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Association of Gingival Crevicular Fluid Biomarkers During Periodontal Maintenance With Subsequent Progressive Periodontitis

Richard A. Reinhardt^{*}, Julie A. Stoner[†], Lorne M. Golub[‡], Hsi-Ming Lee[‡], Pirkka V. Nummikoski[§], Timo Sorsa^{||}, and Jeffrey B. Payne^{*}

^{*} Department of Surgical Specialties, College of Dentistry, University of Nebraska Medical Center, Lincoln, NE [†] Department of Biostatistics and Epidemiology, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK [‡] Department of Oral Biology and Pathology, School of Dental Medicine, Stony Brook University, Stony Brook, NY [§] Longitudinal Radiographic Assessment Facility, University of Texas Health Science Center at San Antonio, San Antonio, TX ^{||} Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland

Abstract

Background—Analysis of biomarkers in gingival crevicular fluid (GCF) may be helpful in forecasting vulnerability to future attachment loss. This paper sought to correlate GCF biomarkers of inflammation and bone resorption with subsequent periodontal attachment and bone loss in a longitudinal trial of a matrix metalloproteinase (MMP)-inhibitor.

Methods—GCF was collected from two periodontal pockets (mean 5.1 ± SD 1.0 mm) at baseline and annually in postmenopausal females with moderate/advanced periodontitis undergoing periodontal maintenance every 3–4 months during a two-year double-blind, placebo-controlled, randomized clinical trial of subantimicrobial-dose doxycycline (SDD, 20 mg bid). Subjects were randomized to SDD (n = 64) or placebo (n = 64). GCF was analyzed for inflammation markers interleukin-1 β (enzyme-linked immunosorbent assay), total collagenase activity (hydrolysis of synthetic octapeptide), MMP-8 (Western blot), and bone resorption marker carboxyterminal telopeptide cross-link fragment of type I collagen (ICTP; radioimmunoassay). Generalized estimating equations were used to associate these biomarkers, categorized into tertiles, with subsequent clinical attachment (automated disk probe) or interproximal bone loss (radiography). Odds ratio (OR) values compared highest to lowest tertile groups.

Results—Increases in GCF IL-1 β and MMP-8 during the first year of periodontal maintenance were associated with increased odds of subsequent (year 2) periodontal attachment loss (OR = 1.67, p = 0.01 and OR = 1.50, p = 0.02, respectively), driven by the placebo group. Elevated baseline ICTP also was associated with increased odds of 1- and 2-year alveolar bone density loss (OR = 1.98, p = 0.0001) in placebo, not SDD, and bone height loss (OR = 1.38, p = 0.06), again driven by placebo.

Conclusion—These data support the hypothesis that elevated GCF biomarkers of inflammation and bone resorption from a small number of moderate/deep sites hold promise in identifying patients vulnerable to progressive periodontitis, and SDD may modify that risk.

Keywords

Gingival crevicular fluid; doxycycline; periodontitis; type I collagen; interleukin-1; matrix metalloproteinase-8

Introduction

Gingival crevicular fluid (GCF) is a convenient, noninvasive and efficient means to sample biomarkers of inflammation and bone resorption in the oral cavity. GCF represents serum components overlaid with products from local physiological phenomena, such as connective tissue destruction and bone loss, and may have diagnostic value.^{1,2} While individual GCF samples have the possibility of describing the inflammatory events occurring at that site, pooled samples from a small number of sites may characterize broader patient periodontitis vulnerability, and allow periodic assessment during periodontal treatment or maintenance. As we reported previously, GCF was collected from two pockets (5–9 mm) at baseline and then yearly during a 2-year, double-blind, placebo-controlled clinical trial of postmenopausal women on periodontal maintenance therapy.³ Half of the subjects were randomized to receive the matrix metalloproteinase (MMP)-inhibitor sub-antimicrobial dose doxycycline (SDD, 20 mg bid). The primary hypothesis addressed by the trial was that SDD could reduce alveolar bone loss in estrogen-deficient postmenopausal women with moderate-advanced periodontitis and with osteopenia of the lumbar spine or femoral neck.⁴ The study population was 45–70 years of age, had ≥ 9 posterior teeth, and were in good general health without hypersensitivity to tetracyclines or diseases/regular drug therapy that would affect the inflammatory or immune response. Subjects were randomized at study centers in Lincoln, Nebraska and Stony Brook, New York according to smoking status, and were $> 96\%$ white Caucasian. GCF analysis included inflammation markers interleukin (IL)-1 β , MMP-8, and total collagenase activity. Also, a carboxyterminal telopeptide cross-link fragment of type I collagen (ICTP) released during bone resorption was measured. These specific markers were chosen primarily for the following reasons: IL-1 β is easily measured in GCF and stimulates bone-resorbing IL-6, which is reduced by doxycycline analogs in vitro;⁵ collagenase degrades collagen which is the major structural protein of the periodontium, and MMP-8 is the primary collagenase in GCF and these biomarkers and alveolar bone loss are reduced by doxycycline analogs;⁶ SDD has been shown to reduce GCF ICTP, a breakdown product of type I collagen.⁷ Success of periodontal therapy and stability during maintenance depend on preserving clinical attachment levels and crestal alveolar bone, especially in the interproximal areas. These measurements are typically obtained during maintenance appointments, and when destruction is noted (progressive periodontitis), more aggressive therapy is initiated. The use of biomarkers to identify vulnerable patients before periodontal destruction occurs may tailor more aggressive treatment or pharmacotherapy to subjects at the proper time. The first step in this process is to identify which biomarkers are associated with subsequent periodontal destruction. Therefore, the purpose of this paper was to examine the relationship between inflammatory and bone resorption biomarkers in GCF taken at baseline and after one year of periodontal maintenance, and subsequent periodontal attachment and bone loss in a previously-reported clinical trial.^{3, 4, 8}

Materials and Methods

Participants

One hundred and twenty-eight osteopenic postmenopausal females (not taking estrogen) with moderate-advanced periodontitis on 3–4 month periodontal maintenance participated in this study. Inclusion/exclusion criteria, randomization procedures, and clinical trial design are

described in detail elsewhere.⁴ Patient recruitment and follow-up flow diagram may be found in Figure 1. Half of the subjects were randomized each into SDD or placebo groups. All were provided with daily calcium (1200 mg) and vitamin D (400 IU).

Gingival Crevicular Fluid

GCF samples taken at baseline and after one year of SDD/placebo treatment and periodontal maintenance were used in this exploratory analysis. As described previously in detail,³ 10-second paper strip samples from two periodontal pockets (two deep posterior interproximal pockets with bleeding on probing at screening; 5–9 mm) were immediately frozen, stored and later pooled for analysis of biomarkers.

Pooled samples per subject were eluted into 400 µl Tris buffer with protease inhibitors and assayed for IL-1β, ICTP, total collagenase activity, and MMP-8 as described previously.³ In summary, IL-1β was measured in 50 µl aliquots using an enzyme immunoassay based on a double-antibody sandwich technique.[¶] ICTP was determined with 100 µl aliquots and a radioimmunoassay.[#] Total collagenase activity was analyzed with 70 µl in a substrate reaction^{**} and high performance liquid chromatography.^{††} Total MMP-8 levels were tested with Western blots from 100 µl GCF portions.^{3,7}

Clinical and Radiographic Outcomes

Relative clinical attachment levels (RCAL) were measured at posterior interproximal sites at baseline and every 6 months over the 2-year trial using an automated probe,^{‡‡} as described recently.⁸ Sites that varied from baseline by ≥ 2.0 mm were remeasured to confirm. Thresholds defining significant changes at the site level for RCAL relative to baseline were ≥ 1.5 mm defining progression, ≤ -1.5 mm defining improvement and values in between thresholds defining no change based on two standard deviations of replicate measures.^{8,10–12}

Four posterior bitewing radiographs were taken at baseline, 1 and 2 years using a cephalostat^{§§} to standardize projection geometry. Films were digitized and computer analysis was performed at a central facility.^{||} Measurements included computer-assisted densitometric image analysis (CADIA) to evaluate bone density in the interproximal alveolar area, and alveolar bone height (BH) was measured from the cemento-enamel junction (CEJ) or restoration margin. The thresholds defining changes for the CADIA measures were ± 17 at a crestal site or ± 14 at a subcrestal site, and for the alveolar bone height were ± 0.4 mm based on two standard deviations of replicate measures. All techniques were explained in detail in the initial study report.⁴

Statistical Analysis

One-year changes in GCF biomarkers and baseline levels were modeled as categorical variables (tertiles of low, medium, and high). Given the limited number of sites that demonstrated changes in RCAL or alveolar bone, we also considered 3 categories of the distribution of RCAL/radiographic changes (progression, no change, and improvement) as summarized in the previous section. Generalized estimating equations methodology was used to fit cumulative logistic regression models to investigate the association between GCF biomarker level (independent factor) and RCAL or alveolar bone changes (dependent factor)

¶Biosource, Camarillo, CA.

#Immunodiagnostic Systems, Fountain Hills, AZ.

**Bachem, King of Prussia, PA.

††Waters Alliance 2695 System, Milford, MA.

‡‡Florida Probe Corp., Gainesville, FL.

§§Quint Sectograph, Los Angeles, CA.

||Longitudinal Radiographic Assessment Facility, San Antonio, TX.

over time. The models were adjusted for baseline measures (RCAL or BH), randomized treatment group, smoking status, study center, and assay batch. Interaction terms were used to determine whether the association between GCF biomarker levels and RCAL/bone changes over time differed by randomized treatment group or time point (12-month versus 24-month change). Estimates were provided for SDD and placebo groups separately when there was a significant interaction between biomarker levels and study drug. Estimates were combined when there was no significant interaction, but for exploratory purposes, treatment group-specific estimates are provided in the tables. No significant differences between 12- and 24-month outcome measurements that impacted the estimated associations were noted in any analysis of baseline biomarker levels and, therefore, combined estimates over 12-month and 24-month changes in RCAL/bone measures were reported when investigating associations with baseline biomarker levels. Odds of more progressive disease were presented for subjects in the highest tertile group (highest baseline level or greatest increase in biomarker levels) relative to the lowest tertile group and tests for linear trend across the 3 biomarker categories were performed.

Baseline biomarker values were evaluated for associations with changes in RCAL, CADIA and BH after one and two years. In addition, changes in GCF biomarkers over the first year of periodontal maintenance (also categorical variables by tertiles essentially showing reduction, constant, and increased values) were correlated to changes in RCAL, CADIA and BH after two years. Spearman rank correlation coefficients were used to summarize the association between continuous changes in biomarker levels at 12-months.

Results

The mean baseline probing depth for GCF-sampled sites was 5.1 mm (± 1.0 mm standard deviation), compared to 3.8 ± 1.1 mm for posterior interproximal sites not selected for GCF sampling. Selection of GCF sites occurred at the screening visit because GCF specimen sampling had to occur before baseline probing. At the baseline visit, 21% of GCF sites measuring ≥ 5 mm at screening had < 5 mm probing depths. Three percent of both GCF-sampled and non-sampled sites demonstrated progressive periodontitis at 12 and 24 months. There was no significant association between baseline IL-1 β values and subsequent changes in RCAL (test for linear trend, $p=0.4$). However, there was a significant association between 1-year changes in IL-1 β and 2-year changes in RCAL (odds ratio [OR] = 1.67, 95% confidence interval [CI]: 1.13 to 2.46, $p = 0.01$) for the treatment groups combined, but the association was primarily driven by the placebo group (Table 1). Those subjects taking placebo and showing essentially any increase in IL-1 β (> 0.10 pg IL-1 β /pool) during the first year of periodontal maintenance were nearly twice as likely to show progressive periodontitis compared to those in the lowest IL-1 β tertile. There was no significant association in the SDD group. There was no significant association between baseline IL-1 β and 1- and 2-year CADIA (test for linear trend, $p = 0.7$) or between 1-year changes in IL-1 β and 2-year CADIA (test for linear trend, $p = 0.6$). The same was true for IL-1 β and BH changes (test for linear trend, $p = 0.9$ for each).

Baseline or 1-year change in ICTP was not associated with subsequent RCAL changes (tests for trends, $p=0.2$ and $p=0.4$, respectively). When bone changes were considered, the association between baseline ICTP and 1-year and 2-year CADIA changes differed significantly by study drug group ($p = 0.03$, test for interaction). Among placebo subjects, baseline ICTP was significantly associated with 1-year and 2-year CADIA changes (OR = 1.98, 95% CI: 1.39 to 2.82, $p = 0.0001$), but this association was not evident in maintenance patients taking SDD (Table 2). A similar pattern existed between baseline ICTP and 1-year and 2-year BH (OR = 1.38, 95% CI: 0.99 to 1.91, $p = 0.06$) for the treatment groups combined (Table 3), but the association was driven primarily by placebo subjects (OR = 1.89, 95% CI: 1.17 to 3.05, $p =$

0.009). Therefore, patients on periodontal maintenance taking no host-modulation therapy and with high baseline GCF ICTP were nearly twice as likely to show progressive periodontal bone density and height loss. No significant associations were noted between 1-year changes in ICTP and 2-year CADIA (test for trend, $p = 0.5$) or BH (test for trend, $p = 0.2$).

There were no significant associations between baseline or 1-year changes in collagenase activity and subsequent RCAL, CADIA or BH changes (tests for linear trends ≥ 0.1). Total MMP-8 in baseline GCF also was not associated with 1- or 2-year RCAL outcomes (test for trend, $p = 0.1$). However, 1-year increases in GCF total MMP-8 in combined treatment groups were associated with a 50% increase in the odds of progressive periodontitis (RCAL) after 2 years, compared to subjects in the lowest tertile of MMP-8 (OR = 1.50, 95% CI: 1.06 to 2.13, $p = 0.02$), where the association was driven primarily by placebo subjects (Table 4). This association was absent among patients on SDD. These patterns were similar to those shown with GCF IL-1 β (Table 1). No significant associations existed between GCF total MMP-8 and subsequent CADIA or BH changes (test for trend, $p \geq 0.1$ for each).

Since an increase in GCF IL-1 β and total MMP-8 seemed to have similar impacts on subsequent RCAL loss, the interaction of the two biomarkers was studied to test if simultaneous increases in both would provide a more powerful association with subsequent attachment loss. The 1-year changes in IL-1 β and MMP-8 were positively correlated ($r = 0.34$, $p = 0.0002$). However, there was no significant indication that the association between 1-year changes in IL-1 β and 2-year changes in RCAL were modified by 1-year changes in MMP-8 (IL-1 β by MMP-8 total interaction, $p = 0.2$). After adjusting for baseline RCAL measurements, randomization stratification factors, assay batch and the 1-year change in total MMP-8, subjects with increased IL-1 β (> 0.10 pg IL-1 β /pool) had slightly lower odds of periodontitis progression (OR = 1.54 compared to the lowest tertile, $p = 0.03$) than with IL-1 β without MMP-8 adjustments (OR = 1.67, $p = 0.01$) when both treatment groups are combined, although differences were primarily seen for placebo subjects (Tables 1, 5). When the MMP-8 1-year change was likewise adjusted for 1-year IL-1 β change, subjects with increasing MMP-8 (compared to the lowest tertile) no longer had a significantly increased odds of progressive periodontitis (OR = 1.36, $p = 0.1$). These data indicated that increases in GCF IL-1 β were of primary importance in driving 2-year losses in RCAL, and combining the two indicators did not improve IL-1 β predictive associations with subsequent periodontal breakdown.

Discussion

IL-1 β is a proinflammatory and bone-resorbing cytokine that has been shown to be associated with progressive periodontitis in previous studies. Some of the populations had untreated periodontitis,^{13–15} others were refractory to periodontal therapy,¹⁶ while others included children infected with periodontal pathogens.¹⁷ The current study evaluated postmenopausal females on 3–4 month periodontal maintenance who were very stable periodontally over a 2-year period. Only 2–3% of sites showed loss in RCAL,⁸ and 5–10% of sites lost bone height. This was somewhat unexpected, since estrogen-deficient subjects appeared to have a higher incidence of progressive periodontitis than estrogen-sufficient subjects in previous reports.¹⁰ However, the current population was highly compliant with attending their 3–4 month periodontal maintenance visits due to study payment of maintenance costs and use of follow-up calls to make certain that subjects had seen their dental provider for these appointments. In addition, all subjects were supplied with recommended daily doses of vitamin D and calcium, thereby reducing the impact of estrogen-deficiency osteopenia. The sites sampled for GCF were generally not the sites showing progressive periodontitis, unlike most of the site-specific studies above.^{13–16} In fact, 3% of both GCF-sampled (2/subject) and non-sampled sites (up to 46/subject) showed progressive periodontitis at 12 and 24 months. The GCF samples in this study were pooled to increase the ability to detect all of the biomarkers in each subject and to

serve as a patient-based diagnostic tool for subsequent progressive periodontitis. Two 10-second GCF samples were obtained in 2–3 minutes as part of the periodontal maintenance appointment. Ten-second samples of GCF collected from deep pockets (paper strips were inserted until resistance was felt) and sensitive bioassays were used to capture biomarker values for each patient,³ as confirmed in earlier papers.⁷ On the basis of pooled samples, placebo patients showing an increase in GCF IL-1 β from the previous year's maintenance visit were nearly twice as likely to experience progressive periodontitis in the next year. Maintenance patients taking SDD had reduced odds of periodontitis progression (OR=0.81, 95% CI: 0.67–0.97, $p=0.03$),⁸ and numerically lower GCF IL-1 β levels.³ Although associated with progressive attachment loss, GCF IL-1 β was not associated with bone changes, unlike salivary IL-1 β and radiographic bone loss in aggressive periodontitis.¹⁷

Since ICTP is a breakdown product of type I collagen and a marker of bone resorption,¹⁸ it should have greater association with bone changes. This proved to be true. Placebo patients with high GCF ICTP prior to study maintenance appointments had nearly twice the frequency of bone density and height loss after one and two years (Tables 2·3). No associations were seen between ICTP and RCAL. While GCF ICTP has been shown to be predictive of experimental periodontal bone destruction in a dog model,¹⁹ the current study represents a rare comparison of GCF ICTP and subsequent human periodontal bone loss. Scaling and root planing plus local minocycline microspheres were reported to reduce both GCF ICTP and IL-1,²⁰ as did SDD added to supra- and subgingival scaling.⁷ In the current study, SDD added to periodontal maintenance caused a 16% reduction in median ICTP levels ($p=0.08$).³ These same patients who adhered to the study protocol showed a 36% reduction in the odds of progressive bone height loss among SDD versus placebo subjects ($p=0.03$).⁴ SDD also abrogated the association between GCF ICTP and bone changes. These data suggest that pooled GCF ICTP from just two sites may help identify patients on periodontal maintenance who are more vulnerable to periodontal bone loss, and the potential for SDD to prevent this breakdown should be studied further.

There was some evidence in this study that GCF ICTP is associated with bone loss at distant sites. Subjects with the highest tertile of baseline GCF ICTP had 2.11% ($p=0.03$) lower mean T-scores in lumbar spine bone mineral density (BMD) after 2 years than the lowest GCF ICTP tertile (data not shown). Is GCF acting as a surrogate for serum in this instance? Does GCF ICTP have possibilities as an easily-obtained diagnostic fluid for systemic bone changes? Answers to these questions await future studies.

The current study failed to show any association between GCF collagenase activity and subsequent periodontal attachment or bone loss. This was somewhat surprising as previous studies have indicated that sites with recent (past) attachment loss had a higher frequency of active collagenase than control sites, and that doxycycline therapy also inhibited recurrent periodontal destruction.²¹ A separate study suggested that neutral protease activity was a good predictor of “prior” attachment loss.²² Golub et al. also have demonstrated that SDD reduces collagenase activity and attachment loss in the current^{3·8} and other populations.⁹

The more specific biomarker MMP-8 appeared to be more associated with clinical outcomes in the present study. MMP-8 is the predominant collagenase in GCF, primarily a neutrophil product.⁷ Increases in MMP-8 over the first year of maintenance were associated with a 50% increase in the odds of progressive attachment loss among both treatment groups ($p=0.02$), driven primarily by the placebo group (Table 4). SDD reduced the odds of higher GCF MMP-8 by 60% ($p=0.006$)³ along with a 19% reduction in the odds of more progressive periodontitis ($p=0.03$). Specifically, 41% of placebo subjects showed an increase (>0.08 total MMP-8 scanning units) and 23% showed a reduction (<-0.8 total MMP-8 scanning units) during the first year of maintenance, while 27% of subjects on SDD demonstrated a similar increase and

34% of SDD patients showed a reduction. This reduction is in keeping with SDD's MMP inhibiting effects, yet raises the question as to why 27% of SDD subjects still experienced MMP-8 increases. There was no indication that those patients who were less compliant in taking their SDD, vitamin D and calcium supplements (adherent < 80% of the time) had different changes in 1-year MMP-8 or IL-1 β ($p > 0.5$). However, the vast majority of subjects were well controlled on periodontal maintenance and baseline levels of MMP-8 were relatively low, so reductions in MMP-8 with SDD were probably less likely than if baseline levels were higher, as with untreated periodontitis. Among sites from subjects who demonstrated elevated MMP-8 levels based on pooled samples, the probability of site-level progressive periodontitis was small (3.43% placebo, 3.85% SDD) and was extremely low (1.65% placebo, 2.06% SDD) among sites from subjects who demonstrated reductions in MMP-8 levels based on pooled samples. Previous studies have shown that scaling and root planing (SRP) causes reductions in GCF MMP-8 and attachment loss, while sites with elevated MMP-8 had poor responses to SRP.²³ Responses also have been better when SRP is accompanied by periodontal maintenance therapy.²⁴ Other drug therapies also have been shown to improve periodontal outcomes and reduce GCF MMP-8. Cyclooxygenase (COX)- inhibitor meloxicam with SRP reduced GCF MMP-8 more than SRP alone²⁵ and enamel matrix derivative reduced MMP-8 in healing intrabony defects.²⁶ Further explanations as to how some SDD subjects showing increased MMP-8 in pooled GCF samples could have avoided progressive periodontitis may include: 1) these GCF sites lacked other local risk factors for progressive periodontitis beyond elevated MMP-8, such as plaque retentive root anatomy, occlusal discrepancies, or root dentin or cementum irregularities; 2) subjects lacked systemic risk factors, such as genetic or heritable factors, diseases predisposing subjects to periodontitis (diabetes, obesity, rheumatoid arthritis) or predisposing habits (smoking, alcoholism); and 3) certain sites with elevated MMP-8 (or IL-1 β) also could have had elevated tissue inhibitors of metalloproteinases and IL-1 receptor antagonists or lack of other proinflammatory/catabolic mediators, which were not measured but could have offset the effect of elevated single biomarkers.^{27–29} Biochemical mediators of progressive periodontitis involve complex interactions and mechanisms, yet using certain mediators as markers of periodontitis risk may be valuable.

The combination of increased GCF IL-1 β and MMP-8 after one year did not improve the ability to distinguish subjects who would experience periodontal attachment loss one year later (compared to elevated IL-1 β alone, Tables 1 and 5). A previous cross-sectional study indicated that elevated salivary IL-1 β or MMP-8 increased the probability of having existing periodontitis by 15.4 and 11.3 times, respectively.³⁰ When both IL-1 β and MMP-8 were elevated, periodontitis was 45.5 times more likely. However, in the current longitudinal study GCF IL-1 β increases were most important in driving future attachment loss, and no synergism between IL-1 β and MMP-8 existed. The fact that elevations in IL-1 β appeared to drive the RCAL loss is consistent with the hypothesis that the inflammatory response (represented by IL-1 β) induces MMP-8 production along with other destructive enzymes leading to subsequent connective tissue destruction. Proinflammatory cytokines can stimulate MMP-8 secretion from fibroblast, epithelial and plasma cells.^{2,31}

Modeling changes in GCF biomarkers as a categorical variable was appealing because non-linear trends in the association between changes in biomarkers and changes in RCAL or bone can be detected. Interpretation was more straight-forward than when modeling continuous changes, and extreme changes were less influential because the values were grouped into a category of change values and the exact (extreme) changes were not modeled directly. The limitation of this approach is that if there is truly a linear association between changes in biomarker and changes in RCAL/bone, categorizing biomarkers is a less sensitive approach because some of the linear association information is lost when the values are categorized. However, we addressed this in part by testing for linear trends across the categories of biomarker levels.

One of the major limitations of the current study in addressing the biochemical predictors of attachment loss was the small number of sites that demonstrated periodontitis progression during the 2-year period. Only 74 of 2,727 evaluated placebo sites showed attachment loss over 2 years. Placebo subjects with increasing IL-1 β over 1 year represented 46% of those sites and those with increasing MMP-8 represented 54% of such sites. Conversely, subjects with decreasing IL-1 β (< -0.7 pg IL-1 β /pool) or MMP-8 (< -0.8 scanning units) each possessed only 15% of the progressing sites. These subjects also showed a 4 times (decreasing IL-1 β) and 3.2 times (decreasing MMP-8) greater frequency of sites gaining ≥ 1.5 mm of clinical attachment than sites losing 1.5 mm. Patient-pooled sites with combined reduced biomarkers (reduced IL-1 β and MMP-8) had only 4 progressing sites (1% of the 334 pooled sites associated with combined reduced biomarker levels), and demonstrated 27 sites which improved ≥ 1.5 mm (8% of the 334 sites). These data clearly indicate that use of profiles during periodontal maintenance with small numbers of GCF sampling sites and a limited number of biomarkers (including IL-1 β and MMP-8 potentially using chair-side testing devices³²) has promise in characterizing the risk for future periodontal attachment loss. Further, identification of patients at increased risk for future attachment loss may lead to beneficial interventional pharmacotherapies, such as SDD, prior to recurrent periodontal destruction.

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Figure 1.

Consort Statement Flow Diagram for subject enrollment of the clinical trial associated with this paper. It was originally published in 2007,⁴ and this figure has been reproduced with permission from Wiley-Blackwell.

Table 1
1-year IL-1 β Changes and 2-year Odds of More Progressive Relative Clinical Attachment Level Loss

Tertile	All Subjects				Placebo				SDD			
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	OR	95% CI	p-value	p-value
Reduction (1-year change < -0.70 pg IL-1 β /pool)	1 (reference group)			1 (reference group)			1 (reference group)		1 (reference group)			
Constant (1-year change -0.70 to 0.10 pg IL-1 β /pool)	1.37	0.95 to 1.07	0.09	1.75	0.92 to 3.30	0.09	1.08	0.71 to 1.62				0.7
Increase (1-year change > 0.10 pg IL-1 β /pool)	1.67	1.13 to 2.46	0.01	1.98	1.15 to 3.41	0.01	1.37	0.76 to 2.49				0.3

Table 2

Baseline ICTP and Combined 1-/2-year Odds of More Progressive CADIA Bone Loss.*

Tertile	Placebo			SDD		
	OR	95% CI	p-value	OR	95% CI	p-value
Low (< 700 pg ICTP/pool)	1 (reference group)			1 (reference group)		
Medium (700 to 1300 pg ICTP/pool)	1.40	0.97 to 2.03	0.07	1.10	0.69 to 1.76	0.7
High (> 1300 pg ICTP/pool)	1.98	1.39 to 2.82	0.0001	0.98	0.66 to 1.47	0.9

* There is a significant indication that the association between baseline ICTP and CADIA bone loss differs between the treatment groups (p = 0.03).

Table 3
Baseline ICTP and Combined 1-/2- Year Odds of More Progressive Alveolar Bone Height Loss

Tertile	All Subjects			Placebo			SDD		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Low (< 700 pg ICTP/pool)	1 (reference group)			1 (reference group)			1 (reference group)		
Medium (700 to 1300 pg ICTP/pool)	0.99	0.74 to 1.31	0.9	1.20	0.74 to 1.93	0.5	0.90	0.60 to 1.36	0.6
High (> 1300 pg ICTP/pool)	1.38	0.99 to 1.91	0.06	1.89	1.17 to 3.05	0.009	0.98	0.60 to 1.53	0.9

Table 4
1-year Total MMP-8 Changes and 2-year Odds of More Progressive Relative Clinical Attachment Level Loss

Tertile	All Subjects				Placebo				SDD			
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	OR	95% CI	p-value	p-value
Reduction (1-year change < -0.8 total MMP-8 scanning units)	1 (reference group)			1 (reference group)			1 (reference group)		1 (reference group)			
Constant (1-year change -0.8 to 0.08 total MMP-8 scanning units)	1.24	0.84 to 1.84	0.3	1.34	0.71 to 2.53	0.4	1.14	0.71 to 1.84	1.14	0.71 to 1.84	0.6	0.6
Increase (1-year change > 0.08 total MMP-8 scanning units)	1.50	1.06 to 2.13	0.02	1.77	1.08 to 2.89	0.02	1.18	0.66 to 2.12	1.18	0.66 to 2.12	0.6	0.6

Table 5
1-year IL-1 β or MMP-8 Changes (Adjusted for the Other) and 2-year Odds of More Progressive Relative Clinical Attachment Level Loss

Terile	All Subjects			Placebo			SDD		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
IL-1 β Reduction [*]	1 (reference group)			1 (reference group)			1 (reference group)		
IL-1 β Constant [*]	1.28	0.87 to 1.89	0.2	1.60	0.83 to 3.10	0.2	1.03	0.65 to 1.62	0.9
IL-1 β Increase [*]	1.54	1.04 to 2.28	0.03	1.85	1.02 to 3.34	0.04	1.35	0.74 to 2.43	0.3
MMP-8 Reduction [†]	1 (reference group)			1 (reference group)			1 (reference group)		
MMP-8 Constant [†]	1.15	0.77 to 1.73	0.5	1.15	0.60 to 2.20	0.7	1.16	0.67 to 2.00	0.6
MMP-8 Increase [†]	1.36	0.95 to 1.94	0.1	1.53	0.91 to 2.56	0.1	1.14	0.64 to 2.01	0.7

^{*} See Table 1

[†] See Table 4