminogen activator inhibitor; PA, plasminogen activators; TAOC, total antioxidant capacity; SRP, scaling and root planing; PAF, platelet activating factor; MCP-3, monocyte chemotactic protein-3; IFN-γ, interferon-γ; GAgP, generalized aggressive periodontitis; PD, probing depth; CAL, clinical attachment level; BOP, bleeding on probing PI, plaque index; IL, interleukin; CRP, C-reactive protein; SDD, subantimicrobial dose doxcycline; ICTP, carboxy-terminal telopeptide of type I collagen; GI, gingival index; MMP, matrix metalloproteinase; GSH, reduced glutathione; GSSG, oxidized glutathione; PAR_2, protease activated receptor 2; TNF, tumour necrosis factor; DM, diabetes mellitus; PGE_2, prostaglandin E_2; RANKL, receptor activator of nuclear factor κB ligand; MCP-1, monocyte chemotactic protein-1; TACE, transcatheter arterial chemoembolization; SBI, sulcus bleeding index; IL-1ra, interleukin-1 receptor antagonist; AST, aspartate amino transfarase; PBI, papilla bleeding index; hsCRP, high-sensitive C-reactive protein.

Table 2. Studies evaluating possible biomarkers in saliva samples

Reference	Study design	Study groups	Follow-up period	Clinical parameter	Biological sample	Biological parameter	Results
Aemaimanan et al. (2009)	Cross-sectional	30 localized CP, 30 generalized CP, 30 controls		PD, CAL, BOP	Saliva, subgingival plaque	Alanine aminopeptidase Dipeptidyl peptidase IV, *P. gingivalis*	Dipeptidyl peptidase IV but not alanine aminopeptidase activity is associated with CP, presence of *P. gingivalis*
Aurer et al. 1999	Cross-sectional	20 RPP, AP,			Saliva	IL-6	IL-6 increased in RPP, CRP decreased in AP
Baron et al. (1999)	Cross-sectional	8 PD patients, 16 controls			Saliva	Cystatins Total cystatin activity	Total cystatin activity decreased in patients
Cutando et al. (2006)	Cross-sectional	37 patients, variable PD		CPI	Whole saliva	Melatonin	Inverse relation between salivary melatonin and PD severity
De la Pena et al. (2007)	Cross-sectional	175 volunteers		Number of teeth, PD	Saliva	Lactate dehydrogenase	Increased in periodontitis, correlated with PD, may be a biomarker
Diab-Ladki et al. 2003	Cross-sectional	17 PD, 20 controls			Saliva	TAC, total protein content	TAC decreased, an imbalance between oxidants and antioxidants
Garito et al. (1995)	Cross-sectional	69 Periodontal disease patients		PD	Saliva	PAF	Salivary PAF correlated with PD severity
Ghallab & Shaker 2010)	Intervention	22 CP, 22 healthy (half smoker, half non-smoker)	At baseline, 1 month after SRP	PI, GI, PD, CAL only in CP	Saliva	sCD44	Highest in smoker CP, decreased after SRP in both smokers and non-smokers
Gheren et al. (2008)	Prospective	18 CP, 18 controls	30 days after perio. therapy	PD, CAL, PI, GI	Saliva	Salivary arginase activity	Salivary arginase activity increased in CP, decreased after therapy: a candidate salivary marker of periodontal status
Gürsoy et al. (2009)	Cross-sectional	84 CP, 81 controls			Saliva	Elastase, lactate dehydrogenase IL-6, IL-1β, TNF-α	Only IL-1β can differentiate periodontitis
Haigh et al. (2010)	Cross-sectional, intervention	Severe periodontitis			Saliva	Proteomics 128 proteins	S100 proteins highly related with PD severity
Lamster et al. (2003)	Cross-sectional	380 subjects		PD, CAL, GI	Saliva, blood	β glucuronidase	Periodontal clinical parameters correlated with salivary β glucuronidase
Koss et al. 2009	Cross-sectional	89 mild, moderate, severe CP 29 controls			Whole saliva	Peroxidase, hydroxyproline, sIgA	Peroxidase, hydroxyproline increased, sIgA diminished in CP
Nomura et al. (2006)	Cross-sectional	187 subjects			Saliva	Lactate dehydrogenase, AST, blood urea nitrogen	Salivary lactate dehydrogenase, AST, blood urea nitrogen have potential to screen PD
Özmeric et al. (2000)	Cross-sectional	20 CP, 15 controls		PD, CAL, PI, GI	Saliva	Total protein, arginase	Salivary arginase increased in CP
Su et al. (2009)	Cross-sectional	58 periodontitis 234 controls		CPITN	Whole saliva	ROS, TAC	ROS increased in periodontitis, TAC correlates with disease severity
Takane et al. (2002)	Cross-sectional	78 CP, 17 healthy			Saliva	8-OHdG	Higher in CP, can be a diagnostic marker
Teles et al. (2009)	Cross-sectional	74 CP, 44 healthy			Saliva multiplex	G-MCSF, IL-1β, -2, -4, -5, -6, -8, -10 IFN-γ, TNF-α	No difference between groups. IL-8 correlated with PD, BOP, these cytokines cannot discriminate CP and healthy
Totan et al. (2006)	Cross-sectional	Periodontitis, controls		PD, BOP	Saliva	AST, ALP, aminopeptidases, glucuronidases	Salivary AST, ALP increased with pockets, BOP, suppuration
Wilczynska-Borawska et al. (2006)	Cross-sectional	26 PD, 20 healthy controls		GI, PBI, PI, PD, CAL, number of teeth	Whole saliva	Hepatocyte growth factor	Salivary HGF may be related with severity of PD
Yoshie et al. (2007)	Intervention	49 CP	4 weeks after SRP	PD, CAL, BOP	Saliva	AST, ALT, LDH	All decreased after SRP, they can be useful markers for inflammation, destruction

CP, chronic periodontitis; PD, probing depth; CAL, clinical attachment level; BOP, bleeding on probing; RPP, rapidly progressive periodontitis; AP, adult periodontitis; IL, interleukin; CRP, C-reactive protein; CPI, community periodontal index; TAC, total antioxidant capacity; PAF, platelet activating factor; PI, plaque index; GI, gingival index; sCD44, soluble CD44; SRP, scaling and root planing; TNF, tumour necrosis factor; sIgA, secretory immunoglobulin A; AST, aspartate amino transferase; CPITN, community periodontal index of treatment needs; ROS, reactive oxygen species; IFN-γ, interferon-γ; ALP, alkaline phosphatase; PBI, papilla bleeding index; HGF,human gingival fibroblasts; LDH, laktate dehydrogenase.

periodontal pathogens that colonize in the subgingival area (Pussinen et al. 2007). Increased circulating levels of cytokines were reported in chronic periodontitis patients compared with the clinically healthy control subjects (Mooney & Kinane 1994). Data from the selected studies evaluating the potential utility of serum components as diagnostic markers for periodontal tissue destruction are presented in Table 3. Researchers have mainly focused on C-reactive protein (CRP), fibrinogen, and serum amyloid A (SAA) concentrations in serum in relation to periodontitis.

Molecular markers

The ratio of two molecules has gained attention for being indicative of the regulation of normal bone resorption and deposition activities that occur during bone remodelling, that is the ratio of receptor activator of NF-κB ligand (RANKL) to osteoprotegerin (OPG) (Cochran 2008, Leibbrandt & Penninger 2008). RANKL, receptor activator of NF-κB (RANK), and OPG interactions are important in coordinating osteoclastogenesis and alveolar bone resorption (Suda et al. 1999). RANKL is expressed by osteoblasts/stromal cells (Yasuda et al. 1998), fibroblasts (Quinn et al. 2000), and activated T cells (Horwood et al. 1999). It binds directly to RANK on the surface of preosteoclasts and osteoclasts. RANKL stimulates both the differentiation of osteoclast progenitors and activity of mature osteoclasts (Lacey et al. 1998). This ligand can be found as a cell membrane-bound variant (mRANKL) or in a primary soluble (secreted) form, which has been described in activated T cells (Kong et al. 1999). OPG is the naturally occurring inhibitor of osteoclast differentiation. It is a soluble molecule that binds to RANKL with high affinity and blocks RANKL from interacting with RANK (Lacey et al. 1998). As part of an inflammatory response, pro-inflammatory cytokines, such as interleukin-1β (IL-1β), -6, -11, -17, and tumour necrosis factor-α (TNF-α) can induce osteoclastogenesis by increasing the expression of RANKL while decreasing OPG production in osteoblasts/stromal cells (Nakashima et al. 2000). On the contrary, anti-inflammatory mediators such as IL-13 and interferon-γ (IFN-γ) may lower RANKL expression and/or increase OPG expression to inhibit osteoclastogenesis (Nakashima et al. 2000). Moreover, in a cross-sectional study, Sakellari et al. (2008) reported that soluble RANKL levels in GCF samples of the chronic periodontitis patients were higher than the periodontally healthy subjects, which also correlated with *Treponema denticola* and *Porphyromonas gingivalis* levels in the subgingival plaque samples of the same periodontitis patients. However, they failed to show a correlation between GCF RANKL levels and clinical periodontal measurements, namely PD, gingival recession and BOP either cross-sectionally or longitudinally. The concentrations of RANKL and OPG show great variation from study to study, but the ratio of RANKL/OPG has a consistent tendency to increase from periodontal health to periodontitis and to decrease following non-surgical periodontal treatment (Table 4) (Crotti et al. 2003, Mogi et al. 2004, Kawai et al. 2006, Lu et al. 2006, Bostancı et al. 2007, Wara-Aswapati et al. 2007, Buduneli et al. 2008, 2009, Dereka et al. 2010). Thus the ratio of RANKL/OPG shows promise as a discloser of periodontal disease activity.

Alkaline phosphatase (ALP) is a catalysing enzyme that accelerates the removal of phosphate groups in various molecules. ALP was not supported to have a predictive value for periodontal breakdown, but it may serve as a marker in periodontal treatment planning and monitoring (McCauley & Nohutçu 2002, Taba et al. 2005). Aspartate amino transferase (AST) was found to be increased in GCF of severe periodontitis patients decreasing after initial periodontal treatment (Tsalikis et al. 2001).

GCF oncostatin M levels were highest in chronic periodontitis patients when compared with gingivitis patients and healthy control subjects (Thorat Manojkumar et al. 2010). Serum oncostatin M levels were also associated with PD and CAL in chronic periodontitis patients (Pradeep et al. 2010). The positive correlations between oncostatin M levels and PD as well as CAL suggest that it may be a useful biomarker in periodontitis.

Cathepsin B is a proteinase which is synthesized mainly by macrophages in GCF and levels of cathepsin B may distinguish gingivitis from periodontitis (Kennett et al. 1997). Furthermore, GCF levels of cathepsin B correlate significantly with clinical parameters before and also after periodontal treatment, suggesting a use for this enzyme in treatment planning and monitoring (Chen et al. 1998).

Cathepsin K, a cysteine protease, is capable of hydrolysing extracellular bone matrix proteins, is highly expressed in osteoclasts, and it is a well-known marker of osteoclast activity (Motyckova et al. 2001). Eley & Cox (1996a, b) have suggested that GCF cathepsin B level may indeed serve as a predictor of attachment loss. Mogi & Otogoto (2007) investigated GCF levels of cathepsin K and RANKL in 20 mild, 24 moderate, and 22 severe chronic periodontitis patients in comparison with 19 healthy control subjects. Cathepsin K was below the detection limit in the healthy control group, whereas it was detectable in all periodontitis samples. In a recent intervention study, Garg et al. (2009) reported that GCF levels of cathepsin K was higher in chronic periodontitis patients than gingivitis patients and healthy controls, whereas it was decreased significantly after scaling and root-planing. The high levels of cathepsin K in periodontitis samples together with the positive correlation between cathepsin K and RANKL levels suggest that both of them contribute to osteoclastic bone resorption in periodontitis, although no significant correlation could be found between the biomarkers and clinical periodontal measurements.

Carboxyterminal telopeptide pyridinoline cross-links of type I collagen (ICTP) is released into the periodontal tissues as a consequence of collagen degradation and alveolar bone resorption (Seibel 2003). Type I collagen composes 90% of the organic matrix of bone and is the most abundant collagen in osseous tissue (Narayanan & Page 1983). Studies assessing the role of ICTP levels in GCF or peri-implant crevicular fluid as a diagnostic marker of periodontal disease activity have had promising results so far (Oringer et al. 1998, 2002). ICTP was suggested to predict future bone loss, to correlate with clinical parameters and putative periodontal pathogens and also to reduce following periodontal therapy, thus leading to an accurate assessment of tissue breakdown (Giannobile 1999). Because of its specificity and sensitivity for bone resorption, ICTP represents a potentially valuable diagnostic marker for periodontal disease (Giannobile et al. 2003).

Osteocalcin (OC) is a calcium-binding protein of bone and the most abundant non-collagenous protein of the mineralized tissue (Lian & Gundberg

Table 3. Studies evaluating possible biomarkers in serum/plasma samples

Reference	Study design	Study groups	Follow-up period	Clinical parameter	Biological sample	Biological parameter	Results
Al-Ghamdi & Anıl (2007)	Cross-sectional	30 smoker CP, 30 non-smoker CP, 30 healthy			Serum	Immunoglobulins	Smoking decreases Ig content
Amarasena et al. (2008)	Follow-up	266 elderly	6 years Progression: CAL increase >3mm	PD, CAL	Serum	Albumin, Ca, IgG, A, M	Serum Ca correlated with PD, may be a risk factor for progression
Behle et al. (2009)	Intervention	30 CP	Before, 4-weeks after SRP		Plasma	19 biomarkers, multiplex	Overall reduction in systemic inflammation, great personal variation
De Queiroz et al. (2008)	Cross-sectional	17 CP, 8 healthy			Serum	24 cytokines with multiplex	RANTES differed between groups
Duerte et al. (2010)	Intervention	14 healthy controls, 14 CP, 14 GAgP	At baseline and 6 months after SRP		Serum	IL-17, IL-4, IL-23, IFN-γ, TNF-α	IL-17, TNF-α levels were higher in GAgP than CP, healthy both at baseline and after SRP
Furugen et al. (2008)	Cross-sectional	158 Japanese elders			Serum	Adiponectin, resistin, IL-6, TNF-α	Increased serum cortisol is associated with BOP
Glas et al. (2008)	Cross-sectional	105 CP, 122 healthy			Plasma	Surfactant protein D	Increased in CP; may be a biomarker for CP
Guentsch et al. (2009)	Cross-sectional	425 non-smoker, systemically healthy adults		Number of teeth with PD>4–6 mm	Serum	IL-6, TNF-α	Serum IL-6 is associated with periodontal disease severity
Ishisaka et al. (2008)	Cross-sectional	467 adults		PD, CAL, BOP	Serum	Cortisol	Serum cortisol associated with PD, CAL
Iwasaki et al. (2008)	Follow-up	600 elderly	4 years	CAL	Serum	Albumin	Serum albumin may be a risk predictor for progression
Liu et al. (2010)	Cross-sectional	40 CP, 40 healthy		PD, CAL, BOP	Serum	CRP, lipid profile	CRP higher in CP, HDL lower in CP. correlated with clinical parameters
Marcaccini et al. (2009)	Intervention	25 CP, 20 healthy	3 months after SRP	PD, BOP, CAL	Serum, plasma	hsCRP, IL-6, CD40 ligand, MCP-1, sP-selectin, sVCAM-1, sICAM-1	SRP decreased CRP and IL-6, others were not affected
Nakajima et al. (2010)	Intervention	78 CP, 40 healthy	At baseline, after SRP		Serum	hsCRP, IL-6, TNF-α	CRP, IL-6 higher and TNF lower in CP, IL-6, CRP decreased after SRP
Nibali et al. (2007)	Cross-sectional	302 CP, 183 healthy			Serum	Inflammatory, metabolic markers	CP may be related with systemic inflammation, dysmetabolic state
Nicu et al. (2009)	Cross-sectional	105 CP, 57 healthy			Serum	CRP, sCD14	sCD14 increased in CP
Offenbacher et al. (2009)	RCT	151 SRP, 152 community care	6 months	PD, CAL, BOP, subgingival calculus	Serum	CRP	Serum CRP was not affected by SRP
Pradeep et al. (2010)	Intervention	20 healthy, 20 gingivitis, 20 CP	CP patients also 6–8 weeks after SRP	PD, CAL, GI	Serum	Oncostatin M	Highest in CP, decreased after SRP, not detectable in gingivitis, healthy. Correlated with PD, CAL
Raunio et al. (2007)	Cross-sectional	52 subjects		PD, CAL, BL	Mouth-wash, serum	IL-6	Serum IL-6 increased with periodontal disease severity
Raunio et al. (2009)	Cross-sectional	56 CP, 28 healthy			Serum	sCD14	Higher in CP, correlated with periodontal disease extent
Renvert et al. (2009)	RCT	28 CP test (anti-inflammatory therapy), 29 CP placebo		PD, BOP	Serum	hsCRP, IL-6, IL-1β, IL-8, IL-12, TNF-α, IFN-γ	CRP, IFN, IL-6 decreased, others did not change
Trindade et al. (2008)	Cross-sectional	29 CP, 12 AgP, 22 gingivitis, 26 healthy			Serum	Antibody levels against *P. gingivalis*	Serum Ig titres correlated with clinical periodontal diagnosis
Yoshihara et al. (2009)	Cross-sectional	148 subjects at age 77		CAL	Serum	Osteocalcin, bone-specific alkaline phosphatase	Negative correlation between CAL>6 mm and osteocalcin

BL, bone level; CP, chronic periodontitis; CAL, clinical attachment level; PD, probing depth; Ig, immunoglobulin; RANTES, regulated upon activation normal T-cell expressed and secreted; GAgP, generalized aggressive periodontitis; SRP, scaling and root planing; IL, interleukin; IFN-γ, interferon-γ; TNF, tumour necrosis factor; BOP, bleeding on probing CRP, C-reactive protein; HDL, high-density lipoprotein; MCP, monocyte chemotactic protein; sVCAM, soluble vascular cell adhesion molecule; sICAM, soluble intercellular adhesion molecule.

Table 4. Studies evaluating RANKL, OPG, ICTP in gingival crevicular fluid (GCF), saliva or serum samples

Reference	Study design	Study groups	Follow-up period	Clinical parameter	Biological sample	Biological parameter	Results
Bostancı et al. (2007)	Cross-sectional	21 healthy, 22 gingivitis, 28 CP, 25 GAgP, 11 CP immunosuppressed			GCF	RANKL, OPG	RANKL levels increased, OPG decreased in periodontitis *versus* gingivitis, healthy. RANKL/OPG ratio may predict disease occurrence
Bostancı et al. (2008)	Cross-sectional	21 healthy, 22 gingivitis, 28 CP, 25 GAgP, 11 CP immunosuppressed			GCF	TACE	GCF TACE levels were higher in periodontitis, TACE showed (+) correlation with PD, CAL, GCF RANKL concentration
Buduneli et al. (2008)	Cross-sectional	67 untreated CP, 44 maintenance patients			Saliva	sRANKL, OPG	Salivary sRANKL, OPG may be to be affected by smoking and showed significant differences between treated *versus* untreated CP
Buduneli et al. (2009)	Intervention	10 smoker, 10 non-smoker CP	4 weeks after SRP		GCF	IL-17, sRANKL, OPG	OPG decreased, IL-17 increased, sRANKL always similar
Frodge et al. (2008)	Cross-sectional	35 moderate to severe CP, 39 healthy controls			Saliva	TNF-α, ICTP, RANKL	TNF-α detected in all subjects, ICTP, RANKL were detected only in a minority. TNF-α was higher in CP than controls. Subjects with salivary TNF-α levels above a threshold had more sites with BOP, PD >4 mm, CAL >2 mm. Salivary TNF-α may be a biomarker for periodontal destruction
Golub et al. (2008)	Prospective, controlled	CP, Post-menopausal women SDD: 64 Placebo: 64	2 years		GCF	ICTP, MMP-8, MMP-1, MMP-13, IL-1β	SDD decreased collagenase activity, ICTP
Golub et al. (2010)	Prospective	128 postmenopausal women with CP SDD or placebo in maintenance	2 years		Serum	Osteocalcin, ICTP, CTX, bone-specific alkaline phosphatase	SDD decreased significantly serum biomarkers of bone resorption
Gürlek et al. (2009)	Cross-sectional	34 smokers, 22 non-smokers, 11 ex-smokers			Saliva	ICTP, OC, cotinine	ICTP was similar in study groups, OC was lower in smoker than the other groups, correlated negatively with pack-years
Mogi et al. (2004)	Cross-sectional				GCF	Cathepsin K, RANKL	Both increased in CP
Oringer et al. (2002)	Intervention	48 CP; SRP+placebo, SRP+minocycline	Before, 1,3,6 months after SRP		GCF	ICTP, IL-1	ICTP, IL-1 correlated with clinical data, may be disease markers
Özçaka et al. (2010)	Cross-sectional	58 CP, 47 healthy controls			plasma	ICTP, osteocalcin	CP and healthy control groups were similar in plasma ICTP, OC levels
Palys et al. (1998)	Cross-sectional	7 healthy, 8 gingivitis 21 CP		BOP, PD, CAL, PI	GCF subgingival plaque	ICTP, 40 subgingival taxa	Positive relation between ICTP and putative pathogens
Sakellari et al. (2008)	Cross-sectional	35 CP, 38 periodontally healthy		PD, recession, BOP	GCF, sub-gingival plaque	Free sRANKL, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythensis Treponema denticola*	sRANKL was higher in CP, correlated with *Treponema denticola*, *P. gingivalis* but not with clinical parameters
Silva et al. (2008)	Longitudinal	56 CP moderate to severe, followed until progression			GCF	RANKL, MCP-1, TNF-α, IL-1β, MMP-13	Higher RANKL, IL-1β, MMP-13 activity in active sites

CP, chronic periodontitis; GAgP, generalized aggressive periodontitis; GCF, gingival crevicular fluid; RANKL, receptor activator of nuclear factor κB ligand; OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor κB ligand; IL, interleukin; TNF, tumour necrosis factor ICTP, carboxy-terminal telopeptide of type I collagen; BOP, bleeding on probing PD, probing depth; CAL, clinical attachment level; SDD, subantimicrobial dose doxycycline; MMP, matrix metalloproteinase; CTX, carboxy-terminal collagen crosslinks; OC, osteocalcin; SRP, scaling and root planing; PI, plaque index; MCP, monocyte chemotactic protein.

1988). Osteoblasts, odontoblasts, and chondrocytes produce OC (Gallop et al. 1980). Serum level of OC is considered as a marker of bone formation (Christenson 1997, Giannobile et al. 2003). Lower serum levels of OC were reported in periodontitis patients compared with healthy subjects suggesting lower osteoblastic activity and bone formation ability (Shi et al. 1996). Yoshihara et al. (2009) reported that serum OC levels correlated with CAL in elderly Japanese subjects. On the other hand, indifferent plasma levels of ICTP and OC were reported in a recent study comparatively evaluating chronic periodontitis patients and healthy control subjects (Özçaka et al. 2010). There is no consensus, yet on the potential usage of OC for assessment of periodontal diagnosis and prognosis.

Osteonectin is a single-chain polypeptide that binds strongly to hydroxyapatite and other extracellular matrix molecules like collagens. It may be a sensitive marker for detection of periodontal disease status. However, no clinical study reporting osteonectin levels in biological fluids in relation with periodontitis could be found in the present literature search.

Osteopontin (OPN), a glycosylated phosphoprotein, is a bone matrix component produced by osteoblasts, osteoclasts, and macrophages as a multifunctional cytokine. It is a single-chain polypeptide which is concentrated at clear zone attachment areas of plasma membrane where osteoclasts are attached to the underlying mineral surface that is, the clear zone attachment areas of the plasma membrane (Rodan 1995). Kido et al. (2001) showed the presence of OPN in GCF and also showed that GCF OPN levels increased parallel to the increase in PD. Sharma & Pradeep (2006) reported that GCF OPN concentrations increased proportionally with the progression of disease and decreased significantly following nonsurgical periodontal treatment. The same authors reported that chronic periodontitis patients exhibited the highest GCF and plasma OPN levels which decreased significantly following initial periodontal treatment (Sharma & Pradeep 2007). The GCF OPN levels correlated with plasma levels and clinical attachment loss suggesting a role in the pathogenesis of periodontitis. Thus, OPN seems to be a promising biomarker for periodontal disease progression.

Matrix metalloproteinases (MMPs) are considered as modifiers of host response and it has been suggested that their role and involvement should not be interpreted solely as surrogate promoters of tissue destruction, but also as defensive or protective factors against inflammation as a whole (Sorsa et al. 2004, 2006). MMPs represent a structurally related but genetically distinct superfamily of proteases acting not only in physiological development and tissue remodelling but also in pathological tissue destruction (Sorsa et al. 2004). MMPs can be divided into five major groups: collagenases (MMP-1, -8, -13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, -10, -11), membrane-type MMPs (MMP-14, -15, -16, -17), and others (Sorsa et al. 2004). MMPs can collectively degrade almost all components of extracellular matrix and basement membrane and their excess activity lead to periodontal tissue destruction. The interplay of cell–cell and cell–matrix interactions involving the production of enzymes, activators, inhibitors, cytokines, and growth factors regulate not only connective tissue remodelling but also connective tissue matrix destruction (Reynolds & Meikle 1997). MMPs produced by resident cells including fibroblasts, macrophages, neutrophils, and epithelial cells, are controlled by tissue inhibitors of MMPs (TIMPs). MMPs can also process bioactive non-matrix substrates such as cytokines, chemokines, growth factors, and immune modulators thereby mediating anti-inflammatory and pro-inflammatory processes (Sorsa et al. 2006, Kuula et al. 2009). Upon bacterial insult, triggered leucocytes migrate to the site of inflammation and release MMP-8 and MMP-9, which are activated locally (Sorsa et al. 2006). TIMPs regulate the activities of these enzymes and TIMP-1 is more effective on interstitial collagenases. An imbalance between MMPs and TIMPs result in the pathological tissue destruction observed in periodontitis (Table 5) (Aiba et al. 1996, Bıyıkoğlu et al. 2009, Kardeşler et al. 2010).

Salivary TIMP-1 concentration has been reported to be lower in periodontitis patients than the healthy controls, while collagenase activity was higher, which exhibited reciprocal changes after initial periodontal therapy (Hayakawa et al. 1994). Furthermore, saliva samples from healthy subjects consisted mainly procollagenase whereas active collagenase predominated in diseased subjects. Greater collagenase activity has been found also in GCF samples of periodontitis patients than the healthy subjects (Villela et al. 1987). In a prospective study on chronic periodontitis patients comparing active *versus* inactive sites, significantly higher GCF levels of MMP-13 were reported in active sites, suggesting that it could serve as a marker of disease progression (Hernandez et al. 2006).

Ramseier et al. (2009) reported from a recent cross-sectional study that salivary concentrations of MMP-8, -9, and OPG combined with red complex bacteria predicted periodontal disease. In another recent study (Hernandez-Rios et al. 2009), active sites in moderate to advanced chronic periodontitis patients followed for 2 months were defined and GCF levels of MMP-9 and -13 were suggested as useful biomarkers for periodontitis progression. Another cross-sectional study reported that GCF MMP-8, -9 levels correlated with disease activity in chronic periodontitis patients (Beklen et al. 2006). Supporting data came from an intervention study comprising 28 chronic periodontitis patients and 22 controls (Marcaccini et al. 2009). The authors reported higher MMP-3, -8, -9, and gelatinolytic activities in plasma of chronic periodontitis patients which decreased significantly 3 months after non-surgical periodontal treatment. Similar data on GCF MMP-8 levels were also reported by Chen et al. (2000). Kinane et al. (2003) also reported that GCF MMP-8 levels decreased significantly 3 months after non-surgical periodontal therapy in 20 chronic periodontitis patients. Various treatment modalities have been investigated to determine their potential to control the activities of MMPs in periodontal tissue destruction (Buduneli et al. 2002, 2007, Golub et al. 2008, Vardar-Şengül et al. 2008).

GCF MMP-8 levels in shallow crevices were reported to be associated with CAL and thus it was suggested as a prognostic marker (Passoja et al. 2008). Higher MMP-8 levels in saliva and higher MMP-9 levels in GCF of chronic periodontitis patients were detected in comparison with gingivitis patients and healthy control subjects (Rai et al. 2008). Significant positive correlations were found between periodontal disease severity and GCF MMP-8, -9 activities together with negative correlations with TIMP-1, -2 levels (Pozo et al. 2005). Accordingly, GCF MMP-8 activation was found to be the highest in chronic periodontitis patients compared with healthy subjects and

Table 5. Studies evaluating matrix metalloproteinases in GCF, saliva and serum/plasma samples

Reference	Study design	Study groups	Follow-up period	Clinical parameter	Biological sample	Biological parameter	Results
Alfant et al. (2008)	Cross-sectional	44 children with/without AgP CP Healthy children		PD	GCF	MMP-1, -2, -3, -8, -9, -12, -13	MMP levels higher in AgP than the others, especially in diseased sites No correlation with PD
Alpagot et al. (2001)	Prospective	40 CP	6 months	GI, PI; BOP, PD, CAL, suppuration	GCF	MMP-3, TIMP-1	High GCF MMP-3, TIMP-1 may indicate high risk for progression
Beklen et al. (2006)	Cross-sectional	CP			GCF	MMP-3, -8, -9	GCF MMP-8, -9 levels correlated with disease activity
Bildt et al. (2008)	Cross-sectional	8 healthy, 12 CP			GCF	MMPs, TIMPs	MMPs higher, TIMPs lower in CP, with high individual variation
Emingil et al. (2006c)	Cross-sectional	35 GAgP, 29 CP, 20 gingivitis, 21 healthy controls.		PD, CAL, BOP, PI	GCF	MMP-25, MMP-26	MMP-25, -26 levels increased in both periodontitis groups and associated with severity of periodontal inflammation, suggesting that they can have a role in disease progression
Emingil et al. (2006a)	Cross-sectional	20 GAgP, 20 CP, 20 gingivitis, 20 healthy controls		PD, CAL, BOP, PI	GCF	MMP-7, TIMP-1, EMMPRIN	MMP-7 levels were similar in groups. All patient groups had higher EMMPRIN, TIMP-1. Periodontitis groups had higher TIMP-1 than gingivitis. Increased EMMPRIN, TIMP-1 related with severity of periodontal inflammation
Gürsoy et al. (2010)	Cross-sectional	84 CP, 81 controls			Saliva	MMP-8, -14 TIMP-1, ICTP	MMP-8, TIMP-1, ICTP increased in CP and can differentiate periodontitis
Heikkinen et al. (2010)	Cross-sectional	258 boys, 243 girls all adolescents, smoker *versus* non-smokers			Saliva	MMP-8, elastase	Median MMP-8 and elastase were lower in smokers, boys seem to be more susceptible than girls Both biomarkers correlated with BOP
Hernandez et al. (2006)	Prospective	21 CP Active and inactive sites			GCF	MMP-13	Active sites had higher MMP-13; a marker of disease progression
Hernandez-Rios et al. (2009)	Prospective, active sites defined in 2 months	Moderate-advanced CP		PD, CAL, BOP	GCF	MMP-9, MMP-13, TIMP-1	MMP-13 activity significantly elevated in active sites, high levels of ICTP, associated with progression of periodontal breakdown. MMP-9, -13 may be useful biomarkers for periodontitis progression
Kardeşler et al. (2010)	Cross-sectional	12 DM gingivitis, 12 DM periodontitis, 12 H gingivitis, 13 H periodontitis, 24 healthy controls.			GCF	MMP-8, MMP-13, TIMP-1	MMP-8 total amounts were higher in periodontitis, DM-G groups. MMP-13, TIMP-1 total amounts were similar in study groups
Kinane et al. (2003)	Intervention	20 CP, before and after SRP	3 months	PD, CAL	GCF	MMP-8	MMP-8 decreased with SRP
Mäntylä et al. (2003)	Intervention	11 CP, 10 gingivitis, 8 healthy controls	Before and after SRP	Routine clinical parameters	GCF	MMP-8	Chair-side MMP-8 can differentiate CP from gingivitis, healthy, and monitor treatment of CP
Mäntylä et al. (2006)	Prospective	CP Smoker *versus* NS	12 months		GCF	MMP-8	Persistent elevation in MMP-8 may indicate high risk, poor response to therapy
Marcaccini et al. (2009)	Intervention	28 CP, 22 controls	before and 3 months after SRP		Plasma	MMP-2, -3, -8, -9, TIMP-1, -2, total gelatinolytic activity	Higher MMP-3, -8, -9, gelatinolytic activity in CP. MMP-8, -9, gelatinolytic activity decreased after therapy

Miller et al. (2006)	Cross-sectional	28 moderate-severe CP, 29 healthy controls		Saliva	IL-1β, MMP-8, OPG	Elevated IL-1β, MMP-8 but not OPG significantly increased risk of periodontal disease. Their salivary levels may be biomarkers for periodontal disease
Passoja et al. (2008)	Cross-sectional	48 CP patients		PI, PD, CAL, BOP	GCF, serum	MMP-8	MMP-8 in shallow crevices associated with CAL, can be a prognostic marker
Pozo et al. (2005)	Intervention	13CP, 11 healthy	6 months, after SRP	Routine clinical parameters	GCF	MMP-8, -9, TIMP-1, -2	Significant+correlations between periodontal disease severity and MMP activity, – correlations with TIMP-1, -2
Rai et al. (2008)	Cross-sectional	15 healthy 18 gingivitis 20 CP			GCF, saliva	MMP-2, -8, -9	Salivary MMP-8, GCF MMP-9 were higher in CP GCF MMP-2 was lower in CP High correlation with PD, BOP
Ramseier et al. (2009)	Cross-sectional	50 healthy/gingivitis 50 periodontitis			Whole saliva	MMP-8, -9, OPG	MMP-8, -9, OPG combined with red complex predicted periodontal disease
Xu et al. (2008)	Cross-sectional	Healthy, gingivitis, CP			GCF	MMP-8 isoenzymes (fibroblast and PMN-type)	CP exhibited highest activation of GCF MMP-8 isoenzymes

AgP, aggressive periodontitis; CP, chronic periodontitis; PD, probing depth; MMP, matrix metalloproteinase; GI, gingival index; PI, plaque index; BOP, bleeding on probing; CAL, clinical attachment level; TIMP, tissue inhibitors of metalloproteinases; GAgP, generalized aggressive periodontitis; ICTP, carboxy-terminal telopeptide of type I collagen; DM, diabetes mellitus; SRP, scaling and root planing; OPG, osteoprotegerin.

gingivitis patients (Xu et al. 2008). In cross-sectional studies, elevated GCF levels of MMP-7, TIMP-1 (Emingil et al. 2006c), laminin-5 gamma2-chain (Emingil et al. 2006a), MMP-25, -26 (Emingil et al. 2006b) were reported in chronic periodontitis patients compared to gingivitis patients and healthy control subjects. High GCF MMP-13, TIMP-1 levels were suggested to indicate high risk for periodontal disease progression (Alpagot et al. 2001). Similarly, persistent elevation of MMP-8 in GCF samples was regarded as indicating high risk and poor response to periodontal therapy (Mäntylä et al. 2006). Very recently, Gürsoy et al. (2010) also confirmed that salivary MMP-8 and TIMP-1 are increased in chronic periodontitis patients differentiating periodontitis patients from the controls. Furthermore, a chair-side MMP-8 test was indicated to effectively differentiate chronic periodontitis from gingivitis and clinically healthy sites and thus, to monitor treatment of chronic periodontitis patients (Mäntylä et al. 2003).

Neutrophil elastase (NE) is one of the most destructive enzymes with the capability of degrading almost all extracellular matrix components as well as plasma proteins and activating pro-MMPs and inactivating TIMP-1 (Meyle et al. 1992, Eley & Cox 1996a, b, Sorsa et al. 2006, Geraghty et al. 2007). A high concentration of NE is stored in azurophilic granules of PMNs, providing an important step in host defence. When activated, NE can be released rapidly into the extracellular space and cause local tissue damage (Kawabata et al. 2002). Endogenous proteinase inhibitors are important to protect tissues from unregulated proteolysis. Once released in circulation, NE is rapidly inactivated by conjugating with protease inhibitors. Heikkinen et al. (2010) reported that salivary concentrations of MMP-8 and NE correlated with BOP in a cross-sectional study comprising 501 adolescents.

Azurophilic granules of PMNs also contain the enzyme myeloperoxidase (MPO), which can generate a reactive oxidant species including hypochlorous acid (HOCl). MPO is produced by neutrophils whose oxidant products are capable of modifying low-density lipoprotein cholesterol and has been shown to be present in human atheromas and unstable plaques (Nicholls & Hazen 2005). MPO is released into the extracellular environment following neutro-

phil stimulation and/or degranulation (Buchmann et al. 2002). MPO can oxidatively activate MMP-8 and -9 and inactivate TIMP-1 (Sorsa et al. 2006). Decreased plasma MPO levels following periodontal treatment of severe periodontitis patients suggests that MPO may play a major role in pathogenesis of destructive periodontal diseases (Behle et al. 2009). Thus, MPO and NE can potentiate the destructive MMP cascades and indeed MPO has been regarded as a promising marker of periodontal disease activity (Arbes et al. 1999, Yamalık et al. 2000, Wei et al. 2004).

Eley & Cox (1995) have investigated whether GCF levels of dipeptidyl peptidase (DPP) II or IV levels, total activity and concentration could predict progressive attachment loss and suggested that both GCF DPP II and IV may be predictors of periodontal attachment loss. In another early study, Cox & Eley (1992) have also analysed cathepsin B/L-, elastase-, tryptase-, trypsin-, and DPP IV-like activities in GCF samples obtained from chronic periodontitis patients before and also after non-surgical periodontal treatment. GCF protease levels were suggested to reflect the clinical status of periodontal lesions and may thus be of value in monitoring disease activity.

Prostaglandin E_2 (PGE_2), a metabolite of the cyclooxygenase (COX) pathway, is an arachidonic acid metabolite and considered a potent mediator of alveolar bone loss in periodontitis (Offenbacher et al. 1984, 1993). PGE_2 is known to have an activity on fibroblasts and osteoclasts to induce the synthesis of MMPs, IL-1β and other cytokines. PGE_2 has been detected in higher levels in gingival tissue and GCF proportional to the severity of periodontal disease. IL-1β is a central mediator of inflammation and connective tissue destruction in rheumatoid arthritis (Raymond et al. 2006). IL-1β increases matrix degradation also by inducing the production of PGE_2 in synovial cells, as well by its role as a mediator of bone and cartilage destruction (Cutulo 2004). TNF-α is a less potent stimulator of PGE_2 production compared to IL-1β, but these two cytokines synergistically enhance PGE_2 production (Yücel-Lindberg et al. 1999). Several of the cytokines stimulating RANKL expression and bone resorption also enhance the expression of COX-2 and prostaglandin production (Lerner 2006). PGE_2 is also an efficient stimulator of RANKL expression in osteoblasts (Li et al. 2002) as well as on osteoclast progenitor cells (Ono et al. 2005). At persistent high concentrations, PGE_2 stimulates the master osteoclast activator; RANKL expression in stromal cells/osteoblasts and eventually causes enhanced bone resorption. Offenbacher et al. (1986) demonstrated that PGE_2 levels in GCF of patients exhibiting periodontal diseases are significantly higher than those in periodontally healthy subjects, and furthermore, that GCF PGE_2 concentrations are effective for predicting periodontitis progression, i.e. attachment loss, with a high degree of sensitivity and specificity (0.76 and 0.96, respectively). Evidence indicating the role of prostaglandins in periodontal tissue destruction also comes from human studies evaluating potential effects of non-steroidal anti-inflammatory drugs in periodontal treatment (Buduneli et al. 2002, 2010, Vardar et al. 2003).

Pro-inflammatory cytokines also play a significant role in the pathogenesis of periodontal diseases in terms of both soft and hard tissue destruction. Besides IL-1β and TNF-α, IL-6 is also hypothesized to stimulate bone resorption (Ishimi et al. 1990). TNF-α, IL-1β, -6, -8, -18 have been found to be abundantly expressed in human gingiva, and increased levels have been reported in GCF of periodontitis patients (Preiss & Meyle 1994, Boch et al. 2001, Graves & Cochran 2003, Toker et al. 2008, Pradeep et al. 2009a, b, Fitzsimmons et al. 2010, Teles et al. 2010). Increased GCF levels of IL-1β and NE have been reported with increasing level of gingival inflammation also in experimental gingivitis studies and indeed elastase activity has been suggested as an excellent quantitative measure of gingival inflammation (Hermann et al. 2001, Gonzáles et al. 2001). On the contrary, mean salivary levels of granulocyte-macrophage colony-stimulating factor, IL-1β, -2, -4, -5, -6, -8, -10, interferon gamma (IFN-γ), and TNF-α failed to discriminate between periodontal health and disease (Teles et al. 2009). Neutralization of IL-1 and TNF-α by soluble receptors has been reported to decrease osteoclast formation and bone loss in experimental periodontitis (Assuma et al. 1998). Silva et al. (2008) followed 56 moderate to severe chronic periodontitis patients until progression of periodontal destruction was detected and they reported significantly higher GCF levels of RANKL, IL-1β, and MMP-13 in active sites than the inactive sites. Very recently, Rescala et al. (2010) conducted a cross-sectional study comprising 20 chronic periodontitis patients, 17 generalized aggressive periodontitis patients and 10 gingivitis patients. They reported that IL-1β and elastase levels in the GCF samples were higher in deep sites compared with the shallow sites in both of the periodontitis groups and suggested that these biomarkers may indicate periodontal tissue destruction. Furthermore, Frodge et al. (2008) evaluated salivary concentrations of TNF-α, RANKL, and ICTP in 35 subjects with moderate to severe chronic periodontitis in comparison with 39 healthy controls. The authors reported that salivary TNF-α levels were significantly elevated in chronic periodontitis patients suggesting the utility of this biomarker in a panel of salivary parameters that could facilitate the screening, diagnosis, and management of periodontal disease. In another recent study, Yetkin Ay et al. (2009) investigated GCF IL-11 and IL-17 levels in 40 chronic periodontitis patients and 20 healthy controls. It was reported that the IL-11/IL-17 ratio was significantly higher in the healthy control group than the periodontitis patients, whereas shallow sites in the periodontitis patients exhibited higher ratios than the deep sites in the same patients. Thus, an imbalance in the pro- and anti-inflammatory cytokines may be responsible for periodontal breakdown through immune responses.

CRP is a circulating acute-phase protein acting in an innate immune response. Significantly higher serum high-sensitivity CRP (hs-CRP) concentrations were found in periodontitis patients than the healthy controls in various case–control studies (Ebersole et al. 1997, Fredriksson et al. 1999, Loos et al. 2000, Glurich et al. 2002, Craig et al. 2003, Liu et al. 2010). On the other hand, Tüter et al. (2007) found no difference in serum CRP levels between the chronic periodontitis and healthy control groups, and serum CRP levels were not associated with PD. Moreover, periodontal treatment modalities resulted in decreases in serum CRP concentrations (Ebersole et al. 1997, Nakashima et al. 2000, Mattila et al. 2002, Swoboda et al. 2008, Marcaccini et al. 2009, Katagiri et al. 2009, Renvert et al. 2009, Golub et al. 2010). However, Offenbacher et al. (2009) reported that serum CRP concentration was not affected by scaling and root planning.

Serum amyloid A (SAA) is another acute-phase protein, which is associated with high-density lipoprotein (HDL). Elevated SAA concentrations in blood circulation have been found in subjects with both periodontitis and CVDs compared with controls without either disease (Glurich et al. 2002). A positive correlation between SAA level in serum and number of purulent periodontal pockets has been reported (Pussinen et al. 2004). Vuletic et al. (2009) reported that serum SAA concentration decreased after full-mouth extraction in periodontitis patients.

Increased serum levels of IL-1β, TNF, OC, soluble intercellular adhesion molecule (sICAM), IL-6, MMP-9 have been reported in experimental studies in animal models as well as in clinical studies in humans (Raunio et al. 2007, Guentsch et al. 2009, Nakajima et al. 2010). However, Özçaka et al. (2010) failed to find significant differences between chronic periodontitis patients and healthy control subjects in terms of plasma OC and ICTP concentrations. In an intervention study, Marcaccini et al. (2009) reported that chronic periodontitis patients had higher plasma concentrations of MMP-3, -8, -9, and gelatinolytic activity, whereas non-surgical periodontal treatment decreased their levels significantly.

Serum cortisol concentration was reported to be associated with clinical periodontal parameters; PD and CAL (Ishisaka et al. 2008) and with BOP (Furugen et al. 2008). Furthermore, surfactant protein D was suggested to be a biomarker for chronic periodontitis, as it was found to be increased in 105 chronic periodontitis patients in comparison with 122 healthy control subjects (Glas et al. 2008). In a follow-up study, Amarasena et al. (2008) suggested that serum calcium level may be a risk factor for periodontal disease progression, as it correlated with PD. Higher platelet activating factor (PAF) levels were found in both GCF and serum of chronic periodontitis patients than gingivitis patients and healthy control subjects (Zheng et al. 2006, Chen et al. 2009). These elevated levels together with the significant correlations with clinical periodontal measurements suggested that PAF may have a role in the pathogenesis of periodontal destruction.

Conclusions

There exists extensive evidence that molecules in the saliva and in the GCF correlate with tissue inflammation and bone destruction. Among those showing most promise is the ratio of RANKL/OPG, which is a discloser of periodontal disease activity but its utility in predicting future disease is questionable. However, the sensitivity and specificity of these molecules as predictors of future disease, i.e. prognosis, has not been reliably demonstrated. Even after the advent of highly sophisticated methods and almost 30 years of research on finding a consistent and definitive biochemical marker for periodontal disease activity and prognosis, no adequate marker has been successfully identified as yet. The natural history and nature of periodontal disease is one highly complicating factor in this situation as periodontal disease progresses episodically and it is very difficult to define the quiescent and active periods. Moreover, periodontal disease is a multi-factorial disease with several host- and microorganism-related factors acting consecutively. Last but not least, there is a wide range of individual variability in the response to the microbial triggers as well as to the therapeutic measures. Another issue making it difficult to come to a conclusion on the biochemical parameters is the diversity in biological sample collection, storage, and analysis methods used in different studies evaluating the same parameters. We should not be surprized that finding markers for periodontal disease is so elusive: other chronic conditions such as CVDs are similarly complex and despite even greater expenditure and manpower being applied, recent reports reveal that the previously lauded vascular risk factors such as CRP and N-BNP, are in fact not useful (Melander et al. 2009). Interestingly CRP and other risk factors in CVD are more useful as negative predictive factors similar to the use of BOP as a negative predictive factor in periodontal disease (Lang et al. 1986). At present, we do not have a clear understanding of the pathology or the molecular events occurring in the periodontal microenvironment during the tissue destruction process. Certain clinical measures such as clinical attachment loss remain the strongest predictor of future attachment loss and absence of certain clinical inflammatory signs such as BOP are excellent negative predictors of periodontal inflammation. Thus we can conclude that much work remains to identify molecules with clinical utility for estimating current and future destructive periodontal disease activity. We provide the following supportable conclusions, many of which are not novel.

- Clinical measures such as PD, attachment level and BOP are essential for the diagnosis of prevalent periodontal tissue destruction.
- BOP remains to be the most reliable clinical finding correlated with periodontal disease activity.
- An increase in PD over time is associated with loss of attachment and loss of tooth supporting bone. Current PD is indicative of future attachment loss (Van der Velden et al. 2006). Thus, clinical periodontal measurements, mainly PD and BOP continue to be the most reliable parameters not only for the diagnosis but also for determining the prognosis of periodontal diseases, although they do not provide 100% accuracy.
- Investigating the levels of RANKL, OPG, and a range of host bone destruction markers and inflammation related molecules in GCF and saliva may prove to be reliable information on the state of periodontal disease activity however, currently cannot predict future disease activity.
- With the current picture, it is quite clear that highly specific and sensitive biomarkers for diagnosis and monitoring of periodontal diseases are still needed for early and better detection of periodontal tissue destruction.

References

Aemaimanan, P., Sattayasai, N., Wara-aswapati, N., Pitiphat, W., Suwannarong, W., Prajaneh, S. & Taweechaisupapong, S. (2009) Alanine aminopeptidase and dipeptidyl peptidase IV in saliva of chronic periodontitis patients. *Journal of Periodontology* **80**, 1809–1814.

Aiba, T., Akeno, N., Kawane, T., Okamoto, H. & Horiuchi, N. (1996) Matrix metalloproteinases-1 and -8 and TIMP-1 mRNA levels in normal and diseased human gingivae. *European Journal Oral Sciences* **104**, 562–569.

Akalın, F. A., Baltacıoğlu, E., Alver, A. & Karabulut, E. (2007) Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. *Journal of Clinical Periodontology* **34**, 558–565.

Akalın, F. A., Toklu, E. & Renda, N. (2005) Analysis of superoxide dismutase activity levels in gingival and gingival crevicular fluid in patients with chronic periodontitis and periodontally healthy controls. *Journal of Clinical Periodontology* **32**, 238–243.

Alfant, B., Shaddox, L. M., Tobler, J., Magnusson, I., Aukhil, I. & Walker, C. (2008) Matrix metalloproteinase levels in children with aggressive periodontitis. *Journal of Periodontology* **79**, 819–826.

Alpagot, T., Bell, C., Lundergan, W., Chambers, D. W. & Rudin, R. (2001) Longitudinal evaluation of GCF MMP-3 and TIMP-1 levels as prognostic factors for progression of periodontitis. *Journal of Clinical Periodontology* **28**, 353–359.

Amarasena, N., Yoshihara, A., Hirotomi, T., Takano, N. & Miyazaki, H. (2008) Association between serum calcium and periodontal disease progression in non-institutionalized elderly. *Gerodontology* **25**, 245–250.

Arbes, S. J. Jr., Slade, G. D. & Beck, J. D. (1999) Association between extent of periodontal attachment loss and self-reported history of heart attack: an analysis of NHANES III data. *Journal Dental Research* **78**, 1777–1782.

Armitage, G. C. (2004) Analysis of gingival crevice fluid and risk of progression of periodontitis. *Periodontology 2000* **34**, 109–119.

Assuma, R., Oates, T., Cochran, D., Amar, S. & Graves, D. T. (1998) IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. *Journal of Immunology* **160**, 403–409.

Aurer, A., Aurer-Kozelj, J., Stavljenić-Rukavina, A., Kalenić, S., Ivić-Kardum, M. & Haban, V. (1999) Inflammatory mediators in saliva of patients with rapidly progressive periodontitis during war stress induced incidence increase. *Collegium Antropologicum* **23**, 117–124.

Baltacıoğlu, E., Akalın, F. A., Alver, A., Değer, O. & Karabulut, E. (2008) Protein carbonyl levels in serum and gingival crevicular fluid in patients with chronic periodontitis. *Archives of Oral Biology* **53**, 716–722.

Baron, A. C., Gansky, S. A., Ryder, M. I. & Featherstone, J. D. (1999) Cysteine protease inhibitory activity and levels of salivary cystatins in whole saliva of periodontally diseased patients. *Journal of Periodontal Research* **34**, 437–444.

Behle, J. H., Sedaghatfar, M. H., Demmer, R. T., Wolf, D. L., Celenti, R., Klebschull, M., Belusko, P. B., Herrera-Abreu, M., Lalla, E. & Papapanou, P. N. (2009) Heterogeneity of systemic inflammatory responses to periodontal therapy. *Journal of Clinical Periodontology* **36**, 287–294.

Beklen, A., Tüter, G., Sorsa, T., Hanemaaijer, R., Virtanen, I., Tervahartiala, T. & Konttinen, Y. T. (2006) Gingival tissue and crevicular fluid cooperation in adult periodontitis. *Journal of Dental Research* **85**, 59–63.

Bıyıkoğlu, B., Buduneli, N., Kardeşler, L., Aksu, K., Pitkala, M. & Sorsa, T. (2009) Gingival crevicular fluid MMP-8 and -13 and TIMP-1 levels in patients with rheumatoid arthritis and inflammatory periodontal disease. *Journal of Periodontology* **80**, 1307–1314.

Bildt, M. M., Bloemen, M., Kuijpers-Jagtman, A. M. & Von den Hoff, J. W. (2008) Collagenolytic fragments and active gelatinase complexes in periodontitis. *Journal of Periodontology* **79**, 1704–1711.

Boch, J. A., Wara-Aswapati, N. & Auron, P. E. (2001) Interleukin 1 signal transduction-current concepts and relevance to periodontitis. *Journal of Dental Research* **80**, 400–407.

Bostancı, N., Emingil, G., Afacan, B., Han, B., Ilgenli, T., Atilla, G., Hughes, F. J. & Belibasakis, G. N. (2008) Tumor necrosis factor-alpha-converting enzyme (TACE) levels in periodontal diseases. *Journal of Dental Research* **87**, 273–237.

Bostanci, N., İlgenli, T., Emingil, G., Afacan, B., Han, B., Töz, H., Atilla, G., Hughes, F. J. & Belibasakis, G. N. (2007) Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. *Journal of Clinical Periodontology* **34**, 370–376.

Buchmann, R., Hasilik, A., Nunn, M. E., Van Dyke, T. E. & Lange, D. E. (2002) PMN responses in chronic periodontal disease: evaluation by gingival crevicular fluid enzymes and elastase-alpha-1-proteinase inhibitor complex. *Journal of Clinical Periodontology* **29**, 563–572.

Buduneli, E., Vardar-Sengül, S., Buduneli, N., Atilla, G., Wahlgren, J. & Sorsa, T. (2007) Matrix metalloproteinases, tissue inhibitor of matrix metalloproteinase-1, and laminin-5 gamma2 chain immunolocalization in gingival tissue of endotoxin-induced periodontitis in rats: effects of low-dose doxycycline and alendronate. *Journal of Periodontology* **78**, 127–134.

Buduneli, N., Buduneli, E., Kardeşler, L., Lappin, D. & Kinane, D. F. (2005) Plasminogen activator system in smokers and non-smokers with and without periodontal disease. *Journal of Clinical Periodontology* **32**, 417–424.

Buduneli, N., Bıyıkoğlu, B., Sherrabeh, S. & Lappin, D. (2008) Saliva concentrations of RANKL and osteoprotegerin in smoker versus non-smoker chronic periodontitis patients. *Journal of Clinical Periodontology* **35**, 846–852.

Buduneli, N., Buduneli, E. & Kütükçüler, N. (2009) Interleukin-17, RANKL, osteoprotegerin levels in gingival crevicular fluid from smoker versus non-smoker chronic periodontitis patients during initial periodontal treatment. *Journal of Periodontology* **80**, 1274–1280.

Buduneli, N., Buduneli, E., Çetin, E. O., Kırılmaz, L. & Kütükçüler, N. (2010) Clinical findings and gingival crevicular fluid prostaglandin E2 and interleukin-1-beta levels following initial periodontal treatment and short-term meloxicam administration. *Expert Opinion on Pharmacotherapy* **11**, 1805–1812.

Buduneli, N., Vardar, S., Atilla, G., Sorsa, T., Luoto, H. & Baylas, H. (2002) Gingival crevicular fluid matrix metalloproteinase-8 levels following adjunctive use of meloxicam and initial phase of periodontal therapy. *Journal of Periodontology* **73**, 103–109.

Calsina, G., Ramon, J. M. & Echeverria, J. J. (2002) Effects of smoking on periodontal tissues. *Journal of Clinical Periodontology* **29**, 771–776.

Champagne, C. M., Buchanan, W., Reddy, M. S., Preisser, J. S., Beck, J. D. & Offenbacher, S. (2003) Potential for gingival crevice fluid markers as predictors of risk for periodontal diseases. *Periodontology 2000* **31**, 167–180.

Chapple, I. L. C., Brock, G. R., Milward, M. R., ling, N. & Matthews, J. B. (2007) Compromised GCF total antioxidant capacity in periodontitis: cause or effect? *Journal of Clinical Periodontology* **34**, 103–110.

Chapple, I. L. C., Garner, I., Saxby, M. S., Moscrop, H. & Matthews, J. B. (1999) Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatise levels. *Journal of Clinical Periodontology* **26**, 190–198.

Chen, H. Y., Cox, S. W. & Eley, B. M. (1998) Cathepsin B, alpha2-macroglobulin and cystatin levels in gingival crevicular fluid from chronic periodontitis patients. *Journal of Clinical Periodontology* **25**, 34–41.

Chen, H. Y., Cox, S. W., Eley, B. M., Mäntylä, P., Rönkä, H. & Sorsa, T. (2000) Matrix metalloproteinase-8 levels and elastase activities in gingival crevicular fluid from chronic adult periodontitis patients. *Journal of Clinical Periodontology* **27**, 366–369.

Chen, H., Zheng, P., Zhu, J., Zhao, L., El Mokhtari, N. E., Eberhard, J., Lins, M. & Jepsen, S. (2009) Platelet-activating factor levels of serum and gingival crevicular fluid in non-smoking patients with periodontitis and/or coronary heart disease. *Clinical Oral Investigation* **14**, 629–636.

Christenson, R. H. (1997) Biochemical markers of bone metabolism: an overview. *Clinical Biochemistry* **30**, 573–593.

Cochran, D. L. (2008) Inflammation and bone loss in periodontal diseases. *Journal of Periodontology* **79**, 1569–1576.

Cox, S. W. & Eley, B. M. (1992) Cathepsin B/L-, elastase-, tryptase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid. A comparison of levels before and after basic periodontal treatment of chronic periodontitis patients. *Journal of Clinical Periodontology* **19**, 333–339.

Craig, R. G., Yip, J. K., So, M. K., Boylan, R. J., Socransky, S. S. & Haffajee, A. D. (2003) Relationship of destructive periodontal disease to the acute-phase response. *Journal of Periodontology* **74**, 1007–1016.

Crotti, T., Smith, M. D., Hirsch, R., Soukoulis, S., Weedon, H., Capone, M., Ahern, M. J. & Haynes, D. (2003) Receptor activator NF kappaB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. *Journal of Periodontal Research* **38**, 380–387.

Cutando, A., Galindo, P., Gomez-Moreno, G., Arana, C., Bolanos, J., Acuna-Castroviejo, D. & Wang, H. L. (2006) Relationship between salivary melatonin and severity of periodontal disease. *Journal of Periodontology* **77**, 1533–1538.

Cutulo, M. (2004) IL-1Ra: its role in rheumatoid arthritis. *Rheumatismo* **56**, 41–45.

De la Pena, V. A., Diz Dios, P. & Tojo Sierra, R. (2007) relationship between lactate dehydrogenase activity in saliva and oral health status. *Archives of Oral Biology* **52**, 911–915.

De Queiroz, A. C., Taba, M. Jr., O'Connell, P. A., da Nobrega, P. B., Costa, P. P., Kawata, V. K., Trevisan, G. L., Novaes, A. B. Jr., de Souza, S. L., Palioto, D. B. & Grisi, M. F. (2008) Inflammation markers in healthy and periodontitis patients: a preliminary data screening. *Brazilian Dental Journal* **19**, 3–8.

Dereka, X. E., Markopoulou, C. E., Fanourakis, G., Tseleni-Balafouta, S. & Vrotsos, I. A. (2010) RANKL and OPG mRNA level after non-surgical periodontal treatment. *Inflammation* **33**, 200–206.

Dezerega, A., Pozo, P., Hernandez, M., Oyarzun, A., Rivera, O., Dutzan, N., Gutierrez-Fernandez, A., Overall, C. M., Garrido, M., Alcota, M., Ortiz, E. & Gamonal, J. (2010) Chemokine monocyte chemoattractant protein-3 in progressive periodontal lesions in patients with chronic periodontitis. *Journal of Periodontology* **81**, 267–276.

Diab-Ladki, R., Pellat, B. & Chahine, R. (2003) Decrease in the total antioxidant activity of saliva in patients with periodontal diseases. *Clinical Oral Investigations* **7**, 103–107.

Duerte, P. M., da Rocha, M., Sampaio, E., Mestnik, M. J., Feres, M., Figueiredo, L. C., Bastos, M. F. & Faveri, M. (2010) Serum levels of cytokines in subjects with generalized chronic and aggressive periodontitis before and after non-surgical periodontal therapy: a pilot study. *Journal of Periodontology* **81**, 1056–1063.

Dutzan, N., Gamonali, J., Silva, A., Sanz, M. & Vernal, R. (2009) Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-kappa B ligand, interleukin (IL)-17, IL-10 and transforming growth factor-beta during the progression of chronic periodontitis. *Journal of Clinical Periodontology* **36**, 396–403.

Ebersole, J. L., Machen, R. L., Steffen, M. J. & Willmann, D. E. (1997) Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. *Clinical Experimental Immunology* **107**, 347–352.

Eley, B. M. & Cox, S. W. (1995) Correlation between gingival crevicular fluid dipeptidyl peptidase II and IV activity and periodontal attachment loss. A 2-year longitudinal study in chronic periodontitis patients. *Oral Diseases* **1**, 201–213.

Eley, B. M. & Cox, S. W. (1996a) The relationship between gingival crevicular fluid cathepsin B activity and periodontal attachment loss in chronic periodontitis patients: a 2-year longitudinal study. *Journal of Periodontal Research* **31**, 381–392.

Eley, B. M. & Cox, S. W. (1996b) A 2-year longitudinal study of elastase in human gingival crevicular fluid and periodontal attachment loss. *Journal of Clinical Periodontology* **23**, 681–692.

Emingil, G., Kuula, H., Pirila, E., Atilla, G. & Sorsa, T (2006a) Gingival crevicular fluid laminin-5 gamma2-chain levels in periodontal disease. *Journal of Clinical Periodontology* **33**, 462–468.

Emingil, G., Kuula, H., Sorsa, T. & Atilla, G (2006b) Gingival crevicular fluid matrix metalloproteinase-25 and -26 levels in periodontal disease. *Journal of Periodontology* **77**, 664–671.

Emingil, G., Tervahartiala, T., Mantyla, P., Maatta, M., Sorsa, T. & Atilla, G. (2006c) Gingival crevicular fluid matrix metalloproteinase (MMP)-7, extracellular MMP inducer, and tissue inhibitor of MMP-1 levels in periodontal disease. *Journal of Periodontology* **77**, 2040–2050.

Fitzsimmons, T. R., Sanders, A. E., Bartold, P. M. & Slade, G.D (2010) Local and systemic biomarkers in gingival crevicular fluid increase odds of periodontitis. *Journal of Clinical Periodontology* **37**, 30–36.

Fredriksson, M. I., Figueredo, C. M., Gustafsson, A., Bergström, K. G. & Asman, B. E. (1999) Effect of periodontitis and smoking on blood leukocytes and acute-phase proteins. *Journal of Periodontology* **70**, 1355–1360.

Frodge, B. D., Ebersole, J. L., Kryscio, R. J., Thomas, M. V. & Miller, C. S. (2008) Bone remodelling biomarkers of periodontal disease in saliva. *Journal of Periodontology* **79**, 1913–1919.

Furugen, R., Hayashida, H., Yamaguchi, N., Yoshihara, A., Ogawa, H., Miyazaki, H. & Saito, T. (2008) The relationship between periodontal condition and serum levels of resistin and adiponectin in elderly Japanese. *Journal of Periodontal Research* **43**, 556–562.

Gallop, P. M., Lian, J. B. & Hauschka, P. V. (1980) Carboxylated calcium-binding proteins and vitamin K. *New England Journal of Medicine* **302**, 1460–1466.

Gapski, R., Hasturk, H., Van Dyke, T. E., Oringer, R. J., Wang, S., Braun, T. M. & Giannobile, W. V. (2009) Systemic MMP inhibition for periodontal wound repair: results of a multi-centre randomized-controlled clinical trial. *Journal of Clinical Periodontology* **36**, 149–156.

Garg, G., Pradeep, A. R. & Thorat, M. K. (2009) Effect of nonsurgical periodontal therapy on crevicular fluid levels of cathepsin K in periodontitis. *Archives of Oral Biology* **54**, 1046–1051.

Garito, M. L., Prihoda, T. J. & McManus, L. M. (1995) Salivary PAF levels correlate with the severity of periodontal inflammation. *Journal of Dental Research* **74**, 1048–1056.

Geraghty, P., Rogan, M. P., Greene, C. M., Boxio, R. M., Poiriert, T., O'Mahony, M., Belaaouaj, A., O'Neill, S. J., Taggart, C. C. & McElvaney, N. G. (2007) Neutrophil elastase up-regulates cathepsin B and matrix metalloprotease-2 expression. *Journal of Immunology* **178**, 5871–5878.

Ghallab, N. & Shaker, O. (2010) Salivary-soluble CD44 levels in smokers and non-smokers with chronic periodontitis: a pilot study. *Journal of Periodontology* **81**, 710–717.

Gheren, L. W., Cortell, J. R., Rodrigues, E., Holzhausen, M. & Saad, W. A. (2008) Periodontal therapy reduces arginase activity in saliva of patients with chronic periodontitis. *Clinical Oral Investigations* **12**, 67–72.

Giannobile, W. V. (1997) Crevicular fluid biomarkers of oral bone loss. *Current Opinion in Periodontology* **4**, 11–20.

Giannobile, W. V. (1999) C-telopeptide pyridinoline cross-links. Sensitive indicators of periodontal tissue destruction. *Annals of New York Academy of Sciences* **878**, 404–412.

Giannobile, W. V., Al-Shammari, K. F. & Sarment, D. P. (2003) Matrix molecules and growth factors as indicators of periodontal disease activity. *Periodontology 2000* **31**, 125–134.

Glas, J., Beynon, V., Bachstein, B., Steckenbiller, J., Manolis, V., Euba, A., Müller-Myhsok, B. & Folwaczny, M. (2008) Increased plasma concentration of surfactant protein D in chronic periodontitis independent of SFTPD genotype: potential role as a biomarker. *Tissue Antigens* **72**, 21–28.

Glurich, I., Grossi, S., Albini, B., Ho, A., Shah, R., Zeid, M., Baumann, H., Genco, R. J. & De Nardin, E. (2002) Systemic inflammation in cardiovascular and periodontal disease: comparative study. *Clinical Diagnostics and Laboratory Immunology* **9**, 425–432.

Golub, L. M., Lee, H. M., Stoner, J. A., Reinhardt, R. A., Sorsa, T., Goren, A. D. & Payne, J. B. (2010) Doxycycline effects on serum bone biomarkers in post-menopausal women. *Journal of Dental Research* **89**, 644–649.

Golub, L. M., Lee, H. M., Stoner, J. A., Sorsa, T., Reinhardt, R. A., Wolff, M. S., Ryan, M. E., Nummikoski, P. V. & Payne, J. B. (2008) Subantimicrobial-dose doxycycline modulates gingival crevicular fluid biomarkers of periodontitis in postmenopausal osteopenic women. *Journal of Periodontology* **79**, 1409–1418.

Gonzáles, J. R., Herrmann, J. M., Boedeker, R. H., Francz, P. I., Biesalski, H. & Meyle, J. (2001) Concentration of interleukin-1beta and neutrophil elastase activity in gingival crevicular fluid during experimental gingivitis. *Journal of Clinical Periodontology* **28**, 544–549.

Grant, M. M., Brock, G. R., Matthews, J. B. & Chapple, I. L. (2010) Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. *Journal of Clinical Periodontology* **37**, 17–23.

Graves, D. T. & Cochran, D. (2003) The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *Journal of Periodontology* **74**, 391–401.

Guentsch, A., Puklo, M., Preshaw, P. M., Glockmann, E., Pfister, W., Potempa, J. & Eick, S. (2009) Neutrophils in chronic and aggressive periodontitis in interaction with *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. *Journal of Periodontal Research* **44**, 368–377.

Gürlek, Ö., Lappin, D. F. & Buduneli, N. (2009) Effects of smoking on salivary C-telopeptide pyridinoline cross-links of type I collagen and osteocalcin levels. *Archives of Oral Biology* **54**, 1099–1104.

Gürsoy, U. K., Könönen, E., Pradhan-Palikhe, P., Tervahartiala, T., Pussinen, P. J., Suominen-Taipale, L. & Sorsa, T. (2010) Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. *Journal of Clinical Periodontology* **37**, 487–493.

Gürsoy, U. K., Könönen, E., Uitto, V.-J., Pussinen, P. J., Hyvarinen, K., Suominen-Taipale, L. & Knuuttila, M. (2009) Salivary interleukin-1b concentration and the presence of multiple pathogens in periodontitis. *Journal of Clinical Periodontology* **36**, 922–927.

Haigh, B. J., Stewart, K. W., Whelan, J. R., Barnett, M. P., Smolenski, G. A. & Wheeler, T. T. (2010) Alterations in the salivary proteome associated with periodontitis. *Journal of Clinical Periodontology* **37**, 241–247.

Hayakawa, H., Yamashita, K., Ohwaki, K., Sawa, M., Noguchi, T., Iwata, K. & Hayakawa, T. (1994) Collagenase activity and tissue inhibitor of metalloproteinases-1 (TIMP-1) content in human whole saliva from clinically healthy and periodontally diseased subjects. *Journal of Periodontal Research* **29**, 305–308.

Heikkinen, A. M., Sorsa, T., Pitkaniemi, J., Tervahartiala, T., Kari, K., Broms, U., Koskenvuo, M. & Meurman, J. H. (2010) Smoking affects diagnostic salivary periodontal disease biomarker levels in adolescents. *Journal of Periodontology* **81**, 1299–1307.

Heitz-Mayfield, L. J. A. (2005) Disease progression: identification of high-risk groups and individuals for periodontitis. *Journal of Clinical Periodontology* **32** (Suppl. 6), 196–209.

Hernandez, M., Valenzuela, M. A., Lopez-Otin, C., Alvarez, J., Lopez, J. M., Vernal, R. & Gamonal, J. (2006) Matrix metalloproteinase-13 is highly expressed in destructive periodontal disease activity. *Journal of Periodontology* **77**, 1863–1870.

Hernandez-Rios, M., Sorsa, T., Obregon, F., Tervahartiala, T., Valenzuela, M. A., Pozo, P., Dutzan, N., Lesaffre, E., Molas, M. & Gamonol, J. (2009) Proteolytic roles of matrix metalloproteinase (MMP)-13 during progression of chronic periodontitis: initial evidence for MMP-13/MMP-9 activation cascade. *Journal of Clinical Periodontology* **36**, 1011–1017.

Herrmann, J. M., Gonzáles, J. R., Boedeker, R. H., Vonholdt, J. & Meyle, J. (2001) Microassay for the detection of elastase activity in the gingival crevice. *Journal of Clinical Periodontology* **28**, 31–37.

Holzhausen, M., Cortelli, J. R., da Silva, V. A., Franco, G. C., Cortelli, S. C. & Vergnolle, N. (2010) Protease-activated receptor-2 (PAR_2) in human periodontitis. *Journal of Dental Research* **89**, 948–953.

Horwood, N. J., Kartsogiannis, V., Quinn, J. M. W., Romas, E., Martin, T. J. & Gillespie, M. T. (1999) Activated T lymphoctes support osteoclast formation in vitro. *Biochemical and Biophysical Research Communications* **265**, 144–150.

Ikezawa-Suzuki, I., Shimada, Y., Tai, H., Komatsu, Y., Tanaka, A. & Yoshie, H (2008) Effects of treatment on soluble tumour necrosis factor receptor type 1 and 2 in chronic periodontitis. *Journal of Clinical Periodontology* **35**, 961–968.

Ishimi, Y., Miyaura, C., Jin, C. H., Akatsu, T., Abe, E., Nakamura, Y., Yamaguchi, A., Yoshiki, S., Matsuda, T. & Hirano, T. (1990) IL-6 is produced by osteoblasts and induces bone resorption. *Journal of Immunology* **145**, 3297–3303.

Ishisaka, A., Ansai, T., Soh, I., Inenaga, K., Awano, S., Yoshida, A., Hamasaki, T., Sonoki, K., Takata, Y., Nishihara, T. & Takehara, T. (2008) Association of cortisol and dehydroepiandrosterone sulphate levels in serum with periodontal status in older Japanese adults. *Journal of Clinical Periodontology* **35**, 853–861.

Iwasaki, M., Yoshihara, A., Hirotomi, T., Ogawa, H., Hanada, N. & Miyazaki, H. (2008) Longitudinal study on the relationship between serum albumin and periodontal disease. *Journal of Clinical Periodontology* **35**, 291–296.

Jepsen, S., Springer, I. N., Buschmann, A., Hedderich, J. & Açıl, Y. (2003) Elevated levels of collagen cross-link residues in gingival tissues and crevicular fluid of teeth with periodontal disease. *European Journal of Oral Sciences* **111**, 198–202.

Kardeşler, L., Bıyıkoğlu, B., Çetinkalp, S., Pitkala, M., Sorsa, T. & Buduneli, N. (2010) Crevicular fluid matrix metalloproteinase-8, -13, and TIMP-1 levels in type 2 diabetics. *Oral Diseases* **16**, 476–481.

Kardeşler, L., Buduneli, N., Bıyıkoğlu, B., Çetinkalp, Ş. & Kütükçüler, N. (2008) Gingival crevicular fluid PGE2, IL-1beta, t-PA, PAI-2 levels in type 2 diabetes and relationship with periodontal disease. *Clinical Biochemistry* **41**, 863–868.

Katagiri, S., Nitta, H., Nagasawa, T., Uchimura, I., Izumiyama, H., Inagaki, K., Kikuchi, T., Noguchi, T., Kanazawa, M., Matsuo, A., Chiba, H., Nakamura, N., Kanamura, N., Inoue, S., Ishikawa, I. & Izumi, Y. (2009) Multi-center intervention study on glycohemoglobin (HbA1c) and serum, high-sensitivity CRP (hs-CRP) after local anti-infectious periodontal treatment in type 2 diabetic patients with periodontal disease. *Diabetes Research and Clinical Practice* **83**, 308–315.

Kaufman, E. & Lamster, I. B. (2000) Analysis of saliva for periodontal diagnosis. *Journal of Clinical Periodontology* **27**, 453–465.

Kawabata, K., Hagio, T. & Matsuoka, S. (2002) The role of neutrophil elastase in acute lung injury. *European Journal of Pharmacology* **451**, 1–10.

Kawai, T., Matsuyama, T., Hosokawa, Y., Makihira, S., Seki, M., Karimbux, N. Y., Goncalves, R. B., Valverde, P., Dibart, S., Li, Y. P., Miranda, L. A., Ernst, C. W., Izumi, Y. & Taubman, M. A. (2006) B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. *American Journal of Pathology* **169**, 987–998.

Kennett, C. N., Cox, S. W. & Eley, B. M. (1997) Investigations into the cellular contribution to host tissue proteases and inhibitors in gingival crevicular fluid. *Journal of Clinical Periodontology* **24**, 424–431.

Kido, J., Nakamura, T., Asahara, Y., Sawa, T., Kohri, K. & Nagata, T. (2001) Osteopontin levels in gingival crevicular fluid. *Journal of Periodontal Research* **36**, 328–333.

Kinane, D. F. (2001) Causation and pathogenesis of periodontal disease. *Periodontology 2000* **25**, 8–20.

Kinane, D. F. & Chestnutt, I. G. (2000) Smoking and periodontal disease. *Critical Reviews in Oral Biology Medicine* **11**, 356–365.

Kinane, D. F., Darby, I. B., Said, S., Luoto, H., Sorsa, T., Tikanoja, S. & Mäntylä, P. (2003) Changes in gingival crevicular fluid matrix metalloproteinase-8 levels during periodontal treatment and maintenance. *Journal of Periodontal Research* **38**, 400–404.

Kinney, J. S., Ramseier, C. A. & Giannobile, W. V. (2007) Oral fluid-based biomarkers of alveolar bone loss in periodontitis. *Annals of New York Academy of Sciences* **1098**, 230–251.

Koka, S., Forde, M. D. & Khosla, S. (2006) Systemic assessments utilising saliva: part 2 Osteoporosis and use of saliva to measure bone turnover. *International Journal of Prosthodontics* **19**, 53–60.

Kong, Y. Y., Yoshida, H., Sarosi, I., Tan, H. L., Timms, E., Capparelli, C., Morony, S., Oliveirados-Santos, A. J., Van, G., Itie, A., Khoo, W., Wakeham, A., Dunstan, C. R., Lacey, D. L., Mak, T. W., Boyle, W. J. & Penninger, J. M. (1999) OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* **397**, 315–323.

Koss, M. A., Castro, C. E., Salúm, K. M. & López, M. E. (2009) Changes in saliva protein composition in patients with periodontal disease. *Acta Odontologica Latinoamerica* **22**, 105–112.

Kurtiş, B., Develioğlu, H., Taner, I. L., Baloş, K. & Tekin, I. O. (1999) IL-6 levels in gingival crevicular fluid (GCF) from patients with non-insulin dependent diabetes mellitus (NIDDM), adult periodontitis and healthy subjects. *Journal of Oral Sciences* **41**, 163–167.

Kuula, H., Salo, T., Pirilä, E., Tuomainen, A. M., Jauhiainen, M., Uitto, V. J., Tjäderhane, L., Pussinen, P. J. & Sorsa, T. (2009) Local and systemic responses in matrix metalloproteinase 8-deficient mice during *Porphyromonas gingivalis*-induced periodontitis. *Infection and Immunity* **77**, 850–859.

Lacey, D. L., Timms, E., Tan, H.-L., Kelley, M. J., Dunstan, C. R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., Hsu, H., Sullivan, J., Hawkins, N., Davy, E., Capparelli, C., Eli, A., Qian, Y. X., Kaufman, S., Sarosi, I., Shalhoub, V., Senaldi, G., Guo, J., Delaney, J. & Boyle, W. J. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* **93**, 165–176.

Lamster, I. B. & Ahlo, J. K. (2007) Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. *Annals of New York Academy of Sciences* **1098**, 216–229.

Lamster, I. B., Holmes, L. G., Gross, K. B., Oshrain, R. L., Cohen, D. W., Rose, L. F., Peters, L. M. & Pope, M. R. (1994) The relationship of beta-glucuronidase activity in crevicular fluid to clinical parameters of periodontal disease. Findings from a multicenter study. *Journal of Clinical Periodontology* **21**, 118–127.

Lamster, I. B., Holmes, L. G., Williams Gross, K. B., Oshrain, R. L., Cohen, D. W., Rose, L. F., Peters, L. M. & Pope, M. R. (1995) The relationship of b-glucuronidase activity in crevicular fluid to probing attachment loss in patients with adult periodontitis. *Journal of Clinical Periodontology* **22**, 36–44.

Lamster, I. B., Kaufman, E., Grbic, J. T., Winston, L. J. & Singer, R. E. (2003) Beta-glucuronidase activity in saliva: relationship to clinical periodontal parameters. *Journal of Periodontology* **74**, 353–359.

Lang, N. P., Adler, R., Joss, A. & Nyman, S. (1990) Absence of bleeding on probing. An indicator of periodontal stability. *Journal of Clinical Periodontology* **17**, 714–721.

Lang, N. P., Joss, A., Orsanic, T., Gusberti, F. A. & Siegrist, B. E. (1986) Bleeding on probing. A predictor for the progression of periodontal disease? *Journal of Clinical Periodontology* **13**, 590–596.

Leibbrandt, A. & Penninger, J. M. (2008) RANK/RANKL: regulators of immune responses and bone physiology. *Annals of the New York Academy of Sciences* **1143**, 123–150.

Lerner, U. H. (2006) Inflammation-induced bone remodelling in periodontal disease and the influence of post-menopausal osteoporosis. *Journal of Dental Research* **85**, 596–607.

Li, X., Pilbeam, C. C., Pan, L., Breyer, R. M. & Raisz, L. G. (2002) Effects of prostaglandin E2 on gene expression in primary osteoblastic cells from prostaglandin E2 knockout mice. *Bone* **30**, 567–573.

Lian, J. B. & Gundberg, C. M. (1988) Osteocalcin: biochemical considerations and clinical applications. *Clinical Orthopedia Related Research* **226**, 267–291.

Lin, S. J., Chen, Y. L., Kuo, M. Y., Li, C. L. & Lu, H. K. (2005) Measurement of gp130 cytokines ancostatin M and IL-6 in gingival crevicular fluid of patients with chronic periodontitis. *Cytokine* **21**, 160–167.

Liu, J., Wu, Y., Ding, Y., Meng, S., Ge, S. & Deng, H. (2010) Evaluation of serum levels of C-reactive protein and lipid profiles in patients with chronic periodontitis and/or coronary heart disease in an ethnic Han population. *Quintessence International* **41**, 239–247.

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* **21**, 533–551.

Loos, B. G., Craandijk, J., Hoek, F. J., Wertheim-van Dillen, P. M. & van der Velden, U. (2000) Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of Periodontology* **71**, 1528–1534.

Loos, B. G. & Tjoa, S. (2005) Host-derived diagnostic markers for periodontitis: do they exist in gingival crevice fluid? *Periodontology 2000* **39**, 53–72.

Lu, H. K., Chen, Y. L., Chang, H. C., Li, C. L. & Kuo, M. Y. (2006) Identification of the osteoprotegerin/receptor activator of nuclear factor-kappa B ligand system in gingival crevicular fluid and tissue of patients with chronic periodontitis. *Journal of Periodontal Research* **41**, 354–360.

Mäntylä, P., Stenman, M., Kinane, D., Salo, T., Suomalainen, K., Tikanoja, S. & Sorsa, T. (2006) Monitoring periodontal disease status in smokers and nonsmokers using a gingival crevicular fluid matrix metalloproteinase-8-specific chair-side test. *Journal of Periodontal Research* **41**, 503–512.

Mäntylä, P., Stenman, M., Kinane, D. F., Tikanoja, S., Luoto, H., Salo, T. & Sorsa, T. (2003) Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *Journal of Periodontal Research* **38**, 436–439.

Marcaccini, A. M., Meschiari, C. A., Sorgi, C. A., Saraiva, M. C., de Souza, A. M., Faccioli, L. H., Tanus-Santos, J. E., Novaes, A. B. & Gerlach, R. F. (2009) Circulating interleukin-6 and high sensitivity C-reactive protein decrease after periodontal therapy in otherwise healthy subjects. *Journal of Periodontology* **80**, 594–602.

Mattila, K., Vesanen, M., Valtonen, V., Nieminen, M., Palosuo, T., Rasi, V. & Asikainen, S. (2002) Effect of treating periodontitis on C-reactive protein levels: a pilot study. *BMC Infectious Diseases* **2**, 30.

McCauley, L. K. & Nohutçu, R. M. (2002) Mediators of periodontal osseous destruction and remodeling: principles and implications for diagnosis and therapy. *Journal of Periodontology* **73**, 1377–1391.

Melander, O., Newton-Cheh, C., Almgren, P., Hedblad, B., Berglund, G., Engström, G., Persson, M., Smith, J. G., Magnusson, M., Christensson, A., Struck, J., Morgenthaler, N. G., Bergmann, A., Pencina, M. J. & Wang, T. J. (2009) Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. *Journal of American Medical Association* **302**, 49–57.

Meyle, J., Zell, S., Brecx, M. & Heller, W. (1992) Influence of oral hygiene on elastase concentration of gingival crevicular fluid. *Journal of Periodontal Research* **27**, 226–231.

Miller, C. S., King, C. P., Langub, M. C., Kryscio, R. J. & Thomas, M. V. (2006) Salivary biomarkers of existing periodontal disease. A cross-sectional study. *Journal of American Dental Association* **137**, 322–329.

Mogi, M., Otogoto, J., Ota, N. & Togari, A. (2004) Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *Journal of Dental Research* **83**, 166–169.

Mogi, M. & Otogoto, J. (2007) Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. *Archives of Oral Biology* **52**, 894–898.

Mooney, J. & Kinane, D. F. (1994) Humoral immune responses to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in adult periodontitis and rapidly progressive periodontitis. *Oral Microbiology and Immunology* **9**, 321–326.

Motyckova, G., Weilbaecher, K. N., horstmann, M., Rieman, D. J., Fisher, D. Z. & Fisher, D. E. (2001) Linking osteopetrosis and pycnodysostosis: regulation of cathepsin-K expression by the microphthalmia transcription factor family. *Proceedings of National Academy of Science USA* **98**, 5798–5803.

Nakajima, T., Honda, T., Domon, H., Okui, T., Kajita, K., Ito, H., Takahashi, N., Mackawa, T., Tabeta, K. & Yamazaki, K. (2010) Periodontitis-associated up-regulation of systemic inflammatory mediator level may increase the risk of coronary heart disease. *Journal of Periodontal Research* **45**, 116–122.

Nakamura-Minami, M., Furuichi, Y., Ishikawa, K., Mitsuzono-Tofuku, Y. & Izumi, Y. (2003) Changes of alpha1-protease inhibitor and secretory leukocyte protease inhibitor levels in gingival crevicular fluid before and after non-surgical periodontal treatment. *Oral Diseases* **9**, 249–254.

Nakashima, T., Kobayashi, Y., Yamasaki, S., Kawakami, A., Eguchi, K., Sasaki, H. & Sakai, H. (2000) Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. *Biochemical and Biophysical Research Communications* **275**, 768–775.

Narayanan, A. S. & Page, R. C. (1983) Connective tissues of the periodontium: a summary of current work. *Collagen Related Research* **3**, 33–64.

Nibali, L., D'Aiuto, F., Griffiths, G., Patel, K., Suvan, J. & Tonetti, M. S. (2007) Severe periodontitis is associated with systemic inflammation and a dysmetabolic status: a case-control study. *Journal of Clinical Periodontology* **34**, 931–937.

Nicholls, S. J. & Hazen, S. L. (2005) Myeloperoxidase and cardiovascular disease. *Arteriosclerosis and Thrombosis and Vascularisation* **25**, 1102–1111.

Nicu, E. A., Laine, M. L., Morre, S. A., Van der Velden, U. & Loos, B. G. (2009) Soluble CD14 in periodontitis. *Innate Immunity* **15**, 121–128.

Nomura, Y., Tamaki, Y., Tanaka, T., Arakawa, H., Tsurumoto, A., Kirimura, K., Sato, T., Hanada, N. & Kamoi, K. (2006) Screening of periodontitis with salivary enzyme tests. *Journal of Oral Sciences* **48**, 177–183.

Offenbacher, S., Beck, J. D., Moss, K., Mendoza, L., Paquette, D. W., Barrow, D. A., Couper, D. J., Stewart, D. D., Falkner, K. L., Graham, S. P., Grossi, S., Gunsolley, J. C., Madden, T., Maupome, G., Trevisan, M., Van Dyke, T. E. & Genco, R. J. (2009) Results from the Periodontitis and Vascular Events (PAVE) Study: a pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. *Journal of Periodontology* **80**, 190–201.

Offenbacher, S., Heasman, P. A. & Collins, J. G. (1993) Modulation of host PGE2 secretion as a determinant of periodontal disease expression. *Journal of Periodontology* **64**, 432–444.

Offenbacher, S., Odle, B. M. & Van Dyke, T. E. (1984) The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* **21**, 101–112.

Offenbacher, S., Odle, B. M. & Van Dyke, T. E. (1986) The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* **21**, 101–112.

Ono, K., Kaneko, H., Choudhary, S., Pilbeam, C. C., Lorenzo, J. A., Akatsu, T., Kugai, N. & Raisz, L. G. (2005) Biphasic effect of prostaglandin E2 on osteoclast formation in spleen cell cultures: role of the EP2 receptor. *Journal of Bone and Mineral Research* **20**, 23–29.

Oringer, R. J., Al-Shammari, K. F., Aldredge, W. A., Iacono, V. J., Eber, R. M., Wang, H. L., Berwald, B., Nejat, R. & Giannobile, W. V. (2002) Effect of locally delivered minocycline microspheres on markers of bone resorption. *Journal of Periodontology* **73**, 835–842.

Oringer, R. J., Palys, M. D., Iranmanesh, A., Fiorellini, J. P., Haffajee, A. D., Socransky, S. S. & Giannobile, W. V. (1998) C-telopeptide pyridinoline cross-links (ICTP) and periodontal pathogens associated with endosseous oral implants. *Clinical Oral Implants Research* **9**, 365–373.

Özçaka, Ö., Nalbantsoy, A., Bıçakçı, N., Köse, T. & Buduneli, N (2010) Plasma levels of C-telopeptide pyridinoline cross-links of type I collagen and osteocalcin in chronic periodontitis. *Inflammation*, doi: 10.1007/s10753-010-9225-0.

Özmeriç, N. (2004) Advances in periodontal disease markers. *Clinica Chimica Acta* **343**, 1–16.

Özmeric, N., Elgün, S. & Uraz, A. (2000) Salivary arginase in patients with adult periodontitis. *Clinical Oral Investigations* **4**, 21–24.

Palys, M. D., Haffajee, A. D., Socransky, S. S. & Giannobile, W. V. (1998) Relationship between C-telopeptide pyridinoline cross-links (ICTP) and putative periodontal pathogens in periodontitis. *Journal of Clinical Periodontology* **25**, 865–871.

Passoja, A., Ylipalosaari, M., Tervonen, T., Raunio, T. & Knuuttila, M. (2008) Matrix metalloproteinase-8 concentration in shallow crevices associated with the extent of periodontal disease. *Journal of Clinical Periodontology* **35**, 1027–1031.

Perinetti, G., Paolantonio, M., Femminella, B., Serra, E. & Spoto, G. (2008) Gingival crevicular fluid alkaline phosphatise activity reflects periodontal healing/recurrent inflammation phases in chronic periodontitis patients. *Journal of Periodontology* **79**, 1200–1207.

Pozo, P., Valenzuela, M. A., Melej, C., Zaldívar, M., Puente, J., Martínez, B. & Gamonal, J. (2005) Longitudinal analysis of metalloproteinases, tissue inhibitors of metalloproteinases and clinical parameters in gingival crevicular fluid from periodontitis-affected patients. *Journal of Periodontal Research* **40**, 199–207.

Pradeep, A. R., Daisy, H. & Hadge, P. (2009a) Serum levels of monocyte chemoattractant protein-1 in periodontal health and disease. *Cytokine* **47**, 77–81.

Pradeep, A. R., Daisy, H., Hadge, P., Garg, G. & Thorat, M. (2009b) Correlation of gingival crevicular fluid interleukin-18 and monocyte chemoattractant protein-1 levels in periodontal health and disease. *Journal of Periodontology* **80**, 1454–1461.

Pradeep, A. R., Manojkumar, S. T., Garima, G. & Raju, A. (2010) Serum levels of oncostatin M (a gp 130 cytokine): an inflammatory biomarker in periodontal disease. *Biomarkers* **15**, 277–282.

Preiss, D.S & Meyle, J. (1994) Interleukin-1 beta concentration of gingival crevicular fluid. *Journal of Periodontology* **65**, 423–428.

Pussinen, P. J., Jauhiainen, M., Vilkuna-Rautiainen, T., Sundvall, J., Vesanen, M., Mattila, K., Palosuo, T., Alfthan, G. & Asikainen, S. (2004) Periodontitis decreases the antiatherogenic potency of high density lipoprotein. *Journal of Lipid Research* **45**, 139–147.

Pussinen, P. J., Paju, S., Mantyla, P. & Sorsa, T. (2007) Serum microbial- and host-derived markers of periodontal diseases: a review. *Current Medical Chemistry* **14**, 2407–2412.

Rai, B., Kharb, S., Jain, R. & Anand, S. C. (2008) Biomarkers of periodontitis in oral fluids. *Journal of Oral Science* **50**, 53–56.

Ramseier, C. A., Kinney, J. S., Herr, A. E., Braun, T., Sugai, J. V., Shelburne, C. A., Rayburn, L. A., Tran, H. M., Singh, A. K. & Giannobile, W. V. (2009) Identification of pathogen and host-response markers correlated with periodontal disease. *Journal of Periodontology* **80**, 436–446.

Raunio, T., Knuuttila, M., Karttunen, R., Vainio, O. & Tervonen, T. (2009) Serum sCD14, polymorphism of CD14(-260) and periodontal infection. *Oral Diseases* **15**, 484–489.

Raunio, T., Nixdorf, M., Knuuttila, M., Karttunen, R., Vainio, O. & Tervonen, T. (2007) The extent of periodontal disease and the IL-6 − 174 genotype as determinants of serum IL-6 level. *Journal of Clinical Periodontology* **34**, 1025–1030.

Raymond, L., Eck, S., Mollmark, J., Hays, E., Tomek, I., Kantor, S., Elliott, S. & Vincenti, M. (2006) Interleukin-1 beta induction of matrix metalloproteinase-1 transcription in chondrocytes requires ERK-dependent activation of CCAAT enhancer-binding protein-beta. *Journal of Cell Physiology* **35**, 335–340.

Renvert, S., Lindahl, C., Roos-Jansaker, A. M. & Lessem, J. (2009) Short-term effects of an anti-inflammatory treatment on clinical parameters and serum levels of C-reactive protein and proinflammatory cytokines in subjects with periodontitis. *Journal of Periodontology* **80**, 892–900.

Rescala, W., Teles, R. P., Fischer, R. G., Haffajee, A., Socransky, S., Gustafsson, A. & Figueredo, C. (2010) Immunological and microbiological profiles of chronic and aggressive periodontitis subjects. *Journal of Periodontology* **81**, 1308–1316.

Reynolds, J.J & Meikle, M. C. (1997) Mechanisms of connective tissue destruction in periodontitis. *Periodontology 2000* **14**, 144–157.

Rodan, G. A. (1995) Osteopontin overview. *Annals of New York Academy of Sciences* **760**, 1–5.

Rosin, M., Hanschke, M., Splieth, C. & Kramer, A. (1999) Activities of lysosyme and salivary peroxidise in unstimulated whole saliva in relation to plaque and gingivitis scores in healthy young males. *Clinical Oral Investigations* **3**, 133–137.

Şahingür, S. E. & Cohen, R. E. (2004) Analysis of host responses and risk for disease progression. *Periodontology 2000* **34**, 57–83.

Sakellari, D., Menti, S. & Konstantinidis, A. (2008) Free soluble receptor activator of nuclear factor-kappaB ligand in gingival crevicular fluid correlates with distinct pathogens in periodontitis patients. *Journal of Clinical Periodontology* **35**, 938–943.

Savage, A., Eaton, K. A., Moles, D. R. & Needleman, I. (2009) A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *Journal of Clinical Periodontology* **36**, 458–467.

Saxer, U. P. & Mühlemann, H. R. (1975) Motivation and education (in German). *Schweiz Mschr Zahnkeilk* **85**, 905–919.

Seibel, M. J. (2003) Biochemical markers of bone metabolism in the assessment of osteoporosis: useful or not? *Journal of Endocrinology Investment* **26**, 464–471.

Sharma, C. G. & Pradeep, A. R. (2006) Gingival crevicular fluid osteopontin levels in periodontal health and disease. *Journal of Periodontology* **77**, 1674–1680.

Sharma, C. G. & Pradeep, A. R. (2007) Plasma and crevicular fluid osteopontin levels in periodontal health and disease. *Journal of Periodontal Research* **42**, 450–455.

Shi, F., Yu, S. & Xu, L. (1996) Analysis of serum osteocalcin of patients with periodontitis. *Zhonghua Kou Qiang Yi Xue Za Zhi* **31**, 300–302 (in Chinese).

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **24**, 747–759.

Silva, N., Dutzan, N., Hernandez, M., Dezerega, A., Rivera, O., Aguillon, J. C., Aravena, O., Lastres, P., Pozo, P., Vernal, R. & Gamonal, J. (2008) Characterization of progressive periodontal lesions in chronic periodontitis patients: levels of chemokines, cytokines, matrix metalloproteinase-13, periodontal pathogens and inflammatory cells. *Journal of Clinical Periodontology* **35**, 206–214.

Sorsa, T., Tjaderhane, L., Konttinen, Y. T., Lauhio, A., Salo, T., Lee, H.-M., Golub, L. M., Brown, D. L. & Mantyla, P. (2006) Matrix metalloproteinases: Contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Annals of Medicine* **38**, 306–321.

Sorsa, T., Tjaderhane, L. & Salo, T. (2004) Matrix metalloproteinases (MMPs) in oral diseases. *Oral Diseases* **10**, 311–318.

Srinath, R., Acharya, A. B. & Thakur, S. L. (2010) Salivary and gingival crevicular fluid melatonin in

periodontal health and disease. *Journal of Periodontology* **81**, 277–283.

Su, H., Gornitsky, M., Velly, A. M., Yu, H., Benarroch, M. & Schipper, H. M. (2009) Salivary DNA, lipid, and protein oxidation in non-smokers with periodontal disease. *Free Radicals in Biology and Medicine* **46**, 914–921.

Suda, T., Takahashi, N., Udagawa, N., Jimi, E., Gillespie, M. T. & Martin, T. J. (1999) Modulation of osteoclast differentaition and function by the new members of the tumour necrosis factor receptor and ligand families. *Endocrine Reviews* **20**, 345–357.

Surna, A., Kubilius, R., Sakalauskiene, J., Vitkauskiene, A., Jonatis, J., Saferis, V. & Gleiznys, A. (2009) Lysosyme and microbiota in relation to gingivitis and periodontitis. *Medical Science Monitor* **15**, CR66–CR73.

Swoboda, J. R., Kıyak, H. A., Darveau, R. & Persson, G. R. (2008) Correlates of periodontal decline and biologic markers in older adults. *Journal of Periodontology* **79**, 1920–1926.

Taba, M. Jr, Kinney, J., Kim, A. S. & Giannobile, W. V. (2005) Diagnostic biomarkers for oral and periodontal diseases. *Dental Clinics of North America* **49**, 551–571.

Takane, M., Sugano, N., Iwasaki, H., Iwano, Y., Shimuzu, N. & Ito, K. (2002) New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. *Journal of Periodontology* **73**, 551–554.

Tang, T. H., Fitzsimmons, T. R. & Bartold, M. (2009) Effect of smoking on concentrations of receptor activator of nuclear factor êB ligand and osteoprotegerin in human gingival crevicular fluid. *Journal of Clinical Periodontology* **36**, 713–718.

Taubman, M. A., Valverde, P., Han, X. & Kawai, T. (2005) Immune response: The key to bone resorption in periodontal disease. *Journal of Periodontology* **76** (Suppl.), 2033–2041.

Teles, R. P., Gursky, L. C., Faveri, M., Rosa, E. A., Teles, F. R., Feres, M., Socransky, S. S. & Haffajee, A. D. (2010) Relationships between subgingival microbiota and GCF biomarkers in generalized aggressive periodontitis. *Journal of Clinical Periodontology* **37**, 313–323.

Teles, R. P., Likhari, V., Socransky, S. S. & Haffajee, A. D. (2009) Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. *Journal of Periodontal Research* **44**, 411–417.

Thorat Manojkumar, S., Pradeep, A. R., Garg, G. & Raju, A. (2010) Gingival crevicular fluid levels of oncostatin M in periodontal conditions. *Cytokine* **50**, 248–252.

Toker, H., Poyraz, O. & Eren, K. (2008) Effect of periodontal treatment on IL-1beta, IL-1ra, and IL-10 levels in gingival crevicular fluid in patients with aggressive periodontitis. *Journal of Clinical Periodontology* **35**, 507–513.

Tonetti, M. & Claffey, N.on behalf of the European Workshop in Periodontology group C. (2005) Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. *Journal of Clinical Periodontology* **32** (Suppl. 6), 210–213.

Totan, A., Greabu, M., Totan, C. & Spinu, T. (2006) Salivary aspartate aminotransferase, alanine aminotransferase and alkaline phosphatise: possible markers in periodontal diseases? *Clinical, Chemical and Laboratory Medicine* **44**, 612–615.

Trindade, S. C., Gomes-Filho, I. S., Meyer, R. J., Vale, V. C., Pugliese, L. & Freire, S. M. (2008) Serum antibody levels against Porhyromonas gingivalis extract and its chromatographic fraction in chronic and aggressive periodontitis. *Journal of International Academy of Periodontology* **10**, 50–58.

Tsalikis, L., Malaka, E., Pavlitou, E. & Konstantinidis, A. (2001) Aspartate aminotransferase levels in gingival crevicular fluid before and after initial periodontal treatment. *Journal of International Academy of Periodontology* **3**, 68–74.

Türkoğlu, O., Emingil, G., Kütükçüler, N. & Atilla, G. (2009) Gingival crevicular fluid levels of cathelicidin LL-37 and interleukin-18 in patients with chronic periodontitis. *Journal of Periodontology* **80**, 969–976.

Tüter, G., Kurtiş, B. & Serdar, M. (2007) Evaluation of gingival crevicular fluid and serum levels of high-sensitivity C-reactive protein in chronic periodontitis patients with or without coronary artery disease. *Journal of Periodontology* **78**, 2319–2324.

Quinn, J. M. W., Horwood, N. J., Elliott, J., Gillespie, M. T. & Martin, T. J. (2000) Fibroblastic stromal cells express receptor activator of NF-kappa B ligand and support osteoclast differentiation. *Journal of Bone Mineralisation Research* **15**, 1459–1466.

Van der Velden, U. (2005) Purpose and problems of periodontal disease classification. *Periodontology 2000* **39**, 13–21.

Van der Velden, U., Abbas, F., Armand, S. & Loos, B. G. (2006) Java project on periodontal diseases. The natural development of periodontitis: risk factors, risk predictors and risk determinants. *Journal of Clinical Periodontology* **33**, 540–548.

Vardar, S., Baylas, H. & Huseyinov, A. (2003) Effects of selective cyclooxygenase-2 inhibition on gingival tissue levels of prostaglandin $F_{2\alpha}$ and clinical parameters of chronic periodontitis. *Journal of Periodontology* **74**, 57–63.

Vardar-Şengül, S., Buduneli, E., Türkoğlu, O., Buduneli, N., Atilla, G., Wahlgren, J., Sorsa, T. & Baylas, H. (2008) The effects of selective COX-2 inhibitor/celecoxib and omega-3 fatty acid on matrix metalloproteinases, TIMP-1, and laminin-5gamma2-chain immunolocalization in experimental periodontitis. *Journal of Periodontology* **79**, 1934–1941.

Villela, B., Cogen, R. B., Bartolucci, A.A & Birkedal-Hansen, H. (1987) Crevicular fluid collagenase activity in healthy, gingivitis, chronic adult periodontitis and localised juvenile periodontitis patients. *Journal of Periodontal Research* **22**, 209–211.

Vuletic, S., Taylor, B. A., Tofler, G. H., Chait, A., Marcovina, S. M., Schenk, K. & Albers, J. J. (2009) SAA and PLTP activity in plasma of periodontal patients before and after full-mouth tooth extraction. *Oral Diseases* **14**, 514–519.

Wara-aswapati, N., Surarit, R., Chayasadom, A., Boch, J. A. & Pitiphat, W. (2007) RANKL upregulation associated with periodontitis and *Porphyromonas gingivalis*. *Journal of Periodontology* **78**, 1062–1069.

Wei, P. F., Ho, K. Y., Ho, Y. P., Wu, Y. M., Yang, Y. H. & Tsai, C. C. (2004) The investigation of glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1beta in gingival crevicular fluid: implications for oxidative stress in human periodontal diseases. *Journal of Periodontal Research* **39**, 287–293.

Wilczynska-Borawska, M., Borawski, J., Kovalchuk, O., Chyczewski, L. & Stokowska, W. (2006) Hepatocyte growth factor in saliva is a potential marker of symptomatic periodontal disease. *Journal of Oral Sciences* **48**, 47–50.

Xiang, X., Sowa, M. G., Iacopino, A. M., Maev, R. G., Hewko, M. D., Man, A. & Liu, K.-Z. (2010) An update on novel non-invasive approaches for periodontal diagnosis. *Journal of Periodontology* **81**, 186–198.

Xu, L., Yu, Z., Lee, H. M., Wolff, M. S., Golub, L. M., Sorsa, T. & Kuula, H. (2008) Characteristics of collagenase-2 from gingival crevicular fluid and peri-implant sulcular fluid in periodontitis and peri-impalntitis patients: pilot study. *Acta Odontologica Scandinavica* **66**, 219–224.

Yamalık, N., Çağlayan, F., Kılınç, K., Kılınç, A. & Tümer, C. (2000) The importance of data presentation regarding gingival crevicular fluid myeloperoxidase and elastase like activity in periodontal disease and health status. *Journal of Periodontology* **71**, 460–467.

Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A., Tsuda, E., Morinaga, T., Higashio, K., Udagawa, N., Takahashi, N. & Suda, T. (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclast inhibitory factor and is identical to TRANCE/RANKL. *Proceedings of National Academy of Science USA* **95**, 3597–3602.

Yetkin Ay, Z., Sütçü, R., Uskun, E., Bozkurt, F. Y. & Berker, E. (2009) The impact of the IL-11: IL-17 ratio on the chronic periodontitis pathogenesis: a preliminary report. *Oral Diseases* **15**, 93–99.

Yin, X., Bunn, C. L. & Bartold, P. M. (2000) detection of tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 2 (PAI-2) in gingival crevicular fluid from healthy, gingivitis and periodontitis patients. *Journal of Clinical Periodontology* **27**, 149–156.

Yoshie, H., tai, H., Kobayashi, T., Oda-Gou, E., Nomura, Y., Numabe, Y., Ito, K., Kurihara, H. & Kamoi, K. (2007) Salivary enzyme levels after scaling and interleukin-1 genotypes in Japanese patients with chronic periodontitis. *Journal of Periodontology* **78**, 498–503.

Yoshihara, A., Deguchi, T., Hanada, N. & Miyazaki, H. (2009) Relation of bone turnover markers to periodontal disease and jaw bone morphology in elderly Japanese subjects. *Oral Diseases* **15**, 176–181.

Yoshinari, N., Kawase, H., Mitani, A., Ito, M., Sugiishi, S., Matsuoka, M., Shirozu, N., Ishihara, Y., Bito, B., Hiraga, M., Arakawa, K. & Noguchi, T. (2004) Effects of scaling and root planning on the amounts of interleukin-1 and interleukin-1 receptor antagonist and the mRNA expression of interleukin-1beta in gingival crevicular fluid and gingival tissues. *Journal of Periodontal Research* **39**, 158–167.

Yücel-Lindberg, T., Ahola, H., Carlstedt-Duke, J. & Modeer, T. (1999) Signal transduction pathways involved in the synergistic stimulation of prostaglandin production by interleukin-1β and tumour necrosis factor α in human gingival fibroblasts. *Journal of Dental Research* **78**, 61–68.

Zheng, P., Chen, H., Shi, S., Jepsen, S. & Eberhard, J. (2006) Periodontal parameters and platelet-activating factor levels in serum and gingival crevicular fluid in a Chinese population. *Journal of Clinical Periodontology* **33**, 797–802.

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Clinical Relevance

Scientific rationale for the study: Objective and ideal diagnostic methods for periodontal diagnosis of the present as well as future tissue destruction are still being sought.
Principal findings: Clinical periodontal measurements, mainly PD and BOP, continue to be the most reliable parameters not only for the diagnosis but also for determining the prognosis of periodontal diseases. A range of host bone destruction markers and inflammation related molecules in GCF, saliva and blood are not reliable to predict future tissue destruction or disease activity.
Practical implications: It is quite clear that highly specific, sensitive biomarkers for monitoring of periodontal diseases are still needed for better detection of periodontal tissue destruction.