# Smoking Confounds the Periodontal Diagnostics Using Saliva Biomarkers

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**Summary of the key findings: The diagnostic value of saliva MMP-8 was mainly decreased by pack years of smoking, whereas saliva MMP-9, TIMP-1, and MPO were mostly disturbed by time since cessation.**

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**Abstract**

**Aim:** Smoking is a risk factor for periodontal disease due to its complex impact on the inflammatory response in the periodontium. We investigated the effect of smoking on salivary periodontal biomarkers, matrix metalloproteinase (MMP)-8, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 and myeloperoxidase (MPO).

**Materials and Methods:** Saliva biomarkers were analyzed in the Parogene population (n=480) comprising a random cohort of patients undergone coronary angiography and oral examination. The effect of time since cessation and pack years of smoking on biomarkers were investigated.

**Results:** Saliva MMP-8, MMP-9, TIMP-1, and MPO concentrations distinguished periodontitis patients significantly from patients without periodontitis. When the time since cessation was considered, the area-under-the-curve values (p-value) for periodontitis were 0.76 (<0.001), 0.74 (<0.001), 0.70 (<0.001), and 0.76 (<0.001), respectively. Adding information about smoking habits in the models improved slightly the sensitivities of all biomarkers. In logistic regression model saliva MMP-8 was mainly affected by pack years of smoking, while saliva MMP-9, TIMP-1, and MPO were mostly affected by time since cessation, especially if smoking currently or quit recently (<1 year ago).

**Conclusions:** Smoking confounds the salivary diagnostics of periodontitis and should be considered when interpreting the results obtained by potential diagnostic tests.

## INTRODUCTION

Smoking has deleterious effects on oral and systemic health.[1] It is one of the established risk factors for periodontal disease and elevated odds for periodontal progression are observed for both involuntary and active smokers.[2] Smoking disturbs the normal host response,[3] and smokers have more progressed periodontal tissue destruction, i.e. greater attachment loss, deeper probing depths,[4] more tooth loss, and more alveolar bone loss (ABL) compared with non-smokers.[5] Smoking causes vasoconstriction, [6,7] thus less gingival bleeding is observed in patients with periodontitis who smoked. [7] This phenomenon may lead to delayed periodontal diagnosis, because part of the clinical signs of the disease is masked. Additionally, smoking is associated with poor response to periodontal therapy,[8] and its cessation improves response to periodontal treatment.[9] Smoking can increase saliva rate [3] and GCF volume, [10] but it can also decrease the volume of GCF,[11] which can affect the concentrations of inflammatory mediators measured from saliva. Smoking decreases the production of several cytokines and chemokines, thus causing immunosuppressant effects which enhanced susceptibility to periodontitis.[12] Smoking modulates several pathways and measures, thus the overall impact of smoking on periodontium is cumbersome.

Matrix metalloproteinases (MMPs) are extracellular matrix (ECM) degrading enzymes, and these proteases have been investigated abundantly in periodontal diseases. In our recent study we investigated saliva MMP-8, MMP-9, TIMP-1, and MPO as biomarkers of periodontitis.[13] MMP-8,[14-18] MMP-9,[17, 19] and MPO[19,20] measured in oral fluids are elevated, whereas significantly lower concentrations of saliva TIMP-1 have been measured in patients with periodontitis compared with controls.[16, 20]

Even though, saliva MMP-8, MMP-9, and MPO are useful biomarkers, smoking may influence them. The effects of smoking are complex and the results from earlier studies are diverse. For example, MMP-8 concentrations in oral fluids have been reported to be either

higher, lower, or similar based on smoking status. Smokers had significantly elevated MMP-8 levels in saliva,[4] higher MMP-8 protein expression in periodontal tissue,[21] and higher collagenolytic activity[8] when compared with non-smokers. On the contrary, lower levels of MMP-8 in oral fluids are reported in smokers compared with non-smokers.[22-25] In addition, studies where saliva or GCF MMP-8 concentrations did not differ significantly according to smoking status also exists.[2, 26, 27] In the case of MMP-9, total saliva levels were lower in smokers compared to non-smokers,[27] but in other studies saliva or GCF MMP-9 did not differ significantly according to smoking status.[2, 26] On the other hand, saliva TIMP-1 was elevated in smokers compared to non-smokers both in periodontitis and control subjects.[16] Smokers had, especially in serum, elevated MPO compared to non-smokers,[28] but when analyzed in saliva, MPO did not differ significantly according to smoking status.[29]

Smoking habits can be specified by characteristics such as duration, intensity, and time since cessation[26] as well as cotinine levels measured from saliva or other body fluids.[30] Saliva cotinine levels[7] and pack years of smoking[31] have been shown to correlate with periodontal disease severity and progression, i.e. tooth loss and implant failure.[1] Furthermore, the risk of tooth loss reduced as a function of a time after smoking cessation.[1] Even in the short-term, cessation of smoking can result in improved oral health.[9] However, oral health may take several years or decades to improve, and individuals who stopped smoking may never lower the risk of periodontitis to the level compared to individuals who have never smoked. Taken together, we aimed to investigate which smoking dimension; time since cessation or pack years of smoking, have the most notable effect on salivary periodontal biomarkers, MMP-8, MMP-9, TIMP-1, and MPO.

## MATERIALS AND METHODS

### Subjects and Diagnosis

The study population comprised PAROGENE cohort (N=508), which is a subsample of the larger Corogene cohort (N=5809).[32] PAROGENE patients underwent coronary angiography in Helsinki University Hospital, and oral examination was performed to all patients.[33] Concentrations of saliva biomarkers, MMP-8, MMP-9, TIMP-1, and MPO, were available for 480 patients from our earlier study.[13]

The oral examination included clinical measurements, such as periodontal probing pocket depth (PPD) measurements from six points of each toot and bleeding on probing (BOP) registration, and alveolar bone loss (ABL) calculation from radiographic examination as described earlier.[33] If the patient had alveolar bone loss (mild to severe; ABL in cervical third of the root to total ABL) and if periodontal probing pocket depths (PPD) measurement was ≥4 mm in ≥4 sites the patient was categorized as having periodontitis.[13] Patients having no periodontitis included periodontally healthy, gingivitis, and edentulous patients. The periodontitis group included older, diabetic, smoking, dyslipidemic, and more often statin treated patients compared to non-periodontitis group.[13]The cardiac diagnosis was set according to the results from the coronary angiography. The cardiac diagnoses were no coronary artery disease (CAD), stable CAD, acute coronary syndrome (ACS), and "ACS-like, no significant CAD".[33] The cardiac condition had minor effects on the concentrations of the selected biomarkers as presented in our earlier article.[13]

The information about smoking cessation and pack years of smoking was available for 480 and 419 patients, respectively, and the information was self-reported. Due to self-reporting, some data on smoking habits was not recorded. Information on pack years of smoking was lacking from 60 subjects including 41 patients who quit more than a year ago, 10 patients

who quit less than a year ago, and 9 current smokers. Of these patients 22 were non-periodontitis patients and 39 were characterized as having periodontitis. Their cardiac status was grouped in a following way: no CAD (N=9), stable CAD (N=26), ACS (N=23), and "ACS-like, no significant CAD" (N=4). Saliva MMP-8, MMP-9, TIMP-1, or MPO concentrations did not differ significantly in patients with and without information about the pack years. All participants provided written informed consent.

The patients were divided into groups according to smoking cessation in the following way: never smokers (N=227), quit more than a year ago (N=150), quit less than a year ago (N=47), and current smokers (N=56).[9] When information on pack years of smoking was utilized we classified the patients similarly as reported earlier[5]: 0 pack years of smoking (N=227), 1-20 pack years of smoking (N=94), and >20 pack years of smoking (N=98). Pack years of smoking were calculated by number of cigarettes smoked per day divided by 20 and multiplied by the number of years smoked.

**Laboratory determinations**

Laboratory determinations were performed from the supernatants of the stimulated saliva samples[34] with ELISA for MMP-9, TIMP-1, and MPO. MMP-9 **#,** TIMP-1 ******, and MPO †† measurements were performed according to instructions of the manufacturers. Following dilutions for saliva samples were used: 1:20 in MMP-9, 1:10 in TIMP-1, and 1:40 in MPO. Saliva MMP-8 concentrations were measured with time-resolved immunofluorometric assay (IFMA).[35] The inter-assay CV% (N=12) for MMP-8, MMP-9, TIMP-1, and MPO were 7.7 %, 6.0 %, 8.1 %, and 10.9 %, respectively. The detection limits were 0.08 µg/L, 0.04 µg/L, 0.08 µg/L, and 0.05 µg/L, respectively.

**Statistics**

If the variable was normally distributed, means and standard deviations (SD) were used. Variables displaying skewed distribution were presented as medians and interquartile ranges (IQR). Tests used for calculating the statistical significance of the differences were Kruskal-Wallis test, paired samples t-test, and Mann-Whitney test for continuous variables, and Chi-square test for categorical variables. P=0.05 was defined as threshold for statistical significance. Correlations were analyzed by using Spearman's correlation. The diagnostic value (i.e. sensitivity and specificity) of biomarkers were evaluated with C-statistics utilizing predicted probabilities, and the statistical significance between predicted probabilities were calculated by using paired samples t-test.

The sensitivities were calculated according to the cut-offs determined earlier.[13] The reference C-statistics model (Model 1) was adjusted for age, sex, cardiac status, and diabetes. Model 2 was additionally adjusted for time since cessation and Model 3 for pack years of smoking in categories described above.

Three different multivariate logistic regression models were used to determine the association of saliva biomarkers with periodontitis. Model 1 was adjusted for age, sex, cardiac status and diabetes (N=477). Model 2 was adjusted further for smoking cessation (N=476) and Model 3 alternatively for pack years of smoking (N=415). In these logistic regression models gender, cardiac status, diabetes, and smoking were set as categorical variables. The analyses were performed using a statistical software package [##].

## RESULTS

Characteristics of subjects according to smoking habits differed in age, number of teeth, BOP, PPD of 4-5 mm and ≥6 mm, gender, and CAD status (Table 1). Characteristics according to pack years of smoking differed in age, number of teeth, BOP, PPD of 4-5 mm, and gender (Table 2).

Saliva MMP-9 concentrations differed statistically significantly according to smoking habits ($p=0.004$); the most notable difference was between current and never smokers ($p<0.001$) (Table 3). Furthermore, the concentrations differed significantly according to pack years of smoking ($p=0.022$), and these concentrations were significantly lower in subjects >20 pack years compared to never smokers ($p=0.013$) (Table 3). On the contrary, saliva TIMP-1 was significantly higher in subjects >20 pack years of smoking compared to never smokers ($p=0.044$). Saliva MMP-8 concentrations did not differ according to time since cessation or pack years of smoking (Table 3).

The effect of smoking on saliva biomarkers was investigated with different C-statistics models (Table 4). The highest AUC-values were observed in Model 2, in which time since cessation was taken into account, 0.76 ($p<0.001$), 0.74 ($p<0.001$), 0.70 ($p<0.001$), and 0.76 ($p<0.001$) for MMP-8, MMP-9, TIMP-1, and MPO respectively (Table 4). There were only minor differences in sensitivities of biomarkers between different C-statistics models using the set specificities.[13] The most notable differences were observed in saliva MMP-9 and TIMP-1; model taking into account time since cessation improved the sensitivity to 0.72 (from 0.62) and 0.72 (from 0.59), respectively (Table 4). In the pairwise testing of the probabilities, however, only the improvement gained by adding pack years to the MMP-8 model reached the statistical significance.

The categories of smoking habits and pack years of smoking had similar correlations with the saliva biomarkers (Table 5) and they also had highly significant correlations (<0.001) with each other in all groups (coefficients, whole 0.918, periodontitis 0.886, and no-periodontitis 0.955). Saliva MMP-9 correlated negatively with both smoking dimensions in the whole population and the periodontitis subgroup (Table 5). Saliva MMP-8 and MPO correlated negatively with smoking only in the no-periodontitis subgroup, whereas saliva TIMP-1 had a positive correlation with smoking only in the periodontitis subgroup.

Saliva MMP-8, MMP-9, TIMP-1, and MPO associated significantly with periodontitis in logistic regression (Model 1), which was adjusted for age, sex, cardiac status, and diabetes (Table 6). The associations of saliva biomarkers with periodontitis strengthened when adjusting the models further for smoking (Table 6). The strongest association of saliva MMP-8 with periodontitis was achieved when adjusting the model for pack years of smoking (Model 3). On the other hand, the strongest associations of MMP-9, TIMP-1, and MPO were achieved when adjusting the model for smoking moment (Model 2, Table 6). In this model, current smoking and "<1 year since cessation" categories presented significant ORs for all saliva biomarkers, but the category ">1 year since cessation" was non-significant.

## DISCUSSION

In this relatively large study with detailed information on patients' smoking habits we found that smoking exhibits considerable disturbing effect on the performance of MMP-8, MMP-9, TIMP-1, and MPO as saliva biomarkers for periodontitis. When saliva MMP-8 was used to classify the patients into groups with and without periodontitis the results were adversely affected by pack years of smoking, whereas the classification results by using saliva MMP-9, TIMP-1, and MPO concentrations were unfavourably affected by time since cessation.

Interestingly, current smoking and "quit <1 year ago" were strong and significant confounders in logistic regression model, whereas "quit >1 year ago" was a non-significant covariate in all models. The result suggests that the disturbing effect of smoking on the use of these saliva biomarkers is reversible. Thus, time since cessation appeared to be the most prominent dimension of smoking affecting these biomarkers. Pack years of smoking was also a significant covariate in logistic regression models, but dose-dependent response was not observed, even though pack years of smoking is known to correlate significantly with periodontal disease severity.[1]

Saliva MMP-8, MMP-9, TIMP-1, and MPO have been frequently investigated as periodontal biomarkers,[36] and the most investigated is MMP-8 in saliva and other oral fluids.[14, 15, 18, 25, 37] However, more studies revealing factors affecting these biomarkers, including systemic diseases and human habits, are needed. Especially smoking as a strong risk factor for periodontitis and a known confounder of salivary diagnostics deserves more investigations.

The literature on the effect of smoking on saliva MMP-8 and MMP-9 presents conflicting results.[2, 4, 23, 27] This is probably because of the difficulties to estimate the exact amount of all dimensions of smoking (duration, intensity, and time since cessation) [26] and because smoking has direct and complex effects on inflammatory cascades and thereby host-derived biomarkers.[6, 38] Smoking has been associated with higher amount of total white blood cells, and current smoking habit displayed a stronger effect than pack years.[39] The effect was strongest in granulocytes: current smokers had highest amount of granulocytes and significant decreasing trend in granulocyte account with time since cessation was observed, never smokers having the lowest amount.[39] Smoking can also activate lymphocytes,[40] and increase CRP levels.[41] These effects of smoking on inflammatory cells can influence further on MMP production and secretion.

One of the strengths of our study is that both the time since cessation and the pack years of smoking were studied thereby describing both the duration and the intensity of smoking at the same time. The possibility to compare the effect of the two different smoking dimensions provided interesting results. The logistic regression models where the time since cessation was used showed better discrimination abilities (i.e. sensitivities of given specificities) and improved associations of saliva MMP-9, TIMP-1, and MPO with periodontitis. Taking into account the pack years of smoking improved the association of saliva MMP-8 with periodontitis and improved the AUC significantly compared to that obtained without the information on the pack years of smoking.

Time since cessation is a variable used very diversely in earlier studies. Periodontal risk assessment produces a relatively low risk for periodontitis recurrence in former smokers, who have more than 5 years since cessation.[42] However, significant beneficial effects on periodontal parameters are gained already after 12 months after quitting.[9] Thus, alterations in saliva may occur quickly after cessation, because saliva and inflammatory cells are regenerating rapidly.

In our study smoking status is self-reported, thus inaccuracy may appear. All subjects did not wish to inform their amount of smoking, hence pack year information could not be calculated for everyone. Furthermore, we do not have cotinine level, which is used as a validated biomarker of smoking status.[43] Our study design is cross-sectional, and we do not have samples from the same patients in different time points according to time since cessation. Thus, the analyses are retrospective and intra-personal analysis cannot be performed. Our study population and samples are collected at time when electric cigarettes were not commonly used, so that cannot be considered as a confounder. We also lack information about the use of snuff or nicotine replacement products which could probably affect the results. To summarize, smoking has a clear confounding impact on saliva biomarkers, and

smoking needs to be taken into account when using saliva biomarkers in periodontal diagnostics.

## CONCLUSIONS

This is a study to investigate the effect of different smoking dimensions, duration, intensity, and time since cessation, on saliva periodontal biomarker diagnostics. Smoking is a strong risk factor for periodontitis and its effect on inflammatory cascades is complex. The performance of saliva MMP-8 as biomarker was mainly affected by pack years of smoking, whereas the discrimination ability of MMP-9, TIMP-1, and MPO was influenced by time since cessation. Our results suggest that smokers and those who have quit smoking recently may easily present false negative results when biomarkers are measured. Thus, smoking is a crucial factor that needs to be taken into account in biomarker diagnostics.

## COMPLIANCE WITH ETHICAL STANDARDS

Ethical approval: The study was conducted according to the Declaration of Helsinki. The ethical committee of the Helsinki University Central Hospital approved the study design (approval reference number 106/2007). No animal studies were carried out by the authors for this article.

Informed consent: All participants provided informed consent.

## FOOTNOTES

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## IBM SPSS Statistics 24

## CONFLICT OF INTEREST AND SOURCES OF FUNDING STATEMENT:

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**Table 1:** Characteristics of subjects according to the smoking status

| **Parameters** | | **Never smokers (N=227)** | **Quit more than a year ago (N=150)** | **Quit less than a year ago (N=47)** | **Current smokers (N=56)** | **p-value*** |
|---|---|---|---|---|---|---|
| | | | Mean (SD) | | | |
| Age (years) | | 63.9 (9.7) | 65.5 (7.5) | 57.3 (9.2) | 57.4 (6.7) | **<0.001** |
| BMI (kg/m$^2$) | | 27.5 (4.9) | 28.2 (4.6) | 28.0 (5.9) | 29.1 (6.4) | NS |
| Number of teeth | | 22.9 (6.7) | 18.8 (8.5) | 21.5 (6.7) | 20.3 (7.7) | **<0.001** |
| BOP (%, from 4 surfaces) | | 34.3 (17.4) | 39.0 (20.7) | 43.6 (19.3) | 41.8 (19.0) | **0.002** |
| Number of 4-5mm pockets | | 10.8 (10.9) | 10.9 (11.2) | 20.8 (15.3) | 22.9 (17.0) | **<0.001** |
| Number of ≥6mm pockets | | 2.7 (8.4) | 2.9 (8.4) | 5.7 (9.1) | 5.4 (8.1) | **<0.001** |
| | | | N (%) | | | |
| Gender (% men) | | 129 (56.8) | 112 (74.7) | 33 (70.2) | 40 (71.4) | **0.002** |
| Diabetes | | 54 (23.8) | 36 (24.0) | 6 (12.8) | 16 (28.6) | NS |
| Hypertension | | 140 (61.7) | 107 (71.3) | 25 (53.2) | 33 (58.9) | NS |
| Dyslipidemia | | 178 (78.4) | 129 (86.0) | 33 (70.2) | 46 (82.1) | NS |
| Pack years of smoking | 1-20 | - | 57 (38.0) | 18 (38.3) | 19 (33.9) | NS |
| | >20 | - | 51 (34.0) | 19 (40.4) | 28 (50.0) | |
| CAD status | No | 63 (27.8) | 32 (21.3) | 4 (8.5) | 16 (28.6) | **0.003** |
| | Stable | 80 (35.2) | 63 (42.0) | 12 (25.5) | 19 (33.9) | |
| | ACS | 69 (30.4) | 47 (31.3) | 30 (63.8) | 17 (30.4) | |
| | ACS-like, non- | 15 (6.6) | 8 (5.3) | 1 (2.1) | 4 (7.1) | |

| | |
|---|---|
| | significant |
| | CAD |

*Statistical significance tested by using the Kruskal-Wallis test for continuous variables and Chi-square test for categorical variables;
Significant values are bolded; NS = not significant

**Table 2:** Characteristics of subjects according to the pack years of smoking

| **Parameters** | **0 pack years of smoking (N=227)** | **1-20 pack years of smoking (N=94)** | **>20 pack years of smoking (N=98)** | **p-value*** |
|---|---|---|---|---|
| | | Mean (SD) | | |
| Age (years) | 63.9 (9.7) | 62.0 (8.8) | 61.4 (8.2) | **0.023** |
| BMI ($kg/m^2$) | 27.5 (4.9) | 28.1 (5.2) | 28.8 (5.7) | NS |
| Number of teeth | 22.9 (6.7) | 21.0 (7.1) | 18.7 (8.7) | **<0.001** |
| BOP (%, from 4 surfaces) | 34.3 (17.4) | 38.2 (19.4) | 44.5 (20.3) | **<0.001** |
| Number of 4-5mm pockets | 10.8 (10.9) | 15.6 (15.7) | 16.0 (14.7) | **0.032** |
| Number of ≥6mm pockets | 2.7 (8.4) | 3.6 (7.3) | 3.5 (6.7) | NS |
| | | N (%) | | |
| Gender (% men) | 129 (56.8) | 58 (61.7) | 81 (82.7) | **<0.001** |
| Diabetes | 54 (23.8) | 22 (23.4) | 23 (23.5) | NS |
| Hypertension | 140 (61.7) | 67 (71.3) | 60 (61.2) | NS |
| Dyslipidemia | 178 (78.4) | 81 (86.2) | 71 (72.4) | NS |
| CAD status | | | | NS |

| | | | |
|---|---|---|---|
| No | 63 (27.8) | 25 (26.6) | 18 (18.4) |
| Stable | 80 (35.2) | 32 (34.0) | 37 (37.8) |
| ACS | 69 (30.4) | 32 (34.0) | 38 (38.8) |
| ACS-like, non-significant CAD | 15 (6.6) | 5 (5.3) | 5 (5.1) |

Statistical significance tested by using the Kruskal-Wallis test for continuous variables and Chi-square test for categorical variables; Significant values are bolded; NS = not significant

**Table 3:** Saliva biomarker concentrations according to the smoking habits and the pack years of smoking

| Smoking habit | | MMP-8 | MMP-9 | TIMP-1 | MPO |
|---|---|---|---|---|---|
| | | Medians µg/L (IQR) | | | |
| | **Never** | 917 (397-1390) | 242 (104-531) | 178 (110-273) | 1899 (823-4839) |
| | **Quit >1 year ago** | 779 (252-1277) | 200 (65-528) | 180 (121-285) | 1382 (757-3209) |
| | **Quit <1 year ago** | 964 (577-1438) | 219 (79-540) | 210 (121-279) | 2239 (1225-5181) |
| | **Current** | 1072 (373-1522) | 113 (8-296) [§§] | 176 (114-270) | 1274 (562-4459) |
| **p-value [‡‡]** | | NS | p=0.004 | NS | NS |
| **Pack years of smoking** | **0** | 917 (397-1390) | 242 (104-531) | 178 (110-273) | 1899 (823-4839) |
| | **1-20** | 954 (421-1411) | 215 (51-462) | 160 (108-260) | 1607 (825-3983) |
| | **>20** | 822 (334-1306) | 164 (58-449) [¶¶] | 210 (141-283) [¶¶] | 1388 (704-3138) |
| **p-value [‡‡]** | | NS | p=0.022 | NS | NS |

Statistical significance compared to never smokers with the non-parametric Mann-Whitney test, marked as [§§] ($p<0.001$). Statistical significance compared to 0 pack years of smoking with non-parametric Mann-Whitney test, marked as [¶¶] ($p<0.05$). [‡‡] Refers to p-value between categories tested by Kruskal-Wallis test, NS= not significant, IQR= interquartile range from $25^{th}$ to $75^{th}$ percentile.

**Table 4:** ROC data describing the diagnostic ability of saliva biomarkers in periodontitis according to the time since smoking cessation and the pack years of smoking

| C-statistics | | | MMP-8 | MMP-9 | TIMP-1 | MPO |
|---|---|---|---|---|---|---|
| **Crude** | | AUC (95% CI) | 0.69 (0.65-0.74) | 0.64 (0.58-0.69 ) | 0.59 (0.54-0.64) | 0.68 (0.63-0.72) |
| | | p-value | <0.001 | <0.001 | 0.001 | <0.001 |
| | | Cut-off (µg/L)[¶¶] | 792.5 | 188.0 | 189.6 | 1451.5 |
| | | Sensitivity | 0.67 | 0.67 | 0.55 | 0.64 |
| | | Specificity | 0.62 | 0.61 | 0.59 | 0.62 |
| | **Model 1** | AUC (95% CI) | 0.73 (0.68-0.77), <0.001 | 0.68 (0.62-0.73), <0.001 | 0.63 (0.58-0.68), <0.001 | 0.72 (0.67-0.76), <0.001 |
| | | p-value | | | | |
| | | Sensitivity[§§] | 0.72 | 0.62 | 0.59 | 0.69 |
| | | Specificity | 0.62 | 0.61 | 0.59 | 0.62 |
| | **Model 2** | AUC (95% CI) | 0.76 (0.71-0.80), <0.001 | 0.74 (0.70-0.79), <0.001 | 0.70 (0.65-0.75), <0.001 | 0.76 (0.71-0.80), <0.001 |
| | | p-value | | | | |
| | | Sensitivity[§§] | 0.74 | 0.72 | 0.72 | 0.72 |
| | | Specificity | 0.62 | 0.61 | 0.59 | 0.62 |
| | **Model 3** | AUC (95% CI) | 0.74 (0.69-0.79), <0.001 | 0.70 (0.64-0.75), <0.001 | 0.66 (0.60-0.71), <0.001 | 0.72 (0.67-0.77), <0.001 |
| | | p-value | | | | |
| | | Sensitivity[§§] | 0.74 | 0.70 | 0.61 | 0.70 |
| | | Specificity | 0.62 | 0.61 | 0.59 | 0.62 |

Model 1: Adjusted for age, sex, cardiac status, and diabetes

Model 2: Adjusted for age, sex, cardiac status, diabetes, and smoking (never-quit >1 year ago-quit <1 year ago- current smokers)
Model 3: Adjusted for age, sex, cardiac status, diabetes, and pack years of smoking

NS = not significant; [§§] Sensitivity according to the specificity determined by using the cut-off concentration ([¶¶13]) for the whole population.

**Table 5:** Correlations of saliva MMP-8, MMP-9, TIMP-1, and MPO with smoking cessation moment and pack years of smoking

| | | MMP-8 | MMP-9 | TIMP-1 | MPO |
|---|---|---|---|---|---|
| **All** | | | | | |
| | Smoking cessation moment | NS | -0.138, 0.003 | NS | NS |
| | Pack years of smoking | NS | -0.134, 0.006 | NS | NS |
| **Periodontitis** | Smoking cessation moment | NS | -0.215, <0.001 | 0.130, 0.029 | NS |
| | Pack years of smoking | NS | -0.183, 0.004 | 0.202, 0.002 | NS |
| **No periodontitis** | Smoking cessation moment | -0.205, 0.004 | -0.216, 0.002 | NS | -0.211, 0.003 |
| | Pack years of smoking | -0.230, 0.002 | -0.197, 0.009 | NS | -0.232, 0.002 |

Significant Spearman correlation coefficients and p-values are presented; NS= not significant

**Table 6:** The association of saliva biomarkers with periodontitis - the effect of smoking

| | | | MMP-8 | MMP-9 | TIMP-1 | MPO |
|---|---|---|---|---|---|---|
| | | | **OR (95% CI), p-value** | | | |
| **Model 1 (reference)** | Biomarker (µg/L) | | **3.82 (2.52-5.79), <0.001** | **1.84 (1.35-2.49), <0.001** | **0.27 (0.14-0.56), <0.001** | **3.49 (2.39-5.09), <0.001** |
| **Model 2** | Biomarker (µg/L) | | **4.05 (2.65-6.20), <0.001** | **2.03 (1.46-2.83), <0.001** | **0.25 (0.12-0.52), <0.001** | **3.94 (2.64-5.88), <0.001** |
| | Time since cessation | | | | | |
| | | Never | 1 | 1 | 1 | 1 |
| | | Quit >1 year ago | 1.34 (0.84-2.13), NS | 1.45 (0.90-2.35), NS | 1.26 (0.81-1.97), NS | 1.27 (0.80-2.03), NS |
| | | Quit <1 year ago | 4.94 (2.15-11.35), <0.001 | 6.23 (2.57-15.12), <0.001 | 4.63 (2.09-10.26), <0.001 | 4.47 (1.99-10.06), <0.001 |
| | | Current | 5.33 (2.49-11.61), <0.001 | 10.07 (3.69-27.49), <0.001 | 4.04 (1.96-8.31), <0.001 | 6.13 (2.80-13.42), <0.001 |
| | | p-value[§§] | <0.001 | <0.001 | <0.001 | <0.001 |
| **Model 3** | Biomarker (µg/L) | | **4.36 (2.70-7.04), <0.001** | **1.77 (1.27-2.48), 0.001** | **0.26 (0.12-0.57), 0.001** | **3.58 (2.33-5.49), <0.001** |
| | Pack years of smoking | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 1 | 1 | 1 |
| | 1-20 | 2.25 (1.30-3.90), 0.004 | 2.73 (1.51-4.95), 0.001 | 2.14 (1.26-3.63), 0.005 | 2.28 (1.32-3.94), 0.003 |
| | >20 | 2.07 (1.19-3.59), 0.01 | 2.37 (1.33-4.21), 0.003 | 1.85 (1.09-3.12), 0.022 | 2.01 (1.17-3.46), 0.012 |
| | p-value[§§] | 0.003 | <0.001 | 0.006 | 0.003 |

Model 1: Adjusted for age, sex, cardiac status, and diabetes (N=477)
Model 2: Adjusted for age, sex, cardiac status, diabetes, and smoking (never-quit >1 year ago-quit <1 year ago- current smokers (N=476))
Model 3: Adjusted for age, sex, cardiac status, diabetes, and pack years of smoking (N=415)
[§§] refers to p-value between corresponding smoking categories; NS= not significant