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# Associations of oral fluid MMP-8 with periodontitis in Swiss adult subjects

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**Objective**: MMP-8 is a prominent collagenase in periodontal disease. This cross-sectional study examined whether MMP-8 levels in saliva and gingival crevicular fluid (GCF) are associated with periodontitis in a Swiss population.

**Subjects and Methods**: A total of 258 subjects (107 m, 151 f, mean age: 43.5 yr; range: 21–58 yr) acquired from the Swiss bone marrow donor registry participated in the study. Saliva and GCF samples were collected from subjects followed by a thorough dental and periodontal examination. MMP-8 levels were determined with immunofluorometric assay. Associations of MMP-8 levels with periodontal diagnosis, probing pocket depth (PPD) and bleeding on probing were statistically analysed with Pearson chi-square test, Spearman's rho and logistic regression analysis.

**Results**: MMP-8 in GCF correlated with MMP-8 in saliva ($p < .001$). Periodontitis was more common ($p < .001$) among subjects with high levels of MMP-8 in saliva and/or GCF compared with subjects with low levels of MMP-8. Higher MMP-8 levels in GCF and saliva were associated with any periodontal diagnosis (mild, moderate or severe), greater PPD, and bleeding on probing ($p < .05$). When age, gender, smoking, body mass index, number of medications and decayed, missing and filled teeth were adjusted for, all observed associations remained statistically significant. The area under curve of receiver-operating characteristic was 0.67 for saliva and 0.71 for GCF.

**Conclusion**: Elevated MMP-8 levels both in saliva and GCF are associated with periodontitis in a normal adult population.

KEYWORDS
gingival crevicular fluid, MMP-8, normal population, oral fluid, periodontitis, saliva

## 1 | INTRODUCTION

Periodontitis is a slowly progressing chronic polymicrobial infection-induced inflammation. Under inflammatory conditions, endogenous degradation pathways are activated and immune response leads to the release of destructive cellular molecules, that is proteases and reactive oxygen species as well as cytokines and chemokines (Kinane, Preshaw, & Loos, 2011; Sapna, Gokul, & Bagri-Manjrekar, 2014). Consequently, periodontitis has been shown to be associated with susceptibility to certain systemic diseases including diabetes, cardiovascular diseases and stroke (Alfakry, Malle, Koyani, Pussinen, & Sorsa, 2016; Lauhio et al., 2016; Lockhart et al., 2012; Noack et al., 2017; Palm et al., 2013).

Despite increasing evidence of potential links between the periodontal inflammatory burden and systemic health, diagnoses still rely on invasive clinical measurements and radiographic assessments.

These methods are expensive, requiring dental professionals and detecting the disease only in advanced states. Thus, discovering a biomarker with improved diagnostic capability and ease of use is of paramount importance.

Matrix metalloproteinases (MMPs) have an important role in periodontitis via degrading extracellular matrix components (Sorsa, Gieselmann, Arweiler, & Hernandez, 2017; Sorsa, Tjäderhane, & Salo, 2004; Sorsa, Heikkinen et al., 2017). Particularly, elevated levels of active neutrophil collagenase, MMP-8, have been shown to be associated with chronic periodontitis and accelerated disease progression (Ingman et al., 1996; Kinney et al., 2014; Lee, Aitken, Sodek, & McCulloch, 1995; Mäntylä et al., 2003, 2006; Ramseier et al., 2009; Sorsa et al., 2016; Sorsa, Gieselmann et al., 2017; Sorsa, Heikkinen et al., 2017). Due to these observations, MMP-8 is one of the most promising biomarkers of periodontitis (Sorsa, Mäntylä et al., 2011; Sorsa, Tervahartiala et al., 2011; Sorsa, Heikkinen et al., 2017; Sorsa, Gieselmann et al., 2017). Lateral flow MMP-8 point-of-care/chair-side tests have recently been developed for diagnosis and monitoring periodontal diseases and therapy as well as for genetic susceptibility to periodontitis. Thus, MMP-8 oral fluid tests are currently targets for extensive research (Heikkinen et al., 2016, 2017; Johnson et al., 2016; Lorenz et al., 2017; Mäntylä et al., 2003; Nwhator et al., 2014; Sorsa, Heikkinen et al., 2017; Sorsa, Gieselmann et al., 2017).

However, the vast majority of MMP-8 studies have focused on subjects with chronic periodontitis, and far less is known on the associations of MMP-8 on periodontal status among unselected normal population (Leppilahti, 2016). Additionally, MMP-8 tests are mostly conducted using gingival crevicular fluid (GCF) samples. GCF sampling is technically demanding compared with saliva sampling, and dental professionals are needed for performing the sampling to avoid contaminations. Thus, studies are warranted to determine if different MMP-8 tests are comparable, accurate and clinically valid (Heikkinen et al., 2016, 2017; Sorsa, Mäntylä et al., 2011; Sorsa et al., 2016; Sorsa, Gieselmann et al., 2017; Sorsa, Heikkinen et al., 2017; Uitto, Overall, & McCulloch, 2003).

This study examines whether MMP-8 in saliva and/or GCF are associated with periodontitis and could be used to distinguish different periodontal status markers and inflammatory burden among a group of Swiss normal population. Based on the previous findings, our hypothesis is that elevated MMP-8 levels in oral fluids are associated with periodontitis in an unselected adult population.

## 2 | MATERIALS AND METHODS

This observational cross-sectional study was approved by the Ethics Committee of Basel, Switzerland (Ethikkommission beider Basel, Ref. Nr. EK:357/08). The study population was recruited from the Swiss bone marrow donor registry by sending an invitation letter once to subjects in the register of The Blood Transfusion Service SRC Basel, Switzerland; 258 subjects (107 m, 151 f, mean age: 43.5 yr; range: 21–58 yr) participated in the study (Table 1), and informed consent was obtained from each subject.

**TABLE 1** Descriptive information about the study subjects

| | |
|---|---|
| Sex* | |
| Female | 151 (58.5%) |
| Male | 107 (41.5%) |
| Age** | 43.5 (21–58) |
| Smoking* | |
| Never | 150 (58.1%) |
| Former | 70 (27.1%) |
| Current | 38 (14.7%) |
| Any systemic disease* | 84 (32.6%) |
| Diabetes* | |
| No | 255 (98.8%) |
| DMI | 0 |
| DMII | 3 (1.2%) |
| Medications (Nro)** | 0.5 (0–10) |
| Body mass index** | 25.0 (17–49) |
| Decayed, missing and filled teeth** | 14.3 (0–30) |
| Periodontal dg* | |
| None | 138 (53.5%) |
| Mild | 5 (1.9%) |
| Moderate | 82 (31.7%) |
| Severe | 33 (12.8%) |

*n (%)
**mean (range)

Clinical oral examinations were carried out by two calibrated clinicians (MM 204 subjects; AR 54 subjects) in the Department of Preventive Dentistry and Oral Microbiology, School of Dental Medicine, University of Basel, Basel, Switzerland, between 2008 and 2011. A comprehensive anamnesis including systemic diseases, medications, height and weight was taken. Before any clinical assessments, stimulated whole saliva and GCF samples were collected. Paraffin wax stimulated whole saliva (256 samples) was collected as previously described (Mauramo et al., 2014). GCF samples were collected from mesiobuccal aspects of teeth 16, 12, 24, 36, 32 and 44 (i.e. Silness-Löe index teeth) by inserting a paper strip (Periopaper®; ProFlow, Amityville, NY, USA) into the gingival sulcus for 30 s (Sorsa et al., 2016). GCF samples were discarded if strips were contaminated with blood or time of GCF collection was <30 s (GCF samples of 183 subjects are included). The strips and saliva samples were directly placed into a sterile polypropylene tube and kept at −20°C until being analysed.

A thorough dental check-up including decayed, missing and filled teeth (DMFT) index was performed after collecting the samples. A complete periodontal status including probing pocket depth (PPD) and clinical attachment level measurement (CAL) were examined with a pressure-calibrated periodontal probe (Aesculap DB765R (0.2 N), B. Braun Melsungen AG, Hessen, Germany).

Additionally, bleeding on periodontal probing (BOP) present or not was recorded (Ainamo & Bay, 1975). Periodontitis was classified to severe, moderate, mild or no periodontitis according to the Center for Disease Control and Prevention and the American Academy of

Periodontology (Eke, Page, Wei, Thornton-Evans, & Genco, 2012). Briefly, periodontitis was diagnosed severe if ≥2 interproximal sites with CAL ≥6 mm and ≥1 interproximal sites with PPD ≥5 mm; moderate if ≥2 interproximal sites with CAL ≥4 mm or ≥2 interproximal sites with PPD ≥5 mm; and mild if ≥2 interproximal sites with CAL ≥3 mm and ≥2 interproximal sites with PPD ≥4 mm or ≥1 interproximal sites with PPD ≥5 mm.

MMP-8 levels in saliva and GCF samples were determined with time-resolved immunofluorometric assay (IFMA) as previously described (Hanemaaijer et al., 1997; Heikkinen et al., 2010). The monoclonal MMP-8-specific antibodies 8708 and 8706 (Medix Biochemica Oy Ab, Espoo, Finland) were used as a catching and tracer antibody, respectively. Europium chelate was used to label the tracer antibody. The samples were diluted in assay buffer containing 20 mM Tris–HCl, pH 7.5; 0.5 M NaCl; 5 mM CaCl2; 50 μM ZnCl2; 0.5% bovine serum albumin; 0.05% sodium azide; and 20 mg/l diethylenetriamine pentaacetic acid and incubated for 1 hr, followed by incubation for 1 hr with the tracer antibody. Enhancement solution was added, and after 5 min, the fluorescence was measured (1234 Delfia Research Fluoremeter; Wallac, Turku, Finland), and the levels of MMP-8 were expressed as microgram per litre. After the MMP-8 level determination, the MMP-8 levels in GCF samples of a subject were pooled; that is, the mean level of MMP-8 of the six GCF samples/subject was used in the analysis.

### 2.1 | Statistics

Firstly, correlations between MMP-8 levels in saliva and in GCF as well as correlations of MMP-8 levels in GCF collection site with the particular PPD of the same site (teeth 16, 12, 24, 36, 32 and 44) were calculated with Spearman's rank correlation coefficients (rho) along with *p*-values.

Secondly, prevalence percentages with Pearson chi-square test were calculated for periodontitis (yes or no) by MMP-8 levels (categorised low or high, cut-off at mean). Thirdly, to describe the specificity and sensitivity of MMP-8 levels in saliva and GCF as a diagnostic tool to determine periodontitis (yes or no), the area under the curve (AUC) of the receiver-operating characteristic (ROC) were calculated. AUC > 0.9 has high accuracy, while 0.7–0.9 indicates moderate accuracy and 0.5–0.7 low accuracy (Fischer, Bachmann, & Jaeschke, 2003). Additionally, the ROC curve analysis was conducted to determine optimal MMP-8 levels maximising the simultaneous sum of sensitivity and specificity.

Fourthly, uni- and multivariate ordinal logistic regression models were used to determine associations of MMP-8 levels in saliva and in GCF with the response variables, that is periodontitis (none *vs* mild, moderate or severe), bleeding on probing index and PPD in any site of the whole dentition. As there were only five subjects with "mild" periodontitis, the categories "mild" and "moderate" were merged. In the regression analysis, the MMP-8 levels in GCF and saliva were used as log-transformed metric predictors. Age, gender, smoking, body mass index (BMI), number of medications and DMFT were adjusted for. Results were expressed as odds ratios (OR) with 95% confidence intervals and *p*-values. *p*-Values of <.05 are considered as statistically significant.

**TABLE 2** Association of matrix metalloproteinases (MMP)-8 in saliva and gingival crevicular fluid (GCF) with periodontitis

| | Periodontitis | |
|---|---|---|
| | No (%) | Yes (%) |
| MMP-8 in saliva[a] | | |
| Low[b] | 99 (62.7) | 59 (37.3) |
| High[b] | 38 (38.8) | 60 (61.2) |
| MMP-8 in GCF[a] | | |
| Low[b] | 83 (72.2) | 32 (27.8) |
| High[b] | 26 (38.2) | 42 (61.8) |

[a]Pearson chi-square test statistically significant ($p < .001$).
[b]MMP-8 levels are divided into low and high according to mean.

## 3 | RESULTS

### 3.1 | MMP-8 levels in oral fluids and periodontitis

Mean MMP-8 levels in saliva were correlated with mean MMP-8 level in GCF (rho: 0.35; $p < 0.001$). The mean MMP-8 level in saliva was 173.1 ng/ml (range: 2.6–702.5 ng/ml; *SD*: 163.9) and in GCF 86.1 ng/ml (range: 3.3–338.0 ng/ml; *SD*: 74.3). When the mean MMP-8 level was used as a cut-off, the prevalence of periodontitis was 37.3% in the category of low MMP-8 level in saliva and 61.2% in the category of high MMP-8 level in saliva. For MMP-8 in GCF, the respective figures were 27.8% and 61.8%. These differences were statistically significant (Pearson chi-square test <0.001) (Table 2).

To further assess the specificity and sensitivity of MMP-8 in oral fluids to detect periodontitis, the ROC curve analysis was conducted. The most optimal cut-off (maximising the sum of sensitivity and specificity) for MMP-8 levels to determine periodontitis (yes *vs* no) was 73.6 ng/ml for saliva, and 69.2 ng/ml for GCF, corresponding to 0.55 (CI: 0.43–0.85) specificity and 0.76 (CI: 0.45–0.87) sensitivity for saliva and 0.74 (CI: 0.61–0.92) specificity and 0.65 (CI: 0.43–0.79) sensitivity for GCF. The AUC of the ROC for saliva was 0.67 (CI: 0.60–0.74) and for GCF 0.71 (CI: 0.63–0.79) (Figure 1).

### 3.2 | Associations of MMP-8 with different periodontal diagnoses

Logistic regression analysis showed that higher MMP-8 levels in saliva and GCF were associated with the severity of periodontitis. Any (mild, moderate or severe) periodontitis was more common in subjects with higher MMP-8 levels in GCF (OR: 2.59, CI: 1.83–3.78) and in saliva (OR: 1.69; CI: 1.34–2.16). The association was particularly clear for severe periodontitis (MMP-8 in GCF: OR: 3.19; CI: 1.71–6.49, and saliva: OR: 2.52; CI: 1.65–4.11). When age, gender, smoking, BMI, number of medications and DMFT were adjusted for, the associations of MMP-8 in GCF and in saliva with periodontitis remained statistically highly significant (Table 3). Of these confounders, age and current tobacco smoking were statistically significant independent predictors ($p < .05$) for periodontitis in all the regression models.

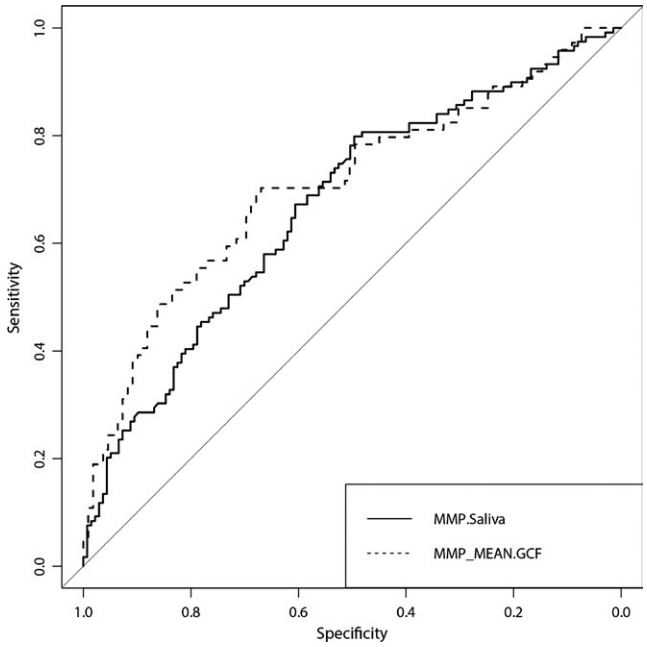


**FIGURE 1** Receiver-operating characteristic curves for saliva and gingival crevicular fluid MMP-8 tests

**TABLE 3** Uni- and multivariable logistic regression model of the associations of mean matrix metalloproteinases (MMP)-8 on periodontal diagnoses. Age, gender, smoking, body mass index, number of medications and decayed, missing and filled teeth were adjusted for. Results are expressed as odds ratios (OR), 95% confidence intervals (CI) and *p*-values

| Model | OR | CI | *p*-Value |
|---|---|---|---|
| Periodontitis (yes *vs* healthy) | | | |
| MMP-8 in gingival crevicular fluid (GCF) | 2.59 | 1.83–3.78 | <.0001 |
| MMP-8 in GCF; Adjusted | 2.27 | 1.47–3.61 | .0003 |
| MMP-8 in saliva | 1.69 | 1.34–2.16 | <.0001 |
| MMP-8 in saliva; Adjusted | 1.58 | 1.19–2.13 | .0021 |
| Severe periodontitis (yes *vs* healthy) | | | |
| MMP-8 in GCF | 3.19 | 1.71–6.49 | .0006 |
| MMP-8 in GCF; Adjusted | 2.86 | 1.30–7.27 | .02 |
| MMP-8 in saliva | 2.52 | 1.65–4.11 | <.0001 |
| MMP-8 in saliva; Adjusted | 2.26 | 1.37–4.02 | .0026 |
| Mild/moderate periodontitis (yes *vs* healthy) | | | |
| MMP-8 in GCF | 2.65 | 1.80–4.06 | <.0001 |
| MMP-8 in GCF; Adjusted | 2.29 | 1.43–3.83 | .0009 |
| MMP-8 in saliva | 1.53 | 1.19–2.00 | .001 |
| MMP-8 in saliva; Adjusted | 1.46 | 1.08–2.01 | .02 |

### 3.3 | Associations of MMP-8 with probing pocket depth

In logistic regression analysis, higher MMP-8 levels in GCF and in saliva were associated with greater PPD anywhere in dentition. Subjects with high MMP-8 levels in GCF had OR of 2.90 (CI: 1.83–4.86; $p < .0001$) for having greater PPD (≥6 mm) when compared to subjects with low MMP-8 levels. Similarly, subjects with higher MMP-8 levels in saliva had OR of 1.93 (CI: 1.43–2.70; $p < .0001$) for having greater PPD. The associations of MMP-8 in GCF and saliva with PPD were statistically significant also when the differences in PPD were less drastic (PPD: ≥4 *vs* <4 or PPD: ≥6 *vs* ≤5). When age, gender, smoking, BMI, number of medications and DMFT were adjusted for, the associations of MMP-8 with PPD were somewhat attenuated but remained statistically significant (Table 4). Of the confounders, age and current tobacco smoking were statistically significant independent predictors ($p < .05$) for greater PPD in all the regression models.

**TABLE 4** Uni- and multivariable logistic regression model of the associations of mean matrix metalloproteinases (MMP)-8 on probing pocket depths (PPD). Age, gender, smoking, body mass index, number of medications and decayed, missing and filled teeth were adjusted for. Results are expressed as odds ratios (OR), 95% confidence intervals (CI), and *p*-values

| Model | OR | CI | *p*-Value |
|---|---|---|---|
| PPD: ≥6 *vs* <4 | | | |
| MMP-8 in gingival crevicular fluid (GCF) | 2.90 | 1.83–4.86 | <.0001 |
| MMP-8 in GCF; Adjusted | 2.58 | 1.36–5.28 | .0056 |
| MMP-8 in saliva | 1.93 | 1.43–2.70 | <.0001 |
| MMP-8 in saliva; Adjusted | 1.55 | 1.02–2.42 | .04 |
| PPD: ≥4 *vs* <4 | | | |
| MMP-8 in GCF | 2.00 | 1.47–2.79 | <.0001 |
| MMP-8 in GCF; Adjusted | 1.60 | 1.09–2.39 | .02 |
| MMP-8 in saliva | 1.49 | 1.20–1.87 | .0005 |
| MMP-8 in saliva; Adjusted | 1.38 | 1.05–1.82 | .02 |
| PPD: ≥6 *vs* ≤5 | | | |
| MMP-8 in GCF | 2.27 | 1.51–3.53 | .0001 |
| MMP-8 in GCF; Adjusted | 2.53 | 1.47–4.59 | .0013 |
| MMP-8 in saliva | 1.71 | 1.30–2.30 | .0002 |
| MMP-8 in saliva; Adjusted | 1.56 | 1.13–2.19 | .0087 |

### 3.4 | Associations of MMP-8 with bleeding on probing

In logistic regression analysis, higher mean MMP-8 levels in GCF and in saliva were associated with BOP. The geometric mean ratios were 1.39 (CI: 1.20–1.61; $p < .0001$) for GCF and 1.21 (CI: 1.09–1.35; $p = .0005$) for saliva. When age, gender, smoking, BMI, number of medications and DMFT were adjusted, the associations of MMP-8 with BOP remained statistically highly significant (adjusted *p*-values for GCF: <.0001; and saliva: .002). None of the confounders were statistically significant predictors for BOP.

## 4 | DISCUSSION

This study examined whether MMP-8 levels in saliva and GCF, that is in oral fluids, are associated with periodontitis. Higher MMP-8 levels

in GCF and saliva were associated with periodontitis, greater PPD and bleeding on probing.

In this study, a group ($n$ = 258) of periodontally unselected Swiss adults was examined. Periodontitis was observed to be statistically significantly more prevalent in subjects with higher levels of MMP-8 in GCF as well as in saliva compared with subjects with lower levels of MMP-8. The logistic regression analyses showed that when the MMP-8 level in GCF or saliva increased by a unit, the odds ratio to have mild to moderate periodontitis increased statistically significantly (GCF OR: 2.6; saliva OR: 1.5). The strongest association was found for severe periodontitis (GCF OR: 3.19; saliva OR: 2.52). Furthermore, associations of MMP-8 with PPD were statistically significant even when the differences in PPD were minor (≥4 *vs* <4). In accordance with these results, several recent studies have likewise demonstrated that MMP-8 levels in oral fluids are associated with periodontitis and greater PPD among adult and adolescent patients (Heikkinen et al., 2016; Kim, Sukhbaatar, Shin, Ahn, & Yoo, 2014; Leppilahti et al., 2011; Noack et al., 2017; Nwhator et al., 2014; Sorsa et al., 2016).

According to these results, MMP-8 could be expected to be relatively sensitive to the inflammatory burden of gingiva. However, high MMP-8 levels were observed also in subjects with clinically healthy gingiva (39%). Additionally, low MMP-8 levels were observed to be relatively frequent (28%) among subjects with periodontitis. These variations can be partly explained by the fact that periodontitis progresses in bursts or alternates between active and inactive states of the disease reflected in oral fluids as alterations in MMP-8 levels (Kim et al., 2014; Lee et al., 1995; Sorsa, Tervahartiala et al., 2011). Thus, the golden standard of periodontal diagnoses, which relies on clinical measurements of periodontal pocket depth and which was also used in this study, may not reflect the current state and activity of the disease. These problems with sensitivity and specificity have been observed and discussed also in other studies (Gul et al. 2016; Leppilahti et al., 2015; Sorsa et al., 2016).

To further assess the clinical relevance of MMP-8 testing in detecting periodontitis, the ROC analysis was performed. The AUC of ROC for saliva was 0.67 and for GCF 0.71, indicating moderate (=AUC > 0.7) diagnostic accuracy for MMP-8 in this population (Fischer et al., 2003). Similar results have been obtained also in other studies in which low to moderate accuracy (AUC 0.63–0.80) has been obtained for MMP-8 in GCF or saliva as a biomarker of periodontitis (Gürsoy et al., 2010; Heikkinen et al., 2016, 2017; Lee et al., 2012; Leppilahti et al., 2011, 2015; Sexton et al., 2011). A recent study using a novel point-of-care mouth rinse MMP-8 test reported impressive 100% specificity (48.3% sensitivity) with no false-positive results for periodontitis (≥1 site with PPD ≥4 mm) (Heikkinen et al., 2016). In this study, less impressive figures were reached. With the optimal MMP-8 levels for maximal simultaneous specificity and sensitivity, 55% specificity and 76% sensitivity for saliva and 74% specificity and 65% sensitivity for GCF were obtained. Another recent study has suggested that combining enzyme profiling of MMP-8 together with elastase and sialidase activity levels provides better diagnostic accuracy compared with single enzyme profiles at least in predicting treatment outcomes of periodontitis (Gul et al. 2016). However, a general limitation of MMP-8 studies and testing is that the affinity of different MMP-8 antibodies to MMP-8 isoforms can vary prominently (Sorsa et al., 2010, 2016; Sorsa, Mäntylä et al., 2011; Sorsa, Tervahartiala et al., 2011). Consequently, it is difficult to determine certain cut-off levels or "normal ranges" for MMP-8 levels. Additionally, comparisons of results may be complicated, particularly if researchers fail to use the same detection or statistical methods in different studies (Leppilahti, 2016). The antibody used in these assays was selected because of its ability to detect the active form of MMP-8 (Sorsa et al., 2010, 2016) that is characteristic in active and progressive stages of periodontitis (Lee et al., 1995; Sorsa et al., 2016). Another strength of this study is that in regression analysis MMP-8 level was used as a metric predictor to avoid cut-off values (ng/ml) to make comparisons more feasible.

Age, gender, smoking, BMI, number of medications and DMFT were included as potential confounders in the regression analysis. These have all been previously shown to be associated with both elevated MMP-8 levels and periodontitis (Genco & Borgnakke, 2013; Hedenbjörk-Lager et al., 2015; Keller, Rohde, Raymond, & Heitmann, 2015; Mäntylä et al., 2003, 2006). In the current analysis, age and current smoking had statistically significant independent associations with periodontal diagnosis and PPD. Particularly, smoking has been shown to be associated with altered MMP levels, and there are smokers who, despite of treatment, repeatedly have particularly high MMP-8 levels in GCF and poor treatment response (Mäntylä et al., 2003, 2006). However, in this study, gender, BMI, number of medications and DMFT did not have independent associations with response variables. As expected, the associations of MMP-8 with the response variables attenuated somewhat after adjusting for the confounders.

The study population was recruited from the Swiss bone marrow donor registry. Thus, a selection bias excluding medically severely compromised subjects must be noted. In general, the subjects were relatively healthy and a weakness of the current study is that type 2 diabetes, a known consensus risk factor for periodontitis (Genco, 1996), could not be included in the analyses, as only three subjects of the study population had diabetes. On the other hand, the study population is comparable to the patients in normal contemporary dental practice, and thus, these results can be generalised into this normal population. Additionally, and strengthening the results, relatively reliable anamnestic data of the subjects were available, and a future aim is to analyse the associations of patient-related confounders including eating, drinking and particularly oral hygiene habits with the MMP-8 levels of these subjects. Previous findings have specifically observed MMP-8 levels to be associated with poor oral hygiene (Heikkinen et al., 2016). Also, the current study, in line with previous studies, demonstrated that elevated MMP-8 levels in saliva and GCF are associated with BOP (Heikkinen et al., 2016; Nwhator et al., 2014). BOP is common when oral hygiene is poor and is a good indicator of active phases of periodontal disease (Lang, Joss, Orsanic, Gusberti, & Siegrist, 1986).

In this study, both GCF and saliva samples were collected. GCF is considered to be a completely distinct exudate or transudate of gingiva and is not mixed with saliva until GCF is flown out of the crevice (Griffiths, 2003). Thus, GCF is not biased by any other possible oral

pathological conditions (Uitto et al., 2003). However, a disadvantage of GCF sampling is that it is technically demanding compared with saliva or mouth rinse sampling, and dental professionals are required to perform the GCF sampling to avoid contaminations. However, in the current study, the mean MMP-8 levels in GCF correlated with MMP-8 levels in saliva. Furthermore, increased levels of MMP-8 in saliva were statistically significantly associated with higher OR of periodontitis, similar to GCF. Thus, this study suggests that with regard to periodontitis, saliva is also a relatively reliable source of MMP-8, but less demanding to collect than GCF. This applies also to mouth rinse sampling (Heikkinen et al., 2016, 2017; Sorsa et al., 2016). Currently, a practical and quantitative chair-side/point-of-care lateral flow oral fluid MMP-8 immunoassay, resembling a pregnancy test, has been developed and successfully validated (Borujeni, Mayer, & Eickholz, 2015; Heikkinen et al., 2016, 2017; Johnson et al., 2016; Lorenz et al., 2017; Sorsa et al., 2016; Sorsa, Gieselmann et al., 2017; Sorsa, Heikkinen et al., 2017).

In conclusion, elevated MMP-8 levels in GCF and saliva were associated with periodontitis, greater PPD and bleeding on probing. According to this study, determination of MMP-8 levels in oral fluids could be used as an adjunctive tool in periodontal diagnostics, particularly in the screening of periodontitis at the population level.

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## CONFLICT OF INTEREST

The authors report no conflicts of interest in respect of this study.

## AUTHORS' CONTRIBUTION

M. Mauramo is the first author, who contributed in all stages including data collection, analysis and writing. A. M. Ramseier contributed to data collection and analysis. E. Mauramo participated in analysis and drafted the paper. A. Buser recruited the study subjects. T. Tervahartiala did the MMP-8 analyses. T. Sorsa participated in study design and drafted the paper. T. Waltimo participated in study design, drafted the paper and was in charge of the study.

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