J Clin Periodontol 2014; 41: 113–120 doi: 10.1111/jcpe.12194

Journal of Clinical Periodontology

Crevicular fluid biomarkers and periodontal disease progression

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Kinney JS, Morelli T, Oh M, Braun TM, Ramseier CA, Sugai JV, Giannobile WV. Crevicular fluid biomarkers and periodontal disease progression. J Clin Periodontol 2014; 41: 113–120. doi: 10.1111/jcpe.12194.

Abstract

Aim: Assess the ability of a panel of gingival crevicular fluid (GCF) biomarkers as predictors of periodontal disease progression (PDP).

Materials and methods: In this study, 100 individuals participated in a 12-month longitudinal investigation and were categorized into four groups according to their periodontal status. GCF, clinical parameters and saliva were collected bi-monthly. Subgingival plaque and serum were collected bi-annually. For 6 months, no periodontal treatment was provided. At 6 months, patients received periodontal therapy and continued participation from 6 to 12 months. GCF samples were analysed by ELISA for MMP-8, MMP-9, Osteoprotegerin, C-reactive Protein and IL-1β. Differences in median levels of GCF biomarkers were compared between stable and progressing participants using Wilcoxon Rank Sum test ($p = 0.05$). Clustering algorithm was used to evaluate the ability of oral biomarkers to classify patients as either stable or progressing.

Results: Eighty-three individuals completed the 6-month monitoring phase. With the exception of GCF C-reactive protein, all biomarkers were significantly higher in the PDP group compared to stable patients. Clustering analysis showed highest sensitivity levels when biofilm pathogens and GCF biomarkers were combined with clinical measures, 74% (95% CI = 61, 86).

Conclusions: Signature of GCF fluid-derived biomarkers combined with pathogens and clinical measures provides a sensitive measure for discrimination of PDP (ClinicalTrials.gov NCT00277745).

Key words: biomarkers; disease progression; gingival crevicular fluid; longitudinal; periodontitis

Accepted for publication 2 November 2013

Despite advances in research methodology and laboratory assays in order to identify factors associated with chronic periodontal disease, it is still unclear how to potentially predict periodontal disease progression (PDP). Periodontitis has been clinically characterized as episodes of acute exacerbations of destruction followed by periods of quiescence and stability (Goodson et al. 1982, 1984, Socransky et al. 1984). The elusive nature of the disease is further complicated by the fact that different teeth within the same patient, as well as different sites around the same tooth can display varying degrees of disease severity, all undergoing PDP.

Clinical measures of periodontitis such as pocket depth (PD), clinical attachment level (CAL) or bleeding on probing (BOP) have limitations to provide the clinician with real-time evaluation of disease status. Furthermore, these clinical measures are poor predictors of future PDP (Lindhe et al. 1983). An ideal diagnostic tool would not only detect the presence and severity of the disease but also predict subsequent clinical course of the infection (McCulloch 1994).

Extensive research has been done in the area of the host response biochemical markers of periodontal disease. It is unlikely that a stand-alone biomarker will be able to fulfil the criteria of predicting future disease destruction. A cross-sectional study demonstrated that the combination of saliva-based biomarkers and periodontal biofilm pathogens suggest

Conflict of interest and source of funding statement

There are no conflicts of interest to declare. This study was supported by NIH (U01-DE014961), NCRR (ULRR0000042), and the Swiss Society of Periodontology. Drs. Braun and Giannobile hold intellectual property related to this work licensed to Micro-Systemic Health, LLC.

potential diagnostic value for identifying periodontal disease status (Ramseier et al. 2009). Later, a longitudinal investigation of the same patient population demonstrated the ability of saliva-derived biomarkers and periodontal pathogens to predict PDP (Kinney et al. 2011).

Gingival crevicular fluid (GCF) is a serum exudate found in the gingival sulcus (McCulloch 1994). As the fluid traverses from the microcirculation across inflamed periodontal tissues, it carries biological molecular markers gathered from the surrounding site. GCF is an attractive oral fluid due to its ease of collection and ability for the clinician to sample multiple sites within the oral cavity simultaneously. In a molecular epidemiologic study, Offenbacher et al. described new clinical categories represented by distinct biological phenotypes based on clinical, microbial, inflammatory and host-response measures for periodontal disease identification. Interestingly, the authors identified that individuals with deep pocket depths and more severe BOP had elevated levels of GCF Interleukin-1β (IL-1β) and Interleukin-6 (IL-6) (Offenbacher et al. 2007).

To date, limited research has been completed in the usage of GCF as a diagnostic measure of periodontal disease. The aim of this follow-up study was to test the utility of GCF biomarkers as a potential predictor of periodontal disease progression.

Material and methods

Patient population

One hundred periodontally healthy and diseased individuals were recruited at the Michigan Center for Oral Health Research clinic between 2005 and 2007 (University of Michigan, Ann Arbor, Michigan). The study was approved by the University of Michigan Health Sciences Institutional Review Board and registered with the NIH clinical registry (ClinicalTrials.gov NCT00277745). Clinical and demographic baseline characteristics of this cohort have been previously published and are described in Table S1 (Ramseier et al. 2009, Kinney et al. 2011). Individuals age 18 years and older were eligible for the study. All individuals possessed ≥20 teeth, had not received periodontal treatment or antibiotic therapy for medical or dental reasons for 3 months prior to the start of the investigation, and were not taking long-term medications affecting periodontal status. Study exclusionary factors included a history of metabolic bone diseases, autoimmune diseases, unstable diabetes or postmenopausal osteoporosis. Pregnant or lactating women were not allowed to participate in the study.

Clinical measures

All teeth except third molars were assessed for periodontal clinical measures by one of three calibrated examiners (CR, JK and TM). Clinical parameters, including PD, CAL and BOP, were measured at six sites per tooth. Other clinical assessments included dichotomous measures of plaque accumulation (PI) and gingival redness index, as previously described by Haffajee et al. (1983). Based on clinical assessments, patients were enrolled into a healthy/gingivitis or periodontitis group. Patients in the healthy and gingivitis group exhibited <3 mm of CAL, no PD >4 mm, and no radiographic alveolar bone loss. Patients in the periodontitis group exhibited a minimum of four sites with evidence of radiographic bone loss, at least four sites with PD > 4 mm, and a minimum of four sites with CAL > 3 mm. Both groups were further classified into subgroups based on additional clinical criteria. Patients with BOP ≤ 20% were categorized as Healthy (H) (n = 18), and those with BOP > 20% were categorized as Gingivitis (G) (n = 32). Within the periodontitis group, those patients with ≤30% of sites with CAL > 3 mm were classified as having Mild Chronic Periodontitis (MP) (n = 28), and those with >30% of sites with CAL > 3 mm were categorized as having Moderate-Severe Periodontitis (SP) (n = 22) (Armitage 1999, Tonetti & Claffey 2005). Standardized periapical digital radiographs (Schick Technologies, Long Island City, NY, USA) were taken in the posterior dentition of all participants by a parallel technique for the determination of alveolar bone height at baseline, 6 and 12 months. Radiographic bone loss was analysed by one calibrated examiner equipped with a computer software measurement tool (Emago®, Oral Diagnostic Systems, Amsterdam, the Netherlands).

Figure 1 illustrates the timeline of the study. There were two phases of the investigation, disease-monitoring phase (baseline to 6 months) and disease-recovery phase (6–12 months). Patients were seen bi-monthly during the study. In order to assess for PDP, no periodontal therapy was provided during the disease-monitoring phase. At 6 months, all patients received periodontal treatment. Those in the healthy and gingivitis groups received prophylaxis and oral hygiene instruction (OHI) and those in the periodontitis groups underwent scaling and root planing (Sc/RP) and OHI. Patients in the healthy and gingivitis groups received a second prophylaxis at 12 months, while those patients in the periodontitis groups received periodontal maintenance at each of the remaining study visits. Tobacco cessation counselling was not provided to patients during the study. Any site undergoing clinical attachment loss of >2 mm from the baseline measurement was deemed as "progressing" and received rescue therapy consisting of localized Sc/RP and local antibiotic delivery (Arestin, OraPharma, Warminster, PA, USA), which has demonstrated to improve periodontal healing compared to Sc/RP alone (Williams et al. 2001).

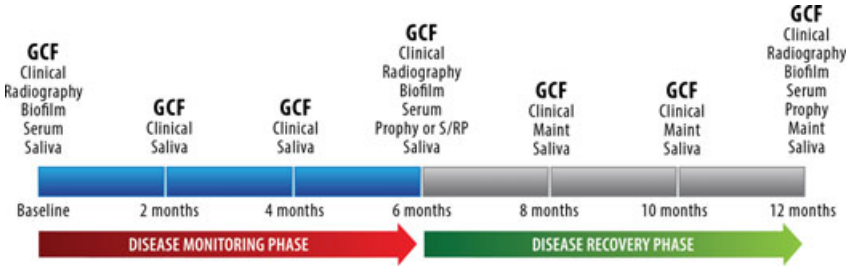

Fig. 1. Timeline of the study illustrating data collection and treatment delivery time points during disease monitoring and recovery phases.

Gingival crevicular fluid collection and analysis

GCF was taken from the mesiobuccal aspect of each site (tooth) for up to 28 teeth per patient. Prior to the collection, supragingival plaque was removed using a sterile instrument. The site was isolated using cotton rolls and dried using a short blast of air directly through the contact (not into the sulcus/pocket). A methylcellulose strip (Pro Flow, Inc., Amityville, NY, USA) was inserted into the sulcus/pocket until light resistance was felt. The strip stayed in position for 30 s. GCF volume was immediately determined using a calibrated Periotron 6000 (Harco Electronics, Tustin, CA, USA). Strips contaminated with blood or exceeding the Periotron maximum detection limit were discarded and the site was re-sampled after 90 s. GCF strips were stored in cryovials at −80°C until extraction.

Eight sites were selected from each participant using an algorithm based on the patient's baseline site measures of CAL, PD, BOP and group classification. Specifically, in patients without periodontitis, sites with PD less than 4 mm and/or CAL less than 3 mm were ranked higher, while in patients with periodontitis, sites with PD greater than 4 mm and/or CAL greater than 3 mm were ranked higher. In patients with gingivitis and periodontitis, sites were ranked even higher if they had BOP. The eight highest-ranked sites from each patient were then selected and their samples were pooled to create a subject-level GCF sample. Each strip was washed five times with 11 μl extraction solution containing a proteinase inhibitor combination of 1% aprotinin and 0.5% phenylmethylsulfonyl fluoride. The total solution volume per strip was 55 μl. Individual strips were centrifuged at 2000 rpm at 4° C for 5 min. then pooled to give a total solution volume per patient of 440 μl (8×55 μl).

The GCF samples were quantitively analysed for the concentration of C-reactive Protein (CRP), Osteoprotegerin (OPG), Collagenase-2 (MMP-8), Gelatinase B (MMP-9), and IL-1β using a Quantibody Human Cytokine Array (RayBiotech, Inc., Norcross, GA, USA).

Whole saliva, serum and microbial plaque biofilm

In order to elucidate the potential value of oral-fluid biomarkers for PDP, a clustering algorithm described in the Statistical Methods section was used. This analysis evaluated the results from GCF biomarkers from this study combined with the results of salivary, serum and pathogens biomarkers previously published (Kinney et al. 2011).

Unstimulated whole saliva was collected at the beginning of each study visit with passive drooling into sterile plastic tubes from all patients (Mandel & Wotman 1976). Samples were further placed on ice, aliquoted, and supplemented with a proteinase inhibitor combination of 1% aprotinin and 0.5% phenylmethylsulfonyl fluoride prior to storage at −80°C. Whole saliva samples were tested for the presence of MMP-8 and -9, Calprotectin, OPG, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, tumour necrosis factor-α (TNF), and interferon-γ and pyridinoline cross-links of type I collagen (ICTP).

Twenty millilitres of whole blood was collected at baseline, 6 months, and 12 months. Once collected, samples were allowed to clot at room temperature for 30 min. then centrifuged for 15 min. at 2600 rpm. Approximately, one millilitre of serum was aliquoted into individual cryovials and stored at −80°C until analysis. Serum samples were tested for the presence of CRP, IL-1β, IL-6, TNF-α, Calprotectin, OPG, ICTP, MMP- 8 and -9.

Subgingival plaque biofilm was collected from the mesiobuccal aspect of all teeth at baseline, 6 and 12 months according to Shelburne et al. 2008 (Shelburne et al. 2008). The detection of *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Treponema denticola* and *Campylobacter rectus* were quantitated by real-time quantitative PCR (qPCR) as described by Mullally et al. (Mullally et al. 2000).

Statistical methods

The level of analysis in this data is the individual patient and all biomarkers were log-transformed prior to analysis to promote normality. Mean longitudinal levels of each GCF biomarker for each of the four patient groups was computed at baseline and each post-baseline time point. Statistical significance of changes from baseline was assessed using the empirical ("robust") standard error from Generalized Estimating Equations to reflect the serial correlation inherent in longitudinal data. Given that there were 24 comparisons for each biomarker (six post-baseline measures for each of four patient groups), statistical significance was defined as a p-value less than 0.002 to maintain an overall false-positive rate of 0.05. Median baseline biomarker levels (non-log-transformed) were computed separately for periodontally stable and progressing patients, and differences between the two groups were analysed using a Wilcoxon Rank Sum test with statistical significance defined as a p-value less than 0.05. We used hierarchical clustering (HC) to divide patients into two groups based solely upon their GCF biomarker levels. HC considers GCF biomarker levels and divides them into two groups such that the two groups have the smallest variability among all possible divisions into two groups, that is, the GCF values within each cluster are closer together than GCF values from different clusters. HC can be specified to generate more than two clusters, but we chose two clusters for ease of interpretation, as these clusters were then compared to the actual clusters of stable and progressing patients. HC was then repeated separately for the plaque pathogen levels, salivary biomarker levels, serum biomarker levels and clinical measures reported (Kinney et al. 2011). An ideal clustering result from HC would be one in which all stable patients were in one cluster and all progressing patients were in the other cluster. Thus, the ability of the HC-generated clusters to discriminate between stable and progressing patients was summarized with sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). All calculations were done with the statistical package R (R Foundation for Statistical Computing, Vienna, Austria); HC was implemented with the library hclus.

Results

Recruitment/enrollment efforts for the study were previously published (Kinney et al. 2011). In total, 148 individuals were screened. Forty-eight failed the clinical screening, leaving 100 participants who were stratified into four groups according to their clinical criteria. One participant from the Moderate-Severe group withdrew at the baseline visit, leaving a total of ninety-nine participants. During disease progression analysis, 83 participants completed the study (15 H, 24 G, 24 MP and 20 SP), with 44 exhibiting PDP during the disease-monitoring period (2 H, 5 G, 21 MP and 16 SP), while 39 demonstrated stability (13 H, 19 G, 3 MP, 4 SP). Results of salivary and pathogen biomarkers associated with periodontal disease progression from this study were previously published (Kinney et al. 2011). In general, the orders of magnitude of GCF and saliva biomarker levels were comparable to other published reports for mediators such as MMP-8, MMP-9, IL-1 beta and OPG (Miller et al. 2006, Teles et al. 2009, 2010, Gursoy et al. 2010, Mirrielees et al. 2010, Salvi et al. 2010, Sexton et al. 2011).

Longitudinal plots

The longitudinal plots of mean (±SD) of GCF biomarker levels found among the four groups over 12 months are shown in Fig. 2. All four patient groups had significant reductions of GCF MMP-8 at month 2 compared to baseline ($p < 0.002$). Continued significant reduced levels were seen in the healthy and gingivitis groups at month 4 with only the gingivitis group having sustained significant lower levels of GCF MMP-8 levels at month 6 compared to baseline ($p < 0.002$). During disease recovery phase both mild and moderate/severe disease groups demonstrated significant lower levels of GCF MMP-8 ($p = 0.001$). Healthy and gingivitis groups showed significant lower GCF MMP-8 levels compared to baseline levels only at month 10. Regarding GCF MMP-9 levels, significantly increased expressions were seen in all patient groups at month 6 compared to baseline ($p = 0.001$). In addition, healthy and gingivitis groups had higher levels of GCF MMP-9 at months 8 and 12 compared to baseline ($p < 0.002$). Gingivitis group demonstrated significant decreases in GCF CRP levels at month 4, 8 and 10 compared to baseline ($p = 0.001$). Significant decreases in GCF CRP concentrations were also observed for both periodontitis groups at month 8 with only the moderate/severe periodontitis group showing significantly reduced levels at month 10 compared to baseline ($p = 0.002$). Regarding GCF OPG levels, healthy and gingivitis groups had significantly higher GCF OPG levels at month 6 compared to baseline ($p < 0.002$) with no further significant findings. Both mild and moderate/severe periodontitis groups showed significant decreased concentrations of GCF IL-1β at months 10 and 12, respectively, when compared to baseline ($p < 0.001$).

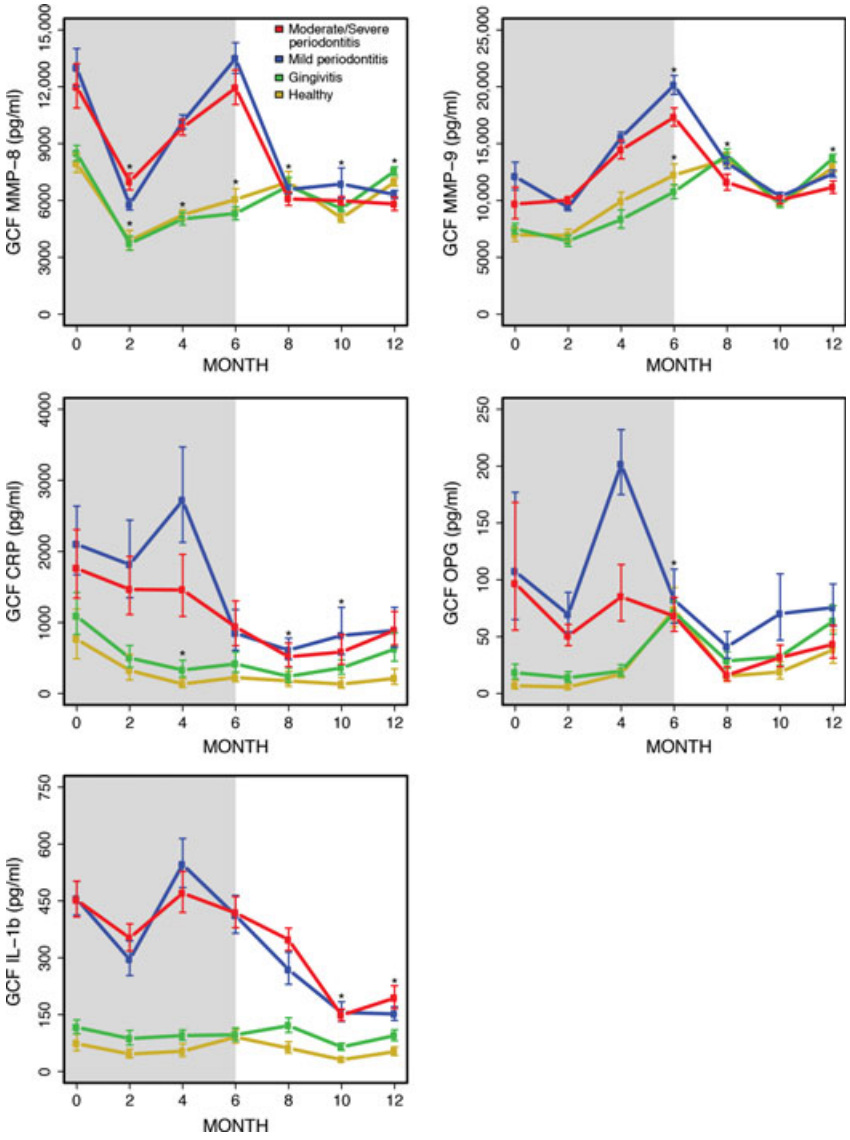

Fig. 2. Panels displaying mean longitudinal levels with standard error bars of each GCF biomarker for each patient group. Statistically significant changes from baseline to post-baseline time point of biomarker concentrations within each patient group are depicted by $*p < 0.002$.

Baseline results comparing individual GCF biomarkers

Median levels of each GCF biomarker for stable and progressing patients are shown in Table 1.

Biomarkers were ordered from least to largest *p*-value, giving a ranking of the relative significance of the biomarkers.

All of the biomarkers except CRP showed significant differences in the median levels of the stable and progressing patients. IL-1β had the most significant difference ($p < 0.001$), indicating statistically significant higher levels of GCF IL-1β in the patients who had disease progression compared to those participants who did not display disease progression. The second GCF biomarker that demonstrated significance between the stable and progressing patient group medians was OPG with a *p*-value of 0.003. A *p*-value of 0.006 was seen in the levels of GCF MMP-8 between stable patients and PDP patients. MMP-9 median levels between the stable and progressing patients also reached significance at a *p*-value of 0.03. CRP was not significantly different between the two groups. When considering the ranking order of these biomarkers, IL-1β was the strongest, followed by OPG, MMP-8 then MMP-9.

Sensitivity/specificity and PPV/NPV

A clustering algorithm, as described in the Statistical Methods section, was used to evaluate the ability of biomarkers in saliva, plaque, serum, GCF or clinical measures, alone or in groups. The algorithm correctly classified patients as either stable or progressing based upon the sensitivity, or proportion of correctly classified progressing patients, and specificity, or proportion of correctly classified stable patients (Table 2). In general, the greatest sensitivity tended to be with saliva biomarkers and greatest specificity with GCF biomarkers (Table 2). However, varying levels of sensitivity and specificity in lower ranges (<60%) were noted for many permutations of the different parameters evaluated. Plaque pathogens provided good sensitivity and specificity for prediction of PDP, 63% (95% CI = 49, 77) and 69% (95% CI = 55, 83) respectively. A high sensitivity was seen when using only salivary biomarkers, 93% (95% CI = 83, 98), but this individual cluster of biomarkers had very low specificity results, 26% (95% CI = 15, 42). Sensitivity levels increased to 72% (95% CI = 58, 84) when plaque pathogens were paired with GCF biomarkers. This pairing of clusters resulted in a slight lowering of specificity, 67% (95% CI = 52, 81). Further increases in sensitivity were reached when periodontal measures were clustered with plaque pathogens and GCF biomarkers, 74% (95% CI = 61, 86). When these three parameters are clustered, the specificity rises slightly to 68% (95% CI = 54, 82). When salivary biomarkers were clustered with periodontal measures, plaque pathogens and GCF, sensitivity levels reduced to 70% (95% CI= 56, 83), but specificity levels increased to the highest, 71% (95% C = 56, 84). Table S2 represents both positive and negative predict value (PPV/NPV) of biomarkers in saliva, plaque, serum, GCF or clinical measures, alone or in groups, associated with periodontal disease progression. Plaque pathogens paired with GCF biomarkers demonstrated a 70% (95% CI =57, 83) PPV and 68% (95% CI = 54, 82) NPV respectively. Further increases in PPV were reached when periodontal measures were clustered with plaque pathogens, salivary and GCF biomarkers, 73% (95% CI = 59, 86). The maximum NPV of 70% (95% CI = 55, 84) was reached when periodontal measures were clustered together with plaque pathogens and GCF biomarkers.

Table 1. Differences in individual GCF biomarkers at baseline between stable and progressing patients.

Biomarker	Stable patients	Progressing patients	*p*-value for difference
IL1-beta	118 (92–998)	482 (15–908)	<0.001
OPG	29 (0–2,640)	172 (0–1,649)	0.003
MMP-8	9,328 (4,695–26, 697)	10, 931 (4,610–23, 772)	0.006
MMP-9	8,378 (2,239–29, 409)	9,323 (3,464–25, 220)	0.03
CRP	1,929 (32–18, 862)	1,624 (8–19, 459)	0.94

Median (range, pg/ml).

Discussion

In this study we sought to examine the ability of a cluster of GCF biomarkers alone or in combination with saliva, plaque pathogens, and/or clinical measures to evaluate a patient's risk of active periodontal disease. Singular analysis of GCF biomarkers offer utility in prediction of PDP; however, greater predictability of PDP is noted when GCF biomarkers are combined with other clinical and biological measures.

A previous study using the same patient population found that anaerobic pathogens *P. gingivalis* (Pg) and *T. denticola* (Td) in combination with MMP-8 and OPG have the capacity to predict a patient's periodontal status (Ramseier et al. 2009). This cohort of patients was then longitudinally followed during a disease-monitoring, non-treatment phase. We then identified clusters of host-response salivary biomarkers and periodontal pathogens that appear to be indicators of periodontal break down (Kinney et al. 2011). In this investigation we now include in the model the findings of the GCF biomarkers for a more comprehensive outcome of using oral fluids and plaque pathogens for disease prediction. To our knowledge, this is the first time such a comprehensive analysis on disease progression has been presented.

Periodontal disease is a multifactorial infection; therefore, when seeking prognostic biomarkers to provide the highest levels of sensitivity and specificity for disease progression, one must look beyond an individual biomarker and consider combinations of valuable host-responses. When we ranked our findings in terms of levels of sensitivity and specificity and PPV/NPV, we found that using only periodontal pathogens as predictors of disease provided us with good sensitivity and specificity results. Our results found that Pg, Tf and Td in conjunction with *E.corrodens* (Er), *F. nucleatun* (Fn) and *P. intermedia* (Pi), equally contributed to well over 50% sensitivity and specificity values, 63% and 69% respectively. Several other investigators have reported the prognostic ability of Pg, *A. actinomycetemcomitans* (Aa), *T. forsythia* (Tf), Td (Haffajee et al.

Table 2. Changes in true positive and negative rates using individual versus clusters of oral fluid biomarkers, plaque pathogens and clinical measures derived at baseline.

Number of Items in Cluster	Salivary Biomarkers	Plaque Pathogens	Serum Biomarkers	Periodontal Measures	GCF Biomarkers	Sensitivity Estimate (%)	95% CI	Specificity Estimate (%)	95% CI
1	■					93	(83, 98)	26	(15, 42)
		■				63	(49, 77)	69	(55, 83)
			■			45	(32, 61)	61	(46, 76
				■		30	(18, 45)	97	(88, 100)
					■	23	(13, 38)	95	(85, 99)
2	■	■				58	(44, 73)	72	(57, 85)
	■		■			47	(33, 62)	45	(31, 62)
	■			■		35	(23, 51)	71	(56, 84)
	■				■	28	(17, 44)	69	(55, 83)
		■	■			47	(33, 62)	61	(46, 76)
		■		■		67	(53, 81)	68	(54, 82)
		■			■	72	(58, 84)	67	(52, 81)
			■	■		59	(45, 74)	59	(44, 75)
			■		■	57	(43, 72)	55	(40, 71)
				■	■	43	(30, 59)	95	(84, 99)
3	■	■	■			47	(33, 62)	45	(31, 62)
	■	■		■		63	(49, 77)	71	(56, 84)
	■	■			■	44	(31, 60)	74	(60, 87)
	■		■	■		58	(44, 73)	43	(29, 61)
	■		■		■	58	(44, 73)	39	(26, 57)
	■			■	■	47	(33, 62)	68	(54, 82)
		■	■	■		67	(53, 81)	68	(53, 82)
		■	■		■	81	(69, 91)	39	(26, 57)
		■		■	■	74	(61, 86)	68	(54, 82)
			■	■	■	66	(52, 79)	57	(42, 73)
4	■	■	■	■		63	(49, 77)	70	(55, 84)
	■	■	■		■	58	(44, 73)	39	(26, 57)
	■	■		■	■	70	(56, 83)	71	(56, 84)
	■		■	■	■	65	(51, 79)	41	(27, 58)
		■	■	■	■	81	(69, 91)	41	(27, 58)
5	■	■	■	■	■	70	(56, 83)	70	(55, 84)

GCF, gingival crevicular fluid; CI, confidence interval; ■, included in clustering algorithm.

1991, Machtei et al. 1997, 1999, Timmerman et al. 2000, Tran et al. 2001, Byrne et al. 2009). It should be noted, however, that other studies have not supported the same findings (Wennstrom et al. 1987, Macfarlane et al. 1988, Listgarten et al. 1991, Silva et al. 2008). Interestingly, GCF biomarkers alone provided us with low sensitivity and high specificity values, 23% and 95% respectively. Although GCF biomarkers, especially IL-1β, demonstrated a significant difference at baseline between progressing and stable patients, when analysed alone they did not demonstrate to be a strong predictor of periodontal disease progression.

Our results show improvements in sensitivity and specificity when periodontal pathogens are combined with GCF biomarkers. Silva et al. examined periodontopathic bacteria and GCF biomarkers and found higher levels of Pg, Aa, Tf and RANK-L, IL-1β, and MMP-13 in patients with active sites. In that study periodontal progression was determined using the tolerance method. Their results found that elevated levels of RANK-L, IL-1β and MMP-13 along with increases in Pg and Aa were indicative of periodontal lesions undergoing attachment loss (Silva et al. 2008).

It appears from our data that the highest level of specificity was reached when combining clusters of salivary and GCF biomarkers with pathogens and clinical measures. In a recent study by Nomura et al. (2012), chronic periodontitis patients were seen longitudinally for 18 months while disease progression was measured. Salivary samples were collected and evaluated for counts of Pg, Pi and Tf and intracellular enzyme levels of aspartate aminotransferase, alanine aminotransferase (ALT), lactate dehydrogenase, alkaline phosphatase and free haemoglobin biomarkers. In their study, periodontal disease progression was set as at least one site with >3 mm loss of attachment compared with baseline. Findings showed that Pg and Pi were both significant predictors of disease progression. However, higher levels of sensitivity and specificity were reached when Pg was combined with salivary ALT.

The use of GCF suggests potential diagnostic value to identify periodontal disease activity and response to therapy. Our results demonstrated that GCF biomarkers itself had good PPV 83% (95% CI = 59, 97) but low NPV 52% (95% CI = 41, 64). The highest PPV and NPV results were reached when GCF biomarkers were combined with plaque pathogens and clinical measures 73% (95% CI = 59, 85) and 70% (95% CI = 55, 84), respectively. The addition of a fourth component, salivary biomarkers, made only slight changes to the PPV 73% (95% CI = 59, 86) and NPV 68% (95% CI = 52, 81) results. Recently, a comprehensive proteomic analysis of

GCF from a periodontally healthy and disease population was performed using liquid chromatography and tandem mass spectrometry, which demonstrated that the bacterial proteins identified in both data sets differed, and no crossover of proteins was observed (Baliban et al. 2012). These results elucidate the potential for human and bacterial biomarkers present within GCF as potential markers of periodontal disease activity. Similarly, Tsuchida et al. described a different methodology for extracting the GCF proteins and found a significantly higher concentration of superoxide dismutase 1, apolipoprotein A-I, and dermicidin to be highly expressed within samples from chronic periodontitis individuals (Tsuchida et al. 2012). Gingival crevicular fluid has also demonstrated a potential value to evaluate periodontal therapy efficacy. Our results showed significant reduction of GCF cytokines after periodontal therapy. It is in accordance with recent results by Oliveira et al., who investigated changes in levels of GCF cytokines after periodontal therapy and found significant reduction in GCF IL-1β, GM-CSF and the ratio IL-1β/IL-10 (De Lima Oliveira et al. 2012).

Although we included serum biomarkers into the overall analysis in our study, we found that serum did not play an important diagnostic role in identifying patients who were susceptible to future periodontal progression (Kinney et al. 2011). Similarly, Takahashi et al. found no significance on serum IL-6 levels on periodontitis patients, although increased expression was found in the gingival tissues (Takahashi et al. 1994). In a recent study, Becerik et al. investigated GCF and plasma acute-phase cytokines levels in different periodontal diseases and demonstrated that plasma cytokine levels are not related to the inflammatory changes occurring in the disease periodontal tissues (Becerik et al. 2012).

Our findings suggest that of the categories of saliva, serum, GCF, clinical measures, and pathogen biomarkers identified, the greatest degree of sensitivity was noted with saliva biomarkers and greatest specificity was with GCF biomarkers on the identification of periodontal disease progression. The overall balance of sensitivity and specificity was most consistent when the five parameters were examined in combination. Clinical implications include improved patient monitoring and control of disease activity. Thus, the identification of patients with multiples sites demonstrating high susceptibility for disease activity would assist the establishment of a personalized approach helping to identify or targeting patient-specific risk factors.

Acknowledgements

The authors appreciate the clinical assistance of Ms. Anna Galloro and Ms. Tina Huffman, the support on microbial analyses from Dr. Charles Shelburne and saliva analyses from Drs. Amy Herr and Anup Singh. We also appreciate the preparation of the figures by Mr. Chris Jung and Mr. Kenneth Rieger (School of Dentistry – University of Michigan).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Patient demographics and clinical parameters stratified by level of disease.

Table S2. Changes in positive and negative predictive values using individual versus clusters of oral fluid biomarkers, plaque pathogens and clinical measures derived at baseline.

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Clinical Relevance

Scientific rationale for the study: Current periodontal diagnostic methods are limited in their ability to provide the clinician with real-time evaluation of disease status. The aim of this study was to assess the ability of a panel of gingival crevicular fluid biomakers as predictors of periodontal disease progression.

Principal findings: Reliable levels of predictability of periodontal disease progression were seen when GCF biomarkers were combined with other clinical and biological measures of disease activity.

Practical implications: Utilization of host oral fluid biomarkers as predictors of periodontal disease progression may offer improved patient monitoring and disease control.