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Monitoring periodontal disease status in smokers and nonsmokers using a gingival crevicular fluid matrix metalloproteinase-8-specific chair-side test

P. Mäntylä[1], M. Stenman[1], D. Kinane[2], T. Salo[3], K. Suomalainen[1], S. Tikanoja[4], T. Sorsa[1]

[1]Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, and Institute of Dentistry, University of Helsinki, Helsinki, Finland, [2]Department of Periodontology, University of Louisville, School of Dentistry, Louisville, KY, USA, [3]Department of Diagnostics and Oral Medicine, Institute of Dentistry, University of Oulu, and Oulu University Central Hospital, Oulu, Finland, and [4]Medix Biochemica Ltd, Kauniainen, Finland

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Background and Objective: With current periodontal diagnostic tools it is difficult to identify susceptible individuals or sites at risk. The aim of this study was to evaluate the efficacy of the matrix metalloproteinase (MMP)-8-specific chair-side dip-stick test in longitudinally monitoring the periodontal status of smoking (S) and nonsmoking (NS) patients with chronic periodontitis, using their gingival crevicular fluid (GCF) MMP-8 concentrations.

Material and Methods: Clinical parameters, MMP-8 test results and concentrations were monitored in 16 patients after initial treatment and in 15 patients after scaling and root planing (SRP), every other month, over a 12-mo time period. Progressing and stable sites, and sites with exceptionally high MMP-8 concentrations, were analysed in smokers and nonsmokers.

Results: SRP reduced the mean GCF MMP-8 levels, test scores, probing depth (PD), attachment loss (AL) and bleeding on probing (BOP). In sites of periodontal disease progression, the distribution of MMP-8 concentrations was broader than in stable sites, indicating a tendency for elevated concentrations in patients with periodontal disease. The mean MMP-8 concentrations in smokers were lower than in nonsmokers, but in smokers' and nonsmokers' sites with progressive disease, MMP-8 concentrations were similar. Sites with exceptionally elevated MMP-8 concentrations were clustered in smokers who also showed a poor response to SRP. In these sites, the MMP-8 concentration did not decrease with SRP and these sites were easily identified by the MMP-8 test.

Conclusion: Persistently elevated GCF MMP-8 concentrations may indicate sites at risk, as well as patients with poor response to conventional periodontal treatment (e.g. SRP). MMP-8 testing may be useful as an adjunct to traditional periodontal diagnostic methods during the maintenance phase.

Dr Päivi Mäntylä, DDS, PhD, Institute of Dentistry, University of Helsinki, PO Box 41, FIN-00014 University of Helsinki, Finland
Tel: +358 9191 27217
Fax: +358 9191 27519
e-mail: paivi.mantyla@helsinki.fi

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Agreement exists that periodontitis is initiated by periodontopathogenic bacteria that colonize subgingivally, causing an inflammatory and destructive host response in certain subjects (1–3). With current periodontal diagnostic tools it is difficult to recognize susceptible individuals or sites. We have no means of predicting when gingivitis is developing into periodontitis or when periodontitis is in a progressive state with increases in pocket probing depths (PD) and further attachment loss (AL) (4). Clinical parameters such as PD and AL, as well as radiological findings, indicate the disease history but not necessarily the status at that particular moment. Bleeding on probing (BOP) has been regarded as a useful negative predictor of gingival health in most cases (5). However, the finding of reduced bleeding sites in smokers (6–10) has confounded this relationship. Therefore, periodontal diagnosis and prognostic assessment is complex, and methods to achieve additional objectivity are needed.

Matrix metalloproteinase-8 (MMP-8; collagenase-2) is a protelytic enzyme secreted mainly by neutrophil leukocytes as a latent pro-enzyme that can be activated by inflammatory irritants of bacterial origin or host inflammation mediators (11,12). MMP-8 is the major collagenase present in inflamed human gingiva, gingival crevicular fluid (GCF) and saliva (1,13,14). In the GCF from the healthy or gingivitis periodontal crevice, MMP-8 is mostly detected as a latent pro-enzyme (15), but in deep periodontal pockets most of the MMP-8 is converted to the active form (1–3).

Smoking is recognized as an important risk factor for oral disease and especially for periodontal health (16,17). Periodontal treatment outcome is often poorer in subjects who are smokers (18,19), with most refractory cases being smokers (20,21). Smoking impairs the normal host defence mechanisms (22,23) and stimulates destructive effects (24,25). Smokers with periodontitis have been reported to have impaired granulocyte function (26). The effect of smoking could thus be monitored by GCF-based biomarkers.

Table 1. Mean ± standard deviation (SD) of probing depth (PD) and attachment loss (AL) values and matrix metalloproteinase-8 (MMP-8) concentrations in different groups of sites at baseline and after scaling and root planing (SRP), and statistical significance for the difference between two time points

		All sites $n = 132$	S sites $n = 80$	NS sites $n = 43$	Progressing S sites $n = 16$	Stable S sites $n = 64$	Progressing NS sites $n = 5$	Stable NS sites $n = 38$	High-responder S sites $n = 8$	Low-responder S sites $n = 72$
PD (mm)	Baseline	4.9 ± 1.8	4.9 ± 1.7	5 ± 2.1	5.8 ± 1.6	4.7 ± 1.6	5 ± 1.6	5 ± 2.2	6.5 ± 1.0	4.7 ± 1.7
	Post-SRP	3.3 ± 1.5	3.6 ± 1.6	2.8 ± 1.3	4.4 ± 1.9	3.4 ± 1.4	2.6 ± 0.5	2.8 ± 1.3	5.8 ± 1.6	3.3 ± 1.4
	p-value	0.00*	0.00*	0.00*	0.007*	0.00*	0.04*	0.00*	NS	0.00*
AL (mm)	Baseline	4.3 ± 2.9	4.4 ± 2.6	4.4 ± 3.5	5.4 ± 2.6	4.1 ± 2.6	3.8 ± 3.1	4.5 ± 3.5	4.8 ± 1.1	4.3 ± 2.7
	Post-SRP	3.3 ± 2.6	3.4 ± 2.4	3.4 ± 2.9	2.5 ± 1.5	3.6 ± 2.6	2.1 ± 2.5	3.6 ± 3	4.2 ± 2.5	3.3 ± 2.4
	p-value	0.00*	0.00*	0.00*	0.001*	0.05*	0.04*	0.000*	Not significant	0.00*
MMP-8	Baseline	2177 ± 2747	1268 ± 2126	3997 ± 3126	1743 ± 2934	1149 ± 1884	3455 ± 1349	4069 ± 3295	1661 ± 1446	1224 ± 2191
(μg/l)	Post-SRP	1339 ± 1617	975 ± 1171	2076 ± 2140	1209 ± 1294	930 ± 1146	1763 ± 1423	2117 ± 2228	1753 ± 1726	889 ± 1075
	p-value	0.001*	Not significant	0.000*	Not significant	Not significant	0.04*	Not significant	Not significant	Not significant

*Difference statistically significant.
NS, nonsmoker; S, smoker.

Over the last decade there has been enormous interest in developing a diagnostic test capable of assessing periodontal disease activity and predicting the progression of periodontitis (3). Gingival inflammation is often present in the absence of progressive periodontal attachment loss. Therefore, it would be important for a test for periodontitis to demonstrate a true association between a surrogate disease parameter and periodontal disease activity. MMP-8 is released from neutrophils in a latent, inactive proform, and becomes induced and activated during periodontal inflammation by independent and/or combined actions of host-derived inflammatory mediators, such as tumour necrosis factor-α (TNF-α) and interleukin-1β, and microbial-derived proteases and reactive oxygen species (ROS) produced by triggered neutrophils (11,12). During active, progressing phases of periodontitis, MMP-8 levels in the GCF are significantly elevated, and MMP-8 will be almost completely converted to the active form (1–3,13,27–29). An association of increased GCF collagenase activity with progressive loss of periodontal connective tissue attachment has been demonstrated, and a significant decrease in GCF MMP-8 activity and levels following successful periodontal treatment has been shown (28–30). Based on these observations, we have developed an easy-to-use chair-side test kit for GCF MMP-8 (14,31–33).

In earlier studies we found that this test and the GCF MMP-8 levels were found to differentiate periodontal health and gingivitis or chronic periodontitis. Moreover, the response to periodontal hygiene phase treatment, consisting of scaling and root planing (SRP), and oral hygiene instructions, could be assessed by this test (28). In the present study, we evaluated MMP-8 concentrations and this prototype test in monitoring the periodontal status of patients with chronic periodontitis who were treated after enrolment and over a 12-mo period of time. We also aimed to clarify the effect of smoking on GCF MMP-8 levels and the diagnostic value of the test and MMP-8 levels in smokers. Our hypothesis is that high levels of MMP-8 are associated with periodontal attachment loss and that the chair-side test would detect these sites.

Material and methods

Study subjects

Sixteen patients with chronic periodontitis gave informed consent to participate in the study. The ethics committee of the Institute of Dentistry of the University of Helsinki approved the study. Determination of clinical periodontal status included PD, AL measurement, BOP and plaque index (PI). In order to be included in the study, the patients with periodontitis had to fulfil the following criteria: (i) no history of systemic disorders; (ii) no history of antibiotics and/or anti-inflammatory drugs within the past 6 mo; (iii) no history of any periodontitis treatment within the past 6 mo; (iv) at least 20 teeth; and (v) at least five sites exhibiting ≥ 4 mm PD and radiographic bone loss. One patient did not participate in the maintenance phase of the study, thus the original number of sites ($n = 132$, 7–10 of each patient) was reduced to 123 during the maintenance phase.

Methods

Collection of GCF and measurement of clinical parameters of predetermined sites from each patient were carried out before any treatment measures, after periodontal treatment consisting of SRP and oral hygiene instructions, and bimonthly during the 12-mo maintenance phase. The baseline for the follow-up was the post-SPR visit. At each visit, GCF was collected and analysed for MMP-8 concentration using the MMP-8-specific periodontal chair-side dipstick test as well as by a time-resolved immunofluorometric assay (IFMA), as previously described (32). The theoretical background of the chair-side test has been described by Sorsa *et al*. (31). Positive test results were recorded as follows: + (a weak blue line); + + (a clear blue line); and + + + (a strong blue line). All clinical measurements were carried out using a manual periodontal probe by a specialist in periodontology (PM).

Patients were further categorized, according to their self-reported smoking status, into smokers (11/10 patients, 89/80 sites) or nonsmokers (five patients, 43 sites). According to daily cigarette consumption (≥ 20 cigarettes per day) and years of smoking (≥ 10 yr), nine of 10 patients were regarded as heavy smokers. An

Table 2. Positive test result and bleeding on probing (BOP) percentages for different groups of sites at baseline and after scaling and root planing (SRP) (percentages of + to + + +/+ + to + + + test results, respectively) and p-values for the difference of percentages

	Test			BOP		
	Baseline %	Post-SRP %	*p*-value	Baseline %	Post-SRP %	*p*-value
All sites ($n = 132$)	50/27	39/10	< 0.001*	70	18	< 0.001*
S sites ($n = 80$)	34/13	30/9	Not significant	63	13	< 0.001*
Progressing S sites ($n = 16$)	31/13	44/13	Not significant	56	19	0.03*
Stable S sites ($n = 64$)	35/13	25/8	Not significant	63	13	< 0.001*
NS sites ($n = 43$)	84/60	58/12	< 0.001*	86	28	< 0.001*
Progressing NS sites ($n = 5$)	100/60	60/20	< 0.001*	100	60	Not significant
Stable NS sites ($n = 38$)	82/61	58/11	< 0.001*	84	26	< 0.001*

*Difference statistically significant.
NS, nonsmoker; S, smoker.

increase of periodontal AL of ≥2 mm during the maintenance phase (post-SRP to 12 mo) was regarded as periodontal disease progression. Sites were further explored with regard to the MMP-8 concentration: smokers' sites with an MMP-8 concentration of ≥4000 μg/l at least twice during the maintenance phase ($n = 8$) were regarded as high-responders and sites with lower concentrations ($n = 72$) as low-responders.

Statistical analyses

Comparisons of GCF MMP-8 concentrations, as well as pocket depths and AL between different groups at different time points, were performed with the Mann–Whitney *U*-test. Comparisons between consecutive measurements in one group were performed with the Wilcoxon test for paired observations. Longitudinal comparisons of MMP-8 concentrations between different groups were analysed with one-way analysis of variance (ANOVA) after logarithmic transformation. Percentages of test-positive and BOP-positive sites were calculated for all groups. In all analyses, the statistical unit was a single site. The level of statistical significance was set at 0.05. All analyses were computed with SPSS 12.0.1 for Windows.

Results

The data were analysed using different approaches: (i) all study subjects' sites (in the cross-sectional study $n = 132$, in the longitudinal maintenance study $n = 123$); (ii) sites grouped according to smoking status (nonsmoker sites $n = 43$; smoker sites $n = 80$) (iii), nonsmoker and smoker sites with AL increase during the maintenance phase (progressing sites: nonsmoker, $n = 5$; smoker, $n = 16$) and nonsmoker and smoker stable sites (nonsmoker, $n = 38$; smoker, $n = 64$); and (iv) smoker sites with exceptionally high GCF MMP-8 concentrations (≥ 4000 μg/l) at least twice during the follow-up (high-responder smoker sites; $n = 8$) and smoker sites with lower concentrations (low-responder smoker sites; $n = 72$).

Cross-sectional study

Sixteen patients (132 sites) participated in the cross-sectional part of the study (evaluation of the initial treatment response, baseline to post-SRP). The mean values of PD, AL and MMP-8, positive MMP-8 chair side dip-stick test results and BOP-positive scores were significantly lower after SRP ($p < 0.05$ for all parameters) (Tables 1 and 2). The positive treatment outcome reflected in PD and AL values and in MMP-8 concentrations after SRP were showed a slightly increased trend during the maintenance phase (Fig. 1).

Longitudinal maintenance phase study

Fifteen patients were followed up every other month for 12 mo after SRP (post-SRP to the 12-mo visit).

Smoker Vs. nonsmoker sites – In smokers' GCF, the mean baseline MMP-8 concentration was statistically significantly lower than in nonsmokers' GCF ($p < 0.001$). The decrease of MMP-8 concentrations after treatment was not statistically significant in smokers' sites but significant in nonsmokers' sites ($p < 0.001$). However, in smokers' sites, the MMP-8 concentrations were elevated during the maintenance phase to the same level as in nonsmokers' sites and were higher in smokers' sites at the end of the follow-up. Mean PD values of smokers' ($n = 80$) and nonsmokers' ($n = 43$) sites were at the same level at baseline (Table 1). The treatment outcome measured as PD was significantly poorer in smokers' sites than in nonsmokers' sites, and the difference between PD values at smokers' and nonsmokers' sites remained during the maintenance phase. In AL values the difference was not as clear (Fig. 2).

Stable and progressing sites – During the maintenance phase, an increase in AL of ≥2 mm was detected in 21 out of 123 (17%) sites. Sixteen (76%) were smokers' sites and five (24%) were nonsmokers' sites. With this criterion, 102 (83%) of 123 sites were regarded as stable (64 smokers' sites and 38 nonsmokers' sites).

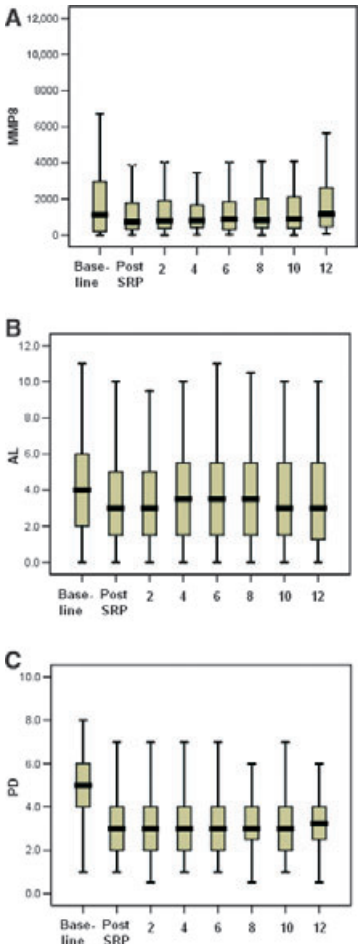

Fig. 1. (A) Matrix metalloproteinase-8 (MMP-8) concentrations [immunofluorometric assay (IFMA) μg/l], (B) attachment losses (AL) (mm) and (C) probing depths (PD) (mm) of all studied sites [baseline to after scaling and root planing (post-SRP), $n = 132$; 2–12 mo, $n = 123$]. Box-plots with the median, quartiles and extreme values are shown. Timeline of measurements: baseline, post-SRP, and maintenance phase 2, 4, 6, 8, 10 and 12 mo.

Stable and progressing smokers' sites – The difference between MMP-8 concentrations in progressing ($n = 16$) and stable smokers' sites ($n = 64$) was not statistically significant at baseline, after SRP or at any maintenance visits. However, progressing smokers' sites had a trend for broader distributions than stable smokers' sites. The mean MMP-8 concentration decreased statistically significantly after SRP in neither group of smokers. In both groups of smokers' sites, the mean AL and PD values showed a statistically significant decrease after

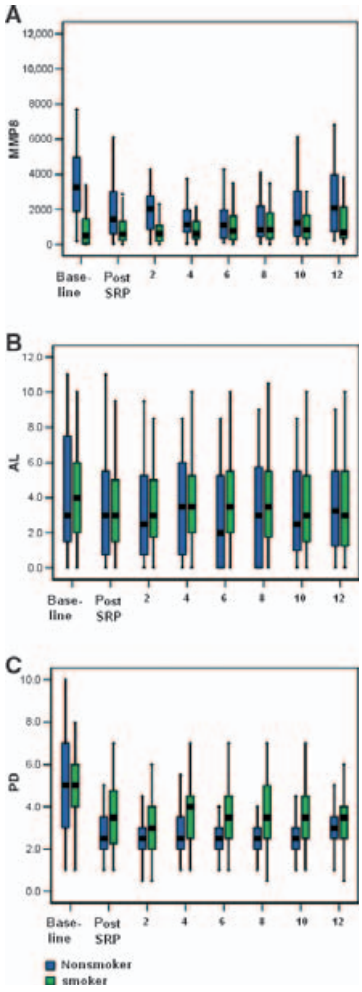

Fig. 2. Smokers' and nonsmokers' sites. (A) Matrix metalloproteinase-8 (MMP-8) concentrations, (B) attachment losses (AL) and (C) probing depths (PD). Baseline to after scaling and root planing (post-SRP), 132 sites [smokers (S), $n = 89$; nonsmokers (NS), $n = 43$); and 2–12 mo, 123 sites (S, $n = 80$; NS, $n = 43$). Box-plots with the median, quartiles and extreme values are shown. Timeline of measurements: baseline, post-SRP, and maintenance phase 2, 4, 6, 8, 10 and 12 mo.

SRP (Table 1). In progressing smokers' sites, pockets were significantly deeper at baseline than in stable smokers' sites ($p < 0.016$) and remained deeper during the whole maintenance phase ($p < 0.05$ for all time points) (Fig. 3).

Stable and progressing nonsmokers' sites – The difference between MMP-8 concentrations in progressing nonsmokers' sites ($n = 5$) and stable nonsmokers' sites ($n = 38$) were statistically not significant at baseline, after SRP or at any maintenance visits. However, progressing nonsmokers' sites had, at every measurement, like progressing smokers' sites, a trend for broader distributions than stable sites. In progressing smokers' sites, the MMP-8 concentrations reached the same or even higher levels as in the respective nonsmokers' sites. (Fig. 3).

Sites with repeatedly elevated MMP-8 concentrations during the maintenance phase – Smokers' and nonsmokers' sites with an MMP-8 concentration of ≥4000 μg/l at two or more time points during the maintenance phase were explored separately. One nonsmokers' site and eight smokers' sites met this criterion. All smokers' sites belonged to two patients, and five out of these eight were progressing sites.

Differences between the MMP-8 concentrations of smokers' sites with repeatedly high MMP-8 concentrations (high-responder smokers' sites, $n = 8$) and smokers' sites with lower concentrations of MMP-8 (low-responder smokers' sites, $n = 72$) were statistically significant at measurements made at 4–12 mo ($p < 0.05$). The concentrations reached exceptionally high values in high-responder sites. In neither group of sites did the MMP-8 concentration decrease significantly after SRP (Table 1). In low-responder sites, pockets were significantly shallower than in high-responder sites at baseline and at all time points of the maintenance phase ($p < 0.05$ for all time points). In these sites, the mean PD decreased statistically significantly after SRP ($p < 0.001$), unlike in high-responder sites ($p = 0.2$). Moreover, the AL showed a significant decrease after SRP in low-responder sites ($p < 0.001$), but not in high-responder sites ($p = 0.6$) (Table 1) (Fig. 4).

MMP-8 chair-side test results

For a positive test-stick result, the threshold was set at an MMP-8 concentration of 1000 μg/l (Fig. 5), and test positive results of individual sites were registered as follows: + (weak blue line), + + (clear blue line) and + + + (strong blue line).

In the data processing of the maintenance phase of the study, the test stick revealed too many test-positive results if all test-positive results were taken into consideration. Therefore, test-positive percentages were calculated and expressed as the following two groups of percentages: all positive results + to + + + and clear positive results + + to + + +.

The test result was more often positive in nonsmokers' sites than in smokers' sites at baseline. Test-positive percentages decreased significantly after SRP in all groups of nonsmokers' sites. The change between baseline and post-SRP test-positive results was not statistically significant in any group of smokers' sites (Table 2).

The percentage of clearly positive test results (+ + to + + +) during the maintenance phase of progressing and stable smokers' sites and of progressing and stable nonsmokers' sites were at the same level (Table 3). The percentage of clearly positive test results in high-responder smokers' sites was higher than in low-responder smokers' sites (Table 3). The sensitivity of the test, based on maintenance phase test results for high-responder and low-responder smokers' sites, was 0.41, with specificity 0.94 and positive likelihood ratio 6.8. In high-responder sites, test-positive percentages increased after SRP and were, at 4–12 mo maintenance visits, statistically significantly ($p < 0.05$ for all time points) higher than in low-responder smokers' sites (Table 4).

Bleeding on probing scores

When percentages of all positive BOP scores per group of sites during the maintenance phase were calculated, the percentages were higher in progressing smokers' and nonsmokers' sites and high-responder smokers' sites than in stable smokers' and nonsmokers' sites and low-responder smokers' sites. Positive BOP percentages were at a

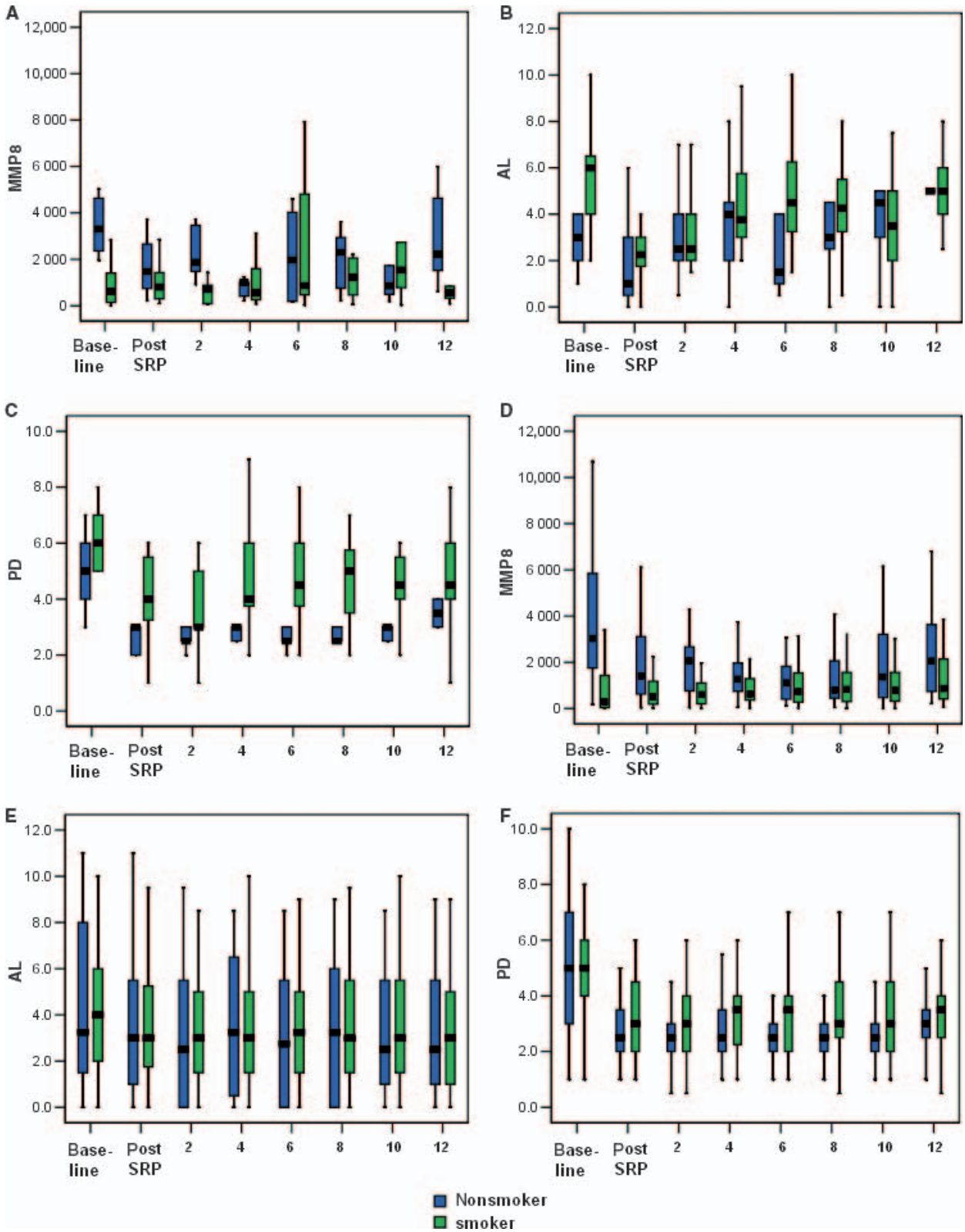

Fig. 3. Comparisons between smokers' (S) and nonsmokers' (NS) sites. (A) Matrix metalloproteinase-8 (MMP-8) concentrations, (B) probing depths (PD) and (C) attachment losses (AL) of progressing S and NS sites (S, $n = 16$; NS, $n = 5$). (D) MMP-8 concentrations, (E) PD and (F) AL of stable S and NS sites (S, $n = 64$; NS, $n = 38$). Box-plots with the median, quartiles and extreme values are shown. Timeline of measurements: baseline, post-SRP, and maintenance phase 2, 4, 6, 8, 10 and 12 mo.

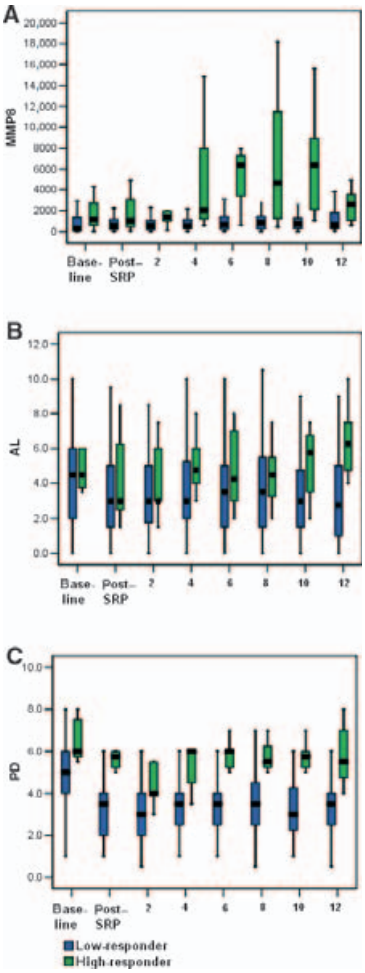

Fig. 4. High-responder smokers (S) sites [matrix metalloproteinase-8 (MMP-8) ≥4000 μg/l at least twice during maintenance, $n = 8$] and low-responder S sites ($n = 72$). (A) MMP-8 concentrations (note a different scale on the *y*-axis compared with the other figures), (B) attachment losses (AL) and (C) probing depths (PD). Boxplots with the median, quartiles and extreme values are shown. Timeline of measurements: baseline, post-SRP, and maintenance phase 2, 4, 6, 8, 10 and 12 mo.

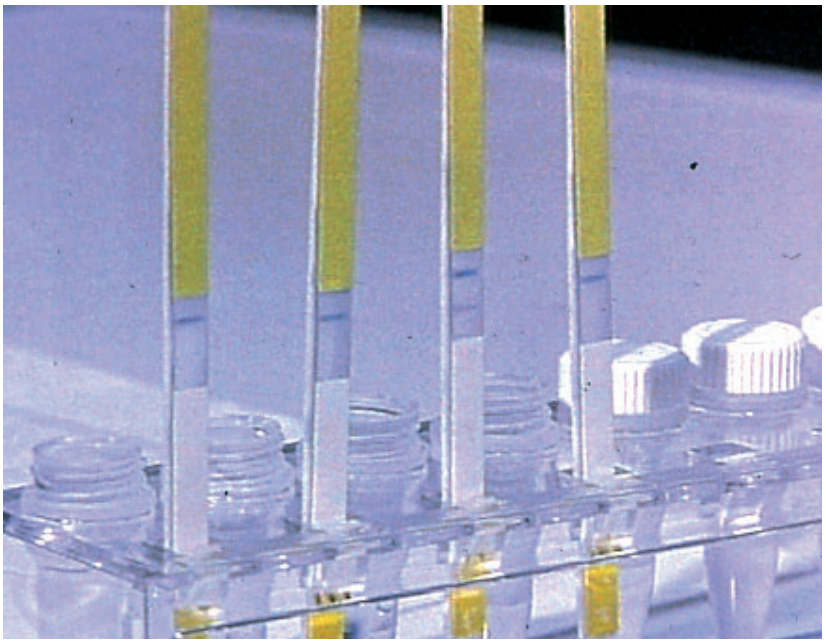

Fig. 5. Demonstration of the matrix metalloproteinase-8 (MMP-8)-specific test stick for the chair-side use. The dip-stick is based on immunochromatography; see the Materials and methods for more details. The appearance of a second blue line in the detection area indicates a gingival crevicular fluid (GCF) MMP-8 concentration of ≥ 1000 μg/l.

Table 3. Clear positive (+ + to + + +) and negative test results of smokers'and nonsmokers' groups of sites during the maintenance phase (after scaling and root planing to 12 mo)

		Progressing	Stable	High-responder	Low-responder
Nonsmokers' sites	+	14%	12%		
	–	86%	88%		
Smokers' sites	+	15%	10%		
	–	85%	90%		
	+			45%	6%
	–			54%	94%

Table 4. Positive test result and bleeding on probing (BOP) percentages of high-responder smokers' sites (n = 8) and low-responder smokers' sites (n = 72) (for the test result percentages of + to + + +/+ + to + + +, respectively)

	Baseline %	Post-SRP %	2 mo %	4 mo %	6 mo %	8 mo %	10 mo %	12 mo %
Test								
$n = 8$	100/13	50/25*	80/40	75/38	88/50	63/50	75/50	75/63
$n = 72$	31/13	26/10*	29/3	16/3	25/9	53/7	27/4	45/11
BOP								
$n = 8$	100	38	20	63	13	63	25	38
$n = 72$	58	11	7	29	26	36	32	14

*The *p*-value for the difference between baseline and after scaling and root planing (post-SRP) is not significant.

similar level in stable smokers' and nonsmokers' sites and low-responder smokers' sites. BOP-positive percentages in progressing smokers' sites and high-responder smokers' sites were slightly higher than in progressing nonsmokers' sites (Table 5).

Discussion

Previous reports have detailed that measurements of MMP-8 concentration (made using IFMA) in the GCF of periodontally healthy individuals and of patients with gingivitis or periodontitis suggested a level of 1000 μg/l as the cut-off concentration for a positive test result in the chair-side GCF MMP-8 test (32). In this longitudinal study, however, it was obvious that the

Table 5. Positive and negative bleeding on probing (BOP) score percentages of smokers' and nonsmokers' groups of sites during the maintenance phase (after scaling and root planing to 12 mo)

		Progressing	Stable	High-responder	Low-responder
Nonsmokers' sites	+	30%	22%		
	–	70%	78%		
Smokers' sites	+	43%	28%		
	–	57%	72%		
	+			38%	22%
	–			62%	78%

cut-off concentration utilized in the prototype of the test was too low to be useful for predictive purposes. We had several false-positive test results when a weak blue line was recorded as positive test result. It is not unusual in treated periodontitis patients' (i.e. patients in the maintenance phase) for sites with no signs of active disease, or with shallow pocket depths, to have GCF MMP-8 concentrations exceeding 1000 μg/l. No normal clinical values for MMP-8 in GCF have been established, but our previous studies suggested that 1000 μg/l, as determined with IFMA, is the concentration which differentiates periodontitis patients from gingivitis subjects and from periodontally healthy individuals (32,33). When test recordings from + + (clear blue line) to + + + (very strong blue line) were accepted as positive results, positive test results targeted to groups of smokers' sites with unstable characteristics during the maintenance phase. In light of our results, repeatedly high concentrations of MMP-8 in the GCF of a maintenance phase patient or fluctuation from low to high concentration with consecutive measurements, especially among smokers, seems to reflect sites in danger of progression of periodontitis and this may be helpful for a dentist when choosing between treatment options.

Progressing smokers' sites exhibited a trend for a broader distribution of MMP-8 concentrations, indicating that a fraction of sites had high MMP-8 concentrations, while some sites had concentrations comparable with stable sites. In stable sites, the distribution of MMP-8 concentrations were narrower and remained stable throughout the study. Beta-glucuronidase activity in the GCF of periodontitis patients shows a similar trend in that it increases with increasing PD, and this can be observed as broadening of the distribution, rather than as a shift in the distribution of the values (34). Periodontitis may progress as bursts of individual sites. Thus, a biomarker which may indicate disease activity may only be elevated for short time periods, and optimal testing depends on timing. Baseline sensitivity of the MMP-8-specific test, related to clinical diagnosis, provided a sensitivity of 0.83 and specificity of 0.96 when MMP-8 concentrations were compared with test-positive results from periodontally healthy subjects, and patients with gingivitis and periodontitis (32). However, sensitivity of the test calculated from longitudinal data becomes poor because of the nature of periodontitis: at the time point of testing, a fraction of the tested sites, even those categorized as progressing, may be quiescent as regards the tested marker. In our material, an exception were smokers' sites with poor treatment response, in which GCF MMP-8 concentrations were elevated practically all the time. Because of the criterion set for the progressing site in our study, we missed sites with minor or fluctuating AL changes. Also in sites categorized as stable, MMP-8 concentrations could temporarily increase and give positive test results.

Despite a general lower mean concentration of MMP-8 in smokers' GCF, progressing smokers' sites showed MMP-8 concentrations as high as respective nonsmokers' sites, and a relatively small group of smokers' sites exhibited the highest MMP-8 concentrations. When these high-responder smokers' sites were compared with low-responder smokers' sites, high-responder smokers' sites were those with the deepest pockets and a poorer response to SRP. Therefore, MMP-8 concentration seems to indicate poor treatment outcome and it eventually identifies patients with refractory periodontitis.

The GCF MMP-8 concentrations of nonsmokers indicate different 'normal levels' than the MMP-8 concentrations of smokers' GCF. Nonsmokers also responded better to conventional periodontal therapy and are more easily maintained. During the maintenance phase of the study, the MMP-8 concentrations of nonsmokers' GCF did not exceed 5000 μg/l while in some smokers the GCF MMP-8 concentration was, at best, > 10,000 μg/l, despite smokers' generally lower levels of MMP-8. Thus, the increase of MMP-8 concentration, especially in smokers' GCF, may be an indicator of progression of periodontitis. Smokers' poorer response to conservative therapy has been recognized (18–20,35). Söder (36) reported that the periodontal pockets of smokers, with high values of MMP-8, are significantly deeper than pockets with low values of MMP-8. In the same study, a positive correlation between PD and levels of MMP-8, both in smoking and nonsmoking patients with refractory periodontal disease, could be detected. Deep periodontal pockets are in constant risk of disease progression, and PD correlates with future loss of attachment (37,38). In our data these were especially smokers' sites. AL did not differ between high-responder smokers' sites and low-responder smokers' sites, suggesting that sites with gingival recession (AL with gingival retraction and thus shallower pocket) are more easily controlled.

Among smokers, less bleeding on probing has been confirmed in several studies (6–10) even in spite of a two- to three-fold greater disease severity determined by the number of deep pockets (8). This could not be confirmed with our material when BOP-positive percentages during the maintenance phase were examined. In

smokers' unstable sites, BOP was more common than in stable sites, and the percentage was slightly higher than in nonsmokers' progressing sites, although the number of nonsmokers' progressing sites was small.

We conclude that repeatedly elevated GCF MMP-8 concentrations can recognize periodontal sites which are at risk for disease progression, especially among smokers. Sites in which the MMP-8 concentration does not decrease with SRP are sites with poor treatment outcome measured using clinical parameters. The MMP-8 specific chair-side test could be useful for monitoring periodontal stability if a higher concentration was chosen as cut-off value. It is worth nothing that, our MMP-8 antibody used in IFMA and the chair-side dip-stick test identifies both latent and active forms of MMP-8 (39). Thus, it is not completely selective or specific for the active form of MMP-8. In the future, MMP-8 dip-stick tests for monitoring periodontal stability should be supplied with an MMP-8 antibody selective and specific for the active form of MMP-8, because the elevation of the active form of MMP-8 in GCF has been shown to be associated with both conversion of gingivitis to periodontitis and progression of periodontitis (15,28,40). Nevertheless, in our previous study (32) gingivitis could be differentiated from periodontitis with a cut-off value of 1000 μg/l, which gives the weak visual blue line in the dip-stick test. This observation permits speculation that gingivitis patients with repeatedly positive MMP-8 test results in our prototype test (with a cut-off value 1000 μg/l) may be patients who are at risk of periodontitis or are already undergoing irreversible minor changes which are difficult to diagnose with standard clinical methods.

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