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Saliva and serum biomarkers in periodontitis and coronary artery disease

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Abstract

Aim: Matrix metalloproteinase (MMP)-8, MMP-9, tissue inhibitor of matrix metalloproteinase (TIMP)-1 and myeloperoxidase (MPO) participate in extracellular matrix breakdown both in periodontium and atherosclerotic plaques. We investigated the diagnostic value of serum and saliva biomarkers in periodontitis and acute coronary syndrome (ACS).

Materials and methods: The population was PAROGENE (n=481), a random cohort of patients with an indication for coronary angiography. All patients underwent a clinical and radiographic oral examination. Groups consisting of periodontitis vs. non-periodontitis, and ACS vs. non-ACS patients were compared.

Results: Saliva MMP-8, MMP-9, and MPO provided significant area-under-curve (AUC) values for periodontitis, 0.69 (<0.001), 0.66 (<0.001), and 0.68 (<0.001), respectively. Serum MMP-8, MMP-9, and MPO levels distinguished ACS from non-ACS patients with AUCs of 0.73 (<0.001), 0.58 (0.03), and 0.68 (<0.001), respectively. Periodontitis confounded the use of serum MMP-9 in diagnostics of ACS. Cardiac status complicated the use of saliva TIMP-1 in periodontal diagnostics. Saliva biomarkers could not be used in ACS diagnosis and serum biomarkers were not useful in diagnosis of periodontitis.

Conclusions: MMP-8, MMP-9, TIMP-1, and MPO are valuable biomarkers for both ACS and periodontitis, but the selection of sample material is crucial: serum is suitable for ACS and saliva for periodontal diagnostic aid.

Clinical relevance

Scientific rationale for study: Enzymes that destruct extracellular matrix play a crucial role in both periodontitis and acute coronary syndrome (ACS). These enzymes can be utilized in diagnostics of these diseases.

Principal findings: Saliva enzymes distinguished patients with and without periodontitis and serum enzymes patients with and without ACS. Periodontitis disturbed the use of serum MMP-9 in ACS diagnostics and cardiac status interfered with the saliva TIMP-1 determinations.

Practical implications: Saliva biomarkers are potentially suitable for diagnostic aid in periodontitis and serum biomarkers in ACS, but periodontal and cardiac status should be taken into account in the analyses.

Introduction

In periodontitis and coronary heart disease (CHD), the extracellular matrix (ECM) breakdown represents a crucial factor in the pathophysiology. Firstly, matrix metalloproteinases (MMPs), especially MMP-8 and MMP-9, degrade ECM in periodontium and regulates remodelling processes in cardiovascular disease (CVD) ie. atherosclerotic plaques. Secondly, the tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) endogenously inhibits MMP-8 and MMP-9 (Visse et al. 2003), reflecting the balance of tissue destruction and protection (Gursoy et al. 2010). Thirdly, myeloperoxidase (MPO) associates with inflammation (Leppilahti et al. 2014), activating oxidatively latent MMP-8 and MMP-9 and inactivating TIMP-1 (Saari et al. 1990). MPO kills bacteria also by generating reactive oxygen species, ie. hypochlorous acid (Strzepa et al. 2017).

Saliva MMP-8, MMP-9, and MPO levels are elevated in patients having periodontitis (Sorsa et al. 2006) and saliva TIMP-1 is also important in periodontitis as being an inhibitor of MMPs (Gursoy et al. 2010). Elevated serum MMP-8 (Noack et al. 2017, Marcaccini et al. 2009) and MMP-9 (Wick et al. 2013) concentrations have been found in periodontitis patients compared to healthy controls, but also opposite findings exist (Nizam et al. 2014).

Elevated serum MMP-9 is associated with atherosclerosis and CVD (Yabluchanskiy et al. 2013). Serum MMP-9 concentrations correlate with atherosclerotic plaque rupture (Fukuda et al. 2006) and myocardial tissue destruction (Newby 2016). Furthermore, serum MMP-8 and TIMP-1 levels are associated with atherosclerotic plaque instability and acute coronary syndrome (ACS) (Pussinen et al. 2013), predicting incident cardiovascular events

(Tuomainen et al. 2014; Tuomainen et al. 2007) and especially fatal events (Tuomainen et al. 2007; Kormi et al. 2017).

Saliva biomarkers have also been investigated in CHD. An activated form of MMP-8 in saliva is significantly elevated in acute myocardial infarction (AMI)-patients compared to non-AMI group, while total saliva MMP-8 concentrations were lower in AMI-patients regardless of the oral status (Buduneli et al. 2011). Elevated saliva MMP-8 concentrations have been associated with CHD in analyses where number of teeth were taken into account as a sign of oral health (Furuholm et al. 2006). After adjusting for periodontal status and smoking, saliva MMP-8 and MPO levels were significantly elevated among non-AMI subjects compared to AMI patients (Rathnayake et al. 2015). In addition, biomarker concentrations may be affected by medications. For example statin administration reduces significantly serum and plasma TIMP-1 (Tziakas et al. 2004, Ferretti et al. 2017) and MMP-9 levels (Tziakas et al. 2004, Andrade et al. 2013), but concerning plasma MMP-9 also opposite findings exist (Ferretti et al. 2017).

Biomarkers with more precise information additionally to clinical parameters in periodontitis and CVD are valuable to investigate. Due to similarities in pathophysiology of extracellular matrix breakdown in periodontitis and CHD giving an indication to the same biomarkers, our study aimed: I) to investigate and compare saliva and serum MMP-8, MMP-9, TIMP-1, and MPO concentrations in periodontitis and in ACS, II) to evaluate the diagnostic value of these biomarkers, III) to determine if periodontitis and ACS confounds the diagnostics.

Materials and Methods

Subjects and Diagnosis

The study population consisted of PAROGENE patients, a subpopulation of the larger Corogene cohort (N=5809) described earlier in detail (Vaara et al. 2012). PAROGENE (N=508) included a cohort of randomly selected patients who underwent coronary angiography in Helsinki University Hospital and clinical oral and radiographic examination in University of Helsinki. PAROGENE cohort is described earlier more extensively (Buhlin et al. 2011). Both saliva and serum samples were available for 481 subjects. Information about cardiovascular medications used before hospitalization and prescribed during hospitalization were collected.

The oral examination included periodontal probing pocket depth (PPD) measurements from six sites of the each tooth and bleeding on probing (BOP) registration (Buhlin et al. 2011). Alveolar bone loss (ABL) was obtained from digital panoramic radiographs and calculated following way: no ABL, mild (ABL in cervical third of the root), moderate (ABL in the mid third of the root), severe (ABL in the apical third of the root), total ABL (Buhlin et al. 2011). A patient was classified as having periodontitis if there was alveolar bone loss (mild to severe) and if PPD was ≥4 mm in ≥4 sites. Patients having no current periodontitis comprised periodontally healthy, gingivitis and edentulous patients.

A patient was diagnosed as having no coronary artery disease (CAD) (N=115) if <50% stenosis of all coronary arteries was seen in angiography. Stable CAD (N=175) diagnosis was set if >50% stenosis was detected in angiography. No CAD and stable CAD patients were merged as non-ACS group (N=286) for further analysis to form a reference group for ACS patients. With this division, seizure CAD can be compared to more stable conditions. ACS patients (N=163) had ≥ 50% stenosis in at least one coronary artery, episode of typical chest pain for ischemia and elevated cardiac enzymes. "ACS-like, no significant CAD"-patients were excluded from further analysis because of their low number (N=28) and because of acute phase response related to takotsubo patients included in this group may be reflected in increases of inflammatory biomarker levels (Parkkonen et al. 2017).

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. All participants provided informed consent.

Laboratory determinations

Stimulated whole saliva samples were collected 37 to 224 days after angiography as described earlier (Hyvärinen et al. 2012). The saliva was centrifuged for 3 min at 9300 g and the supernatants were used for the analyses. Blood samples were taken during the coronary angiography. Samples were stored at −70 C°. Determinations of saliva and serum MMP-9, TIMP-1, MMP-8, and MPO concentrations were performed with following immunoassays. MMP-9-ELISA (GE Healthcare UK Limited, Amersham Place, UK), TIMP-1-ELISA (R&D Systems, Minneapolis, MN, USA) and MPO-ELISA (Immundiagnostik, Bensheim, Germany) were performed according to manufacturer's instructions on diluted samples (1:20

in MMP-9, 1:10 in TIMP-1 and 1:40 in MPO, same dilutions both serum and saliva). The MMP-8 concentrations were determined by an immunofluorometric assay (IFMA) as described earlier (Tuomainen et al. 2007).

The inter-assay coefficient of variability (CV) % (N=12) were 7.7 %, 6.0 %, 8.1 %, and 10.9 %, and the detection limits were 0.08 (µg/L), 0.04 (µg/L), 0.60 (µg/L), and 0.29 (µg/L) for MMP-8, MMP-9, TIMP-1, and MPO, respectively.

Statistics

The distribution of variables was tested before statistical analysis. The normally distributed variables were presented as means and standard deviations (SD). The statistical significance of the differences between the groups was tested by the independent samples t-test. Categorical variables were tested by the Chi-square test. The biomarker concentrations displayed skewed distributions and are presented as medians and interquartile ranges (IQR). Statistical significance was tested by using the Mann-Whitney test. The p-value for statistical significance was defined at 0.05.

Correlation coefficients were calculated by using the Spearman's correlation. The diagnostic sensitivity and specificity of the biomarkers were calculated by receiver operating characteristics (ROC). The sensitivity and specificity are presented by using the cut-off value that was set to the point, in which the distance from the left-upper corner of the unit square was the smallest (Habibzadeh et al. 2016). In the subgroups the sensitivity was determined according to the specificity determined for the whole population.

Multivariate logistic regression models were used to determine the association of saliva biomarkers with periodontitis and serum biomarkers with ACS. The model was adjusted for age, sex, periodontitis or cardiac status, diabetes, and for use of statins (before or after hospitalization). The analyses were performed using IBM SPSS Statistics 22.

Results

Characteristics of the subjects according to cardiac and periodontal status are presented in Table 1. Patients in the non-ACS group had more often dyslipidemia compared to ACS patients. Periodontitis patients were more often older, diabetic, smokers, dyslipidemic, and used more statins (Table 1).

Saliva biomarkers

Concentrations of saliva MMP-8, MMP-9, TIMP-1, and MPO differed significantly between periodontitis and non-periodontitis patients (Table 2); the MMP-8, MMP-9, and MPO were higher, and TIMP-1 concentrations lower in periodontitis. When these saliva biomarker concentrations were examined separately in different cardiac groups, similar significant trends according to periodontitis classification were observed. Only, the difference in saliva TIMP-1 turned to non-significant in non-ACS group (p=0.109). There were no statistically significant differences according to coronary group in saliva MMP-8, MMP-9, TIMP-1, or MPO (Table 2).

The diagnostic ability of biomarkers was investigated by ROC analysis. Saliva MMP-8, MMP-9, and MPO distinguished periodontitis patients from those without periodontitis with AUCs (p-value) 0.69 (<0.001), 0.66 (<0.001), and 0.68 (<0.001), respectively (Table 3). Saliva TIMP-1 distinguished patients without periodontitis from those with periodontitis with AUC (p-value) 0.59 (0.001) (Table 3). Similar significant AUCs were observed, when the ROC analyses were performed separately in cardiac subgroups. Only saliva TIMP-1 did not distinguish periodontitis from non-periodontitis among the non-ACS patients (Table 3).

Furthermore, saliva biomarkers were not useful in ACS diagnostics, except saliva TIMP-1 which distinguished ACS among patients without periodontitis, 0.64 (p=0.005).

By using the ROC-curves, cut-off levels, sensitivity and specificity were determined for each biomarker (Table 3). The only notable difference in these analyses was that, when diagnosing periodontitis, the sensitivity of saliva MMP-9 and MPO was slightly better in ACS patients than in non-ACS patients.

The association of saliva biomarkers and covariates with periodontitis is shown in Table 4. Saliva MMP-8, MMP-9, TIMP-1, and MPO concentrations had significant associations with periodontitis, the association of saliva TIMP-1 being inverse. Among all studied biomarkers, saliva MMP-8 had the strongest association with periodontitis with an OR (95% CI) of 4.07 (2.63-6.29), p<0.001. The use of statins was significantly associated with periodontitis in all models. In addition, age associated with periodontitis in saliva MMP-8, MMP-9, and MPO models, and diabetes the saliva TIMP-1 model.

Serum biomarkers

Concentrations of serum MMP-8, MMP-9, and MPO were significantly higher in patients having ACS compared to non-ACS (Table 5). When analyzed in periodontitis patients only, the difference of serum MMP-9 between non-ACS and ACS lost its significance (Table 5). Furthermore, the serum biomarker concentrations did not differ between patients with and without periodontitis (Table 5).

Serum MMP-8, MMP-9, or MPO provided significant AUCs (p-value) for ACS: 0.73 (<0.001), 0.58 (0.04), and 0.68 (<0.001), respectively (Table 3). Serum MMP-9 turned non-significant in periodontitis patients when diagnosing ACS (Table 3). Serum TIMP-1 did not distinguish ACS patients from non-ACS patients (Table 3). All AUC values of serum biomarkers regarding periodontal diagnosis were non-significant (data not shown). The sensitivity of serum MMP-8 for ACS diagnosis was slightly better in periodontitis compared to non-periodontitis patients. On the contrary, the sensitivity of serum MPO for ACS diagnosis was higher in non-periodontitis compared to periodontitis patients.

The associations of serum biomarkers with ACS are shown in Table 6. All serum biomarkers had significant associations with ACS, the association of serum TIMP-1 being inverse. Among the biomarkers, serum MPO had the strongest association with ACS with an OR of 6.99 (3.09-15.8), p<0.001. From the covariates, only the use of statins before hospitalisation associated significantly with ACS.

Biomarker correlations

The correlations between saliva and serum biomarker concentrations are presented in Table 7. Saliva MMP-8 correlated significantly with saliva MMP-9, TIMP-1, and MPO. Furthermore, saliva MMP-9 had significant correlations with saliva TIMP-1 and MPO. Serum MPO correlated significantly with serum MMP-8 and MMP-9, which correlated significantly with each other. Serum TIMP-1 did not have any significant correlations with other serum biomarkers. When serum and saliva biomarker correlations were analyzed, only serum MMP-8 and saliva TIMP-1 had a significant correlation with each other.

The effect of statins and diabetes

Serum biomarker concentrations were calculated according to use of statins before hospitalisation and saliva concentrations according to use of statins after hospitalisation. Medians of serum MMP-8 (28.1 vs. 60.7 µg/L, $p<0.001$), MMP-9 (148.7 vs. 188.3 µg/L, $p=0.02$) and MPO (294.1 vs. 361.7 µg/L, $p<0.001$) concentrations were significantly lower in statin users compared to non-users. Saliva biomarker concentrations did not differ significantly according to statin use. The use of statins was a significant covariate in the logistic regression models both for saliva biomarkers in periodontitis and serum biomarkers in ACS. In these models the use of statins associated directly and inversely with the risk of having periodontitis and ACS, respectively.

Similarly, biomarker concentrations were calculated in patients with and without diabetes. Diabetic patients had significantly higher medians of saliva MMP-9 (329.6 vs. 189.8 µg/L, $p=0.004$), saliva MPO (1973.0 vs. 1505.0 µg/L, $p=0.019$), serum TIMP-1 (132.3 vs. 112.2 µg/L, $p<0.001$), and serum MPO (363.9 vs. 313.6 µg/L, $p=0.032$). In the logistic regression diabetes associated significantly with periodontitis only in the saliva TIMP-1 model (Table 4).

Discussion

In this relatively large cohort of patients with detailed information of periodontal disease and coronary artery disease status, we found that saliva MMP-8, MMP-9, TIMP-1, and MPO levels associated significantly with periodontitis, while the corresponding serum concentrations associated with ACS. Some of the saliva biomarkers were confounded by the cardiac status and some serum biomarkers were affected by the periodontal status. Saliva biomarkers had no diagnostic value in ACS and serum biomarkers were not able to distinguish periodontitis patients from non-periodontitis.

We determined the cut-off values for all examined biomarkers according to the coordinate on the ROC curve with the minimum distance from the left upper corner of the plot (Habibzadeh et al. 2016). This approach results in a cut-off concentration, which produces the highest sensitivity and specificity at the same time and provides the most objective result. In the future studies or in the clinical practice, a more subjective approach could be used emphasizing either specificity or sensitivity after considering which is more important: the

high proportion of true positive or the low proportion of false negative diagnosis. Considering periodontitis, it might be of interest to lower the threshold in order to identify truly healthy subjects from those requiring more periodontal counselling or treatment. Considering CAD, the biomarkers would be most valuable in differentiating ACS from other acute events with resembling clinical characters.

Serum MMP-8 and MPO were suitable for ACS diagnostic providing sensitivities of 65% and 64% and specificities 73% and 65%, respectively, with significant AUCs ranging between 0.68-0.73. In the subgroup analyses, periodontal disease affected only slightly the sensitivities of these biomarkers in diagnosing ACS. The results of these subgroup analyses were confirmed in the whole population by logistic regression models, where periodontitis diagnosis as a confounder did not have a notable effect on the strong association between these biomarkers and ACS. Serum MMP-8 had the largest AUC values in ACS, while serum MPO had the highest odds for it.

Significant associations between circulating MMP-9 concentrations and ACS have been frequently reported in earlier studies by us (Lahdentausta et al. 2018) and others (Kai et al. 1998; Inokubo et al. 2001; Derosa et al. 2007; Tan et al. 2008; Tsiakas et al. 2004) when compared to healthy controls. In the present population, serum MMP-9 associated significantly with ACS but the biomarker just weakly differentiated the patient groups from each other and the result was clearly affected by periodontal status. In the present study serum TIMP-1 concentrations did not display significant AUC-values in ACS diagnostics. The finding is different in relation to the earlier studies in other populations, in which serum or plasma TIMP-1 differentiated significantly ACS patients from healthy controls (Pussinen et al. 2013, Inokubo et al. 2001, Cavusoglu et al. 2006). The low discrimination power of MMP-9 and TIMP-1 in the present study may be due to the reference group, non-ACS, which is composed of symptomatic patients indicated to coronary angiography (Buhlin et al. 2011). However, the effect of periodontitis on the performance of these biomarkers in ACS diagnostics has not been taken into account in earlier studies, although elevated systemic levels of MMP-8 and MMP-9 have been observed in periodontitis patients (Marcaccini et al. 2009). The levels were reported to decrease after non-surgical periodontal therapy indicating both that periodontitis contributes to circulating MMP concentrations and that periodontitis plays a role in systemic inflammation (Marcaccini et al. 2009).

Among the measured saliva biomarkers MMP-8, MMP-9, and MPO were almost equally powerful in detecting periodontitis. These differences were seen by using medians, ROC-analysis, or logistic regression. Cardiac status had an effect on saliva MPO sensitivity in diagnosing periodontitis. Saliva MMP-8 had the highest AUC values in diagnosing periodontitis and it also presented the highest odds for periodontitis. Similar results have been obtained in previous articles, where chronic periodontitis patients had significantly higher levels of MMP-8, MPO, and activated MMP-9 in gingival crevicular fluid (GCF) compared to controls, and these levels decreased significantly three months after non-surgical periodontal therapy (Marcaccini et al. 2010, Correa et al. 2008, Figueredo et al. 2004). Also saliva and GCF TIMP-1 have been utilized in periodontal diagnostics (Gursoy et al. 2010, Pozo et al. 2005). Although in our study saliva TIMP-1 concentrations were significantly associated with periodontitis, the diagnostics was affected by the cardiac status.

MMP-8, MMP-9, and MPO correlated with each other when measured from the same sample material probably reflecting partially the same source of these molecules. Interestingly, serum and saliva biomarker levels did not correlate with each other indicating that the molecules are locally produced. At the same time the result implies that these saliva biomarkers are not useful in the diagnostics of systemic conditions. Saliva diagnostics in medicine and pharmacotherapy is an area of vivid research to date (Kaczor-Urbanowicz et al. 2017). Saliva biomarkers reflecting CVD are investigated abundantly (Miller et al. 2010), because sampling of saliva is non-invasive, rapid, and cost-effective (Miller et al. 2014). Saliva biomarkers such as, MMP-9, MPO, CRP, IL -1β, are presented to be promising in AMI diagnostics (Christodoulides et al. 2012), and a panel of biomarker combinations in early stage of AMI is investigated (Floriano et al 2009). However, challenges remain: saliva contains a lot of inhibitors and periodontitis-derived enzymes, which can disturb its use in diagnosing systemic diseases (Miller et al 2010).

It is known that many systemic disorders, medications, human habits, and genetics can affect the biomarker levels. Serum MMP-8 concentrations are elevated in diabetic and hypercholesterolemic patients and statins reduce significantly serum MMP-8 and MMP-8/TIMP-1 ratio in these patients (Kadoglou et al. 2014). In our study, statin-users had lower serum MMP-8, MMP-9, and MPO compared to non-users, whereas diabetic subjects had higher saliva MMP-9, saliva MPO, serum TIMP-1, and serum MPO compared to non-diabetic patients.

Limitations of study include the cross-sectional, cohort setup. We could not evaluate the prognostic value of these biomarkers with this study design. The biomarkers offering prognostic information would be important to explore, because, besides radiographs, the clinical oral and periodontal examinations are valuable in diagnosing the present and past periodontitis, but do not provide complete prediction of the future disease course (Miller et al 2010). Some evidence of the predictive value of MMP-8 in periodontitis progression exists (Leppilahti et al. 2015). Multiple conditions that might affect the biomarker levels are not examined here. In addition to periodontally healthy patients, the non-periodontitis group comprised edentulous patients and those with gingivitis or history of periodontitis. Edentulism, which can be regarded as a sign of past periodontitis, is associated with saliva biomarker levels (Palm et al. 2014; Salminen 2014): biomarkers deriving from the host may be even lower than those found in the healthy, when again bacterial biomarkers may be on the same level as in those with mild periodontitis. Our study population consisted of middle-aged and elderly subjects with an indication to coronary angiography and with multiple medications. The non-ACS group included both patients with stable coronary artery disease and those without significant coronary artery disease. Thus, the reference group has also medical concerns, which may be reflected in the results and diminish the differences observed. Taken together, the measured biomarkers most probably differentiate the groups with and without active tissue destruction.

Genetics play a role in periodontal and cardiovascular diseases and contribute directly to serum MMP-8 and MPO levels (Salminen et al. 2017; Reiner et al. 2013). In meta-analysis MMP-8 and MMP-9 polymorphisms were associated with periodontitis susceptibility (Weng et al. 2016). Furthermore, the genetic variation in MMP-8 and MMP-9 genes are associated with risk for vascular diseases and coronary heart disease (Pradhan-Palikhe et al. 2012; Li et al. 2013). To our knowledge the input of heredity in salivary biomarker concentrations has not been studied.

MMP-8, MMP-9, TIMP-1, and MPO can be utilized as diagnostic aid of both periodontitis and ACS: saliva is useful for periodontal and serum for cardiac diagnostics. These biomarkers can be utilized by healthcare professionals in screening, and patients can be guided further to dentists or cardiologists. Periodontitis affects biomarker levels both locally in the oral cavity and systemically, and also acute coronary events are weakly reflected in saliva.

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Table 1: Characteristics of subjects according to cardiac and periodontal status

	Non-ACS (N=290)	ACS (N=163)	p-value[1]	Non-periodontitis (N=196)	Periodontitis (N=285)	p-value[2]
	Mean (SD)			Mean (SD)		
Age (years)	64.1 (8.7)	63.0 (9.5)	NS	62.4 (10.3)	64.1 (8.1)	0.047
BMI (kg/m^2)	27.9 (4.9)	28.2 (5.4)	NS	27.8 (5.0)	28.0 (5.1)	NS
	N (%)			N (%)		
Sex (male)	188 (64.8)	118 (72.4)	NS	120 (61.2)	194 (68.1)	NS
Dyslipidemia	250 (86.2)	120 (73.6)	0.001	149 (76.0)	238 (83.5)	0.044
Use of statins before hospitalization	221 (76.2)	61 (37.4)	<0.001	103 (52.6)	184 (64.6)	0.006

Statins prescribed during hospitalization	244 (84.1)	156 (95.7)	<0.001	160 (81.6)	257 (90.2)	0.003
Hypertension	190 (65.5)	102 (62.6)	NS	123 (62.8)	183 (64.2)	NS
Diabetes	75 (25.9)	36 (22.1)	NS	35 (17.9)	78 (27.4)	0.016
Smoking (ever)	146 (50.3)	94 (57.7)	NS	86 (43.9)	167 (58.6)	0.002
Periodontitis	179 (61.7)	94 (57.7)	NS	-	-	-
ACS	-	-	-	69 (35.2)	94 (33.0)	NS

Statistical significance tested by using the independent samples t-test for continuous variables and Chi-square test for categorical variables; [1] p-value for the difference between non-ACS and ACS; [2] p-value for the difference between no current periodontitis and periodontitis; NS = not significant.

Table 2: Concentrations of saliva MMP-8, MMP-9, TIMP-1, and MPO according to periodontal and cardiac status

		Saliva biomarkers			
		MMP-8	MMP-9	TIMP-1	MPO
		Median (IQR), (µg/L)			
Non-periodontitis (N=196)		591 (170-1087)	131 (23-353)	207 (132-296)	1181 (540-2425)
	Non-ACS (N=111)	591 (196-973)	129 (30-393)	187 (119-284)	1133 (529-2524)
	ACS (N=69)	546 (130-1170)	118 (13-338)	223 (159-301)	1225 (493-2198)
	p^1	NS	NS	NS	NS
		p^2	p^2	p^2	p^2

Periodontitis (N=285)		1089 (606-1547)	<0.001	285 (123-562)	<0.001	159 (107-262)	0.001	2182 (1052-6039)	<0.001
	Non-ACS (N=179)	1073 (606-1534)	<0.001	295 (113-594)	<0.001	163 (104-261)	NS	2018 (1035-5332)	<0.001
	ACS (N=94)	1137 (679-1564)	<0.001	302 (150-510)	<0.001	147 (108-271)	0.002	2287 (1198-8969)	<0.001
	p^1	NS		NS		NS		NS	

Statistical significance tested by using the non-parametric Mann-Whitney test [1] non-ACS compared to ACS; [2] periodontitis compared to groups in the no periodontitis category NS = not significant, IQR= interquartile range from 25th to 75th percentile.

Table 3: ROC data describing the diagnostic ability of saliva and serum biomarkers in periodontitis and ACS.

				MMP-8	MMP-9	TIMP-1	MPO
	To be diagnosed	Subgroup					
SALIVA	Periodontitis		AUC (95% CI)	0.69 (0.65-0.74)	0.66 (0.61-0.71)	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
		ACS	AUC (95% CI)	0.71 (0.62-0.79)	0.69 (0.60-0.77)	0.64 (0.56-0.73)	0.70 (0.62-0.78)
			p-value	<0.001	<0.001	0.002	<0.001
			Sensitivity[1]	0.67	0.69	0.64	0.67
			Specificity	0.62	0.61	0.59	0.62
		Non-ACS	AUC (95% CI)	0.69 (0.63-0.76)	0.65 (0.58-0.72)		0.66 (0.60-0.73)
			p-value	<0.001	<0.001	NS	0.001
			Sensitivity[1]	0.67	0.66		0.60
			Specificity	0.62	0.61		0.62
SERUM	ACS		AUC (95% CI)	0.73 (0.68-0.78)	0.58 (0.52–0.64)		0.68 (0.63-0.73)
			p-value	<0.001	0.04	NS	<0.001
			Cut-off (µg/L)	47.3	170.9		343.8

		Sensitivity	0.65	0.54		0.64
		Specificity	0.73	0.58		0.65
	Periodontitis	AUC (95% CI)	0.73 (0.67-0.80)		0.57 (0.50-0.65)	0.67 (0.60-0.73)
		p-value	<0.001	NS	0.043	<0.001
		Sensitivity[1]	0.67			0.58
		Specificity	0.73			0.65
	Non-periodontitis	AUC (95% CI)	0.74 (0.66-0.82)	0.63 (0.53-0.72)		0.71 (0.63-0.79)
		p-value	<0.001	0.005	NS	<0.001
		Sensitivity[1]	0.62	0.63		0.73
		Specificity	0.73	0.58		0.65

NS = not significant; [1] Sensitivity according to the specificity determined by using the cut-off concentration for the whole population.

Table 4: The association of saliva biomarkers with periodontitis

	Saliva			
	MMP-8	**MMP-9**	**TIMP-1**	**MPO**
	OR (95% CI), p-value			
Biomarker (µg/L)	**4.07 (2.63-6.29), <0.001**	**2.02 (1.46-2.79), <0.001**	**0.28 (0.13-0.57), 0.001**	**3.54 (2.40-5.23), <0.001**
Age (years)	1.03 (1.01-1.06), 0.007	1.03 (1.01-1.06), 0.008	NS	1.04 (1.01-1.06), 0.004
Gender (male)	NS	NS	NS	NS
Cardiac status	NS	NS	NS	NS
Diabetes	NS	NS	1.65 (1.02-2.66), 0.04	NS
Statin use*	1.98 (1.03-3.80), 0.041	2.60 (1.33-5.10), 0.005	2.17 (1.16-4.06), 0.015	2.17 (1.13-4.18), 0.021

***Statins prescribed during hospitalisation**

Table 5: Concentrations of serum MMP-8, MMP-9, TIMP-1, and MPO according to cardiac and periodontal status

		Serum biomarkers							
		MMP-8		MMP-9		TIMP-1		MPO	
		Median (IQR), (µg/L)							
Non-ACS (N=290)		28 (14-54)		153 (87-246)		119 (104-142)		287 (199-402)	
	Non-periodontitis (N=111)	28 (14-54)		150 (85-236)		118 (102-137)		285 (204-381)	
	Periodontitis (N=179)	25 (14-54)		159 (89-246)		119 (105-144)		287 (197-423)	
	p^1	NS		NS		NS		NS	
			p^2		p^2		p^2		p^2
ACS (N=163)		81 (30-153)	p<0.001	190 (93-419)	p=0.004	115 (95-142)	NS	408 (283-631)	p<0.001
	Non-periodontitis (N=69)	98 (31-184)	p<0.001	228 (103-562)	p=0.005	117 (98-148)	NS	408 (318-729)	p<0.001
	Periodontitis (N=94)	79 (27-149)	p<0.001	168 (91-353)	NS	114 (91-138)	p=0.043	423 (270-610)	p<0.001
	p^1	NS		NS		NS		NS	

Statistical significance tested by using the non-parametric Mann-Whitney test [1] periodontitis compared to groups in the no periodontitis category; [2] non-ACS compared to ACS category; NS = not significant, IQR= interquartile range from 25th to 75th percentile.

Table 6: The association of serum biomarkers with ACS

	Serum			
	MMP-8	**MMP-9**	**TIMP-1**	**MPO**
	OR (95% CI), p-value			
Biomarker (µg/L)	**5.32 (3.21-8.81), <0.001**	**1.83 (1.05-3.18), 0.033**	**0.15 (0.03-0.82), 0.028**	**6.99 (3.09-15.82), <0.001**
Age (years)	NS	NS	NS	NS
Gender (male)	NS	NS	NS	NS
Periodontitis	NS	NS	NS	NS
Diabetes	NS	NS	NS	NS
Statin use**	0.22 (0.13-0.34), <0.001	0.18 (0.11-0.28), <0.001	0.17 (0.11-0.26), <0.001	0.19 (0.12-0.30), <0.001

**** Statins used before hospitalisation**

Table 7: Correlations of serum and saliva MMP-8, MMP-9, TIMP-1, and MPO

		Saliva				Serum			
		MMP-8	**MMP-9**	**TIMP-1**	**MPO**	**MMP-8**	**MMP-9**	**TIMP-1**	**MPO**
Serum	**MPO**	NS	NS	NS	NS	0.614, <0.001	0.483, <0.001	NS	1
	TIMP-1	NS	NS	NS	NS	NS	NS	1	
	MMP-9	NS	NS	NS	NS	0.529, <0.001	1		
	MMP-8	NS	NS	0.119, 0.009	NS	1			
Saliva	**MPO**	0.687, <0.001	0.582, <0.001	NS	1				
	TIMP-1	-0.094, 0.04	-0.129, 0.005	1					
	MMP-9	0.611, <0.001	1						
	MMP-8	1							

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