



HAL
open science

Oral rinse MMP-8 point-of-care test identifies patients with strong periodontal inflammatory burden

Jussi Leppilahti, Minna-Maija Ahonen, Marcela Hernández, Sarvan Munjal, Lutz Netuschil, Veli-Jukka Uitto, Timo Sorsa, Päivi Mäntylä

► **To cite this version:**

Jussi Leppilahti, Minna-Maija Ahonen, Marcela Hernández, Sarvan Munjal, Lutz Netuschil, et al.. Oral rinse MMP-8 point-of-care test identifies patients with strong periodontal inflammatory burden. Oral Diseases, 2010, 17 (1), pp.115. 10.1111/j.1601-0825.2010.01716.x . hal-00599812

HAL Id: hal-00599812

<https://hal.science/hal-00599812>

Submitted on 11 Jun 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ORAL DISEASES

Oral rinse MMP-8 point-of-care test identifies patients with strong periodontal inflammatory burden

Journal:	<i>Oral Diseases</i>
Manuscript ID:	ODI-02-10-OM-1554.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	12-Mar-2010
Complete List of Authors:	Leppilahti, Jussi; University of Helsinki, Institute of Dentistry Ahonen, Minna-Maija; University of Helsinki, Institute of Dentistry Hernández, Marcela; Universidad de Chile, Periodontal biology Munjal, Sarvan; dentognostics GmbH Netuschil, Lutz; dentognostics GmbH Uitto, Veli-Jukka; University of Helsinki, Institute of Dentistry; Helsinki University Central Hospital, Department of Oral and Maxillofacial Diseases Sorsa, Timo; University of Helsinki, Institute of Dentistry; Helsinki University Hospital, Department of Oral and Maxillofacial Diseases Mäntylä, Päivi; University of Helsinki, Institute of Dentistry
Keywords:	oral rinse, biomarker, chair-side test, periodontal diagnostics, MMP-8



Deleted: severe periodontitis

1
2 Oral rinse MMP-8 point-of-care test identifies patients with strong periodontal inflammatory
3 burden
4
5

6
7 Leppilähti Jussi M¹, Ahonen Minna-Maija¹, Hernández Marcela³, Munjal Sarvan⁴, Netuschil Lutz⁴,
8 Uitto Veli-Jukka^{1,2}, Sorsa Timo^{1,2}, Mäntylä Päivi¹
9

10
11 ¹Institute of Dentistry, University of Helsinki, Helsinki, Finland

12
13 ²Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki,
14 Finland

15
16 ³Periodontal Biology Laboratory, Faculty of Dentistry, University of Chile, Santiago, Chile

17
18 ⁴dentognostics GmbH, Jena, Germany
19
20
21
22
23
24
25
26
27
28
29
30

31 **Running title:** Oral rinse MMP-8 testing

32 **Key words:** Oral rinse, biomarker, chair-side test, periodontal diagnostics, MMP-8
33
34
35
36
37

38 **Correspondence should be addressed to:**

39 Dr Päivi Mäntylä, DDS, PhD

40 Institute of Dentistry, University of Helsinki

41 P.O.Box 41

42 FIN-00014 University of Helsinki

43 Finland

44 Tel: +358 9 191 27 217

45 Fax: +358 9 191 27 519

46 E-mail: paivi.mantyla@helsinki.fi
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Objective: The aim of the study was to determine whether oral rinse MMP-8 levels, measured by three different methods, TIMP-1 levels and elastase activity differentiate subjects with different periodontal condition; and second, to find out if MMP-8 levels were comparable among the methods used.

Methods: MMP-8 levels were analyzed with an immunofluorometric method (IFMA), dentoAnalyzer and commercial ELISA. Also TIMP-1 levels and elastase activity were measured. For statistical analysis 214 study subjects were categorized into four groups, specified by the presence and number of moderate (4–5 mm) and deep (≥ 6 mm) periodontal pockets, and bleeding on probing percentage.

Results: MMP-8 levels especially measured by dentoAnalyzer and adjusted to the number of teeth per subject differentiated the study group with ~~strong~~ periodontal inflammatory burden from groups with lower levels. This was also verified with receiver operating characteristic (ROC) analysis. Elastase activity associated with higher IFMA and dentoAnalyzer MMP-8 levels. IFMA MMP-8/TIMP and dentoAnalyzer MMP-8/TIMP-1 tended to be higher with the increasing level of periodontal inflammatory burden. TIMP-1 levels decreased with increasing age.

Conclusions: Oral rinse MMP-8 together with TIMP-1 analysis may have potential in complementary periodontal diagnostics. dentoAnalyzer can be applied in quantitative oral rinse chair side biomarker diagnostics.

Deleted: the highest level of

Introduction

Periodontitis is a very common inflammatory disease in humans. Though bacteria are the initiating factor, periodontitis is not a classical infectious disease. Majority of the causative organisms are also present in the healthy mouth (Socransky & Haffajee 2005), and the host response modified by environmental and behavioural factors plays a key role in disease development, progression and in the maintenance of the treatment result. Despite the etiology or type of periodontitis, the products of host response and tissue destruction are similar. Markers of inflammatory process from periodontal tissues migrate into the periodontal pockets within a serum originating fluid, gingival crevicular fluid (GCF) (Uitto et al., 2003), and further from gingival crevices/pockets into oral cavity where GCF associates with saliva. The more inflamed the periodontal tissues and the deeper the periodontal pockets are, the more GCF is excreted and the more it contains inflammatory markers (Uitto et al., 2003). This also means that the inflammatory burden of periodontium may be reflected in saliva (Uitto et al., 1990; Sorsa et al., 1990).

Unlike in medicine, biochemical testing in dentistry has not yet been taken into everyday clinical practice despite the advantages in supplementation of traditional diagnostics. GCF has been used for site specific diagnostic studies of periodontal disease status, and diagnostic tests for host derived markers have been developed (Uitto et al., 2003; Sorsa et al., 2004). Inflammatory markers from GCF analysed individually or in combination may be valuable in the identification of individual sites or teeth at risk of periodontitis progression. Saliva can also be regarded as a diagnostic fluid but unlike GCF, it mirrors more generally the oral status and is more unspecific. Whole saliva contains sloughed epithelial cells from oral mucous membranes, nasopharyngeal discharge, food debris, bacteria and their products and, in dentate subjects, its content is also affected by GCF flow from gingival pockets. An oral rinse sample can be regarded as a fluid containing mainly the GCF from all periodontal pockets (Sorsa et al., 1990; Uitto et al., 1990, 2003). Thus, analysis of oral rinse may be useful in the diagnosis of general periodontal disease status, early identification of subjects at risk of periodontitis (Uitto et al., 2003), evaluation of periodontal treatment results or the need of hygiene visits during maintenance and in the follow up of the effect of modifying medication over host response (for example low-dose doxycycline, LDD) (Reinhardt et al., 2010).

1
2 Matrix metalloproteinases (MMPs) and their inhibitors and polymorphonuclear leukocyte
3 (PMN) elastase could be candidates as diagnostic markers in oral rinse specimens (Uitto et al.,
4 2003; Sorsa et al., 2004). Among MMPs, especially GCF MMP-8 levels have been found to be
5 increased in periodontitis (Mäntylä et al., 2003, 2006). The deeper the periodontal pockets are, the
6 higher are the levels of MMP-8 at individual sites, and the levels decrease by periodontal hygiene
7 phase treatment. GCF MMP-8 levels remain low in treated and stable periodontitis while in subjects
8 with tendency to progression of periodontitis or poor treatment response (refractory periodontitis)
9 GCF indicates repeatedly high MMP-8 levels. Though smoking may decrease GCF MMP-8 levels,
10 there may also be smoking subjects who repeatedly have especially high GCF MMP-8 levels and
11 poor treatment response (Mäntylä et al., 2006).

12
13 Salivary MMP-8 levels have been suggested serving as a biomarker of periodontitis and
14 applicable as a point-of-care test because MMP-8 levels in saliva have been found to correlate with
15 periodontal disease severity (Miller et al., 2006; Ramseier et al., 2009). Herr et al. (2007) have
16 introduced a microfluidic assay for point-of-care rapid quantitation of biomarkers from human
17 saliva which in validation with MMP-8 identified the periodontitis subjects.

18
19 Also salivary elastase-like activity has been concluded to reflect the severity of periodontal
20 destruction because elevated activity levels have been found in untreated periodontitis subjects'
21 saliva, which decrease after periodontitis hygiene phase treatment (Nieminen et al., 1993). The
22 results were similar in the oral water-rinse samples: a significant correlation was noticed between
23 oral rinse elastase activity and the average of community index of periodontal treatment needs
24 (CPITN) (Uitto et al., 1996).

25
26 In our previous study (Sorsa et al., 2010) we analyzed GCF MMP-8 levels by different
27 methods and found that methods which based on the same monoclonal antibody against MMP-8
28 [immunofluorometric method (IFMA), MMP-8 specific chair-side dip-stick test and dentoAnalyzer
29 appliance] gave comparable results. In this current paper we have used IFMA, dentoAnalyzer as
30 well as commercially available Amersham ELISA assay to determine MMP-8 levels in oral rinse
31 samples from subjects with different periodontal health status expressed as different levels of
32 periodontal inflammatory burden. Additionally, TIMP-1 levels and detection of elastase-like activity
33 were screened in oral rinse samples by Amersham ELISA and a test utilising a chromogenic
34 substrate (Nieminen et al., 1993; Uitto et al., 1996), respectively. The aim was to find out if these
35 markers analyzed with the applied methods individually or in combination, have diagnostic

Deleted: measured

1 potential in differentiation of subjects with different periodontal inflammatory burden level. In
2
3 addition we wished to find out whether the results of MMP-8 analysis obtained by used methods
4
5 were comparable.
6
7

Deleted: condition

8 **Materials and Methods**

9 *Patients*

10
11
12 In this cross-sectional study 214 adult patients with age range 44–78 years (mean±sd
13
14 57.2±7.9) were randomly selected from dental public health clinics in cities of Helsinki and Vantaa.
15
16 Patients in need of antibiotic prophylaxis or suffering of a contagious disease were excluded. The
17
18 protocol was clearly explained to all study subjects and institutional review board-approved
19
20 informed consents were signed. The study protocol was approved by the ethical committee of the
21
22 Institute of Dentistry, University of Helsinki and Helsinki University Central Hospital, and the
23
24 experiments were undertaken with the understanding and written consent of each subject and
25
26 according to ethical principles, including the World Medical Association Declaration of Helsinki.

Deleted: born year 1956 or earlier

27
28 All study subjects were examined clinically: bleeding on probing (BOP) was registered
29
30 dichotomously on four tooth surfaces and pocket probing depths (PPD) by Florida-probe device
31
32 (Florida Probe Corporation, USA) on six tooth surfaces. PPD was recorded if ≥ 4 mm. Examinations
33
34 were done by two calibrated general dentists. Background characteristics were recorded by using
35
36 questionnaires. Oral rinse samples were taken from all patients.

37
38 Patients were categorized into four groups according to their periodontal inflammatory
39
40 burden, determined by combining the Periodontal Inflammatory Burden Index (PIBI) described by
41
42 Lindy et al. (2008) and BOP%. PIBI is calculated by adding the number of periodontal sites
43
44 indicating moderate periodontitis (number of periodontal pockets with PPD 4-5 mm = Nmod) to the
45
46 weighted number of periodontal sites indicating advanced periodontitis (number of periodontal
47
48 pockets with PPD ≥ 6 mm multiplied by two = Nadv).

49
50 Periodontal inflammatory markers are conducted into oral rinse within the GCF. Flow of
51
52 GCF is increased in inflamed tissues and is also proportional to the area of inflamed epithelial
53
54 surface of the periodontal sulcus/pocket (Armitage, 1995; Weidlich et al., 2001). BOP% indicates
55
56 inflammation in the periodontal tissues and the PIBI reflects periodontal inflammatory injury
57
58 (Lindy et al., 2008). Based on this we reasoned to integrate PIBI and BOP% into one index (PIBI x
59
60

BOP%) with the purpose to indicate the GCF flow. We hypothesized that the higher the flow of GCF, the higher is the level of inflammatory markers in oral rinses, and that it reflects the periodontal health status.

According to the categorization methods described above we formed four groups of study subjects: (1) 36 subjects were regarded as periodontally healthy (BOP < 10% of teeth surfaces and no deepened periodontal pockets; (2) 21 subjects with BOP \geq 10% of teeth surfaces but no deepened periodontal pockets, mild periodontal inflammatory burden, regarded clinically as gingivitis. Patients diagnosed to have deepened periodontal pockets (PPD \geq 4 mm) were categorized into two groups according the PIBI x BOP %: 104 subjects had PIBI x BOP % 1–100 (group 3; moderate periodontal inflammatory burden) and 53 patients had PIBI x BOP % \geq 100 (group 4; strong periodontal inflammatory burden). Threshold of 100 was chosen to discriminate moderate inflammatory burden (clinically regarded as minor or early periodontitis) from strong inflammatory burden (clinically more advanced disease).

Deleted: B

Deleted: B

Deleted: B

Oral rinse samples

Oral rinse samples were taken as follows: 1 ml of tap water was placed into the subject's oral cavity with a disposable plastic pipette. Subjects rinsed their mouth with tap water for 1 minute and the rinse was collected into collection tubes (Nieminen et al., 1993; Uitto et al., 1996). On the same day an aliquot of the specimen was taken for elastase activity testing and the rest of the sample was frozen on the same day for further MMP-8 and TIMP-1 analyses. (Uitto et al., 1996).

Deleted: At

Deleted: next

Deleted: tests

MMP-8, TIMP-1 and elastase determinations

All samples were analyzed for MMP-8 levels by three different immunological assays: dentoAnalyzer device (Munjal et al., 2007; Sorsa et al., 2010), a time-resolved immunofluorometric assay (Hanemaaijer et al., 1997; Mäntylä et al., 2003, 2006) and a commercial ELISA kit by Amersham (Munjal et al., 2007; Sorsa et al., 2010). Briefly, dentoAnalyzer is designed as a portable user-friendly bench-top instrument. The device applies a sandwich based immunoassay technology using ABICAP filters. TIMP-1 levels were measured with Amersham ELISA (Tuomainen et al., 2007; Rautelin et al., 2009). Amersham ELISA analyses of MMP-8 and TIMP-1 levels were done according to manufacturer's instructions. MMP-8 and TIMP-1 results (ng/ml) were expressed as levels per subject and per total number of teeth/subject.

1
2 Elastase activity test was done based on the method described by Nieminen et al. (1993) and
3 Uitto et al. (1996). The chromogenic substrate succinyl-alanyl-valine-p-nitroanilide (Sigma Co, St
4 Louis, MO, USA) was dissolved in a solution with the following final concentrations: 1 mM
5 substrate, 0.1% dimethyl sulfoxide, 0.2 M NaCl and 0.05 M Tris-HCl buffer, pH 7.5. The enzyme
6 activity is expressed as optical density after subtracting the 0 h value. Optical densities were
7 measured at nominal/categorical scale by comparing the colour of test plates to a coloured slide
8 designed for eye comparison of optical densities. Optical densities were analyzed by two observers
9 to minimize chance of misinterpretation. Optical densities were categorized according to previously
10 published results, which proved good correlation between elastase activity and the number of deep
11 periodontal pockets (Uitto et al., 1996). 0.2 OD was used as a cut off level for a significant positive
12 elastase activity and levels below the threshold of 0.2 OD were regarded as negative (Uitto et al.,
13 1996).

22 *Data analysis*

23
24 Non-parametric Kruskal-Wallis or Mann-Whitney U test for independent samples was used
25 for testing statistical significances of differences between study groups and bivariate non-linear
26 Spearman correlation, for testing of correlations between results obtained by different MMP-8
27 detection methods. An association of nominal variables in crosstables was tested by Cramér V test.
28 The diagnostic accuracy of MMP-8 measuring methods used was evaluated using Receiver
29 Operating Characteristics (ROC) curve analysis. p-values < 0.05 were considered to be statistically
30 significant. Statistical analyses and tests were done by SPSS-software (version 17.0).

37 **Results**

40 *Clinical parameters and demographic data*

41
42 Subject groups, clinical parameters and demographic characteristics are shown in Table 1.
43 Association between consensus risk factors (Genco, 1996) for chronic periodontitis; gender, tobacco
44 smoking and diabetes, and different periodontal indices were tested (Table 2). In general terms,
45 male gender and tobacco smoking were associated with higher values of all periodontal indices
46 used in the study: PIBI, BOP%, and PIBI x BOP% (p < 0.001), except for the association between
47 male gender and BOP%, that was insignificant (p = 0.323). However, diabetes associated with
48
49
50
51
52
53
54
55
56
57
58
59
60

Deleted: x

Deleted: x

1
2 decreased BOP% ($p = 0.016$) in this study population (Table 2). Diabetic subjects were not
3 examined as a separate group, and they could belong to any of the study groups 1–4.
4
5

6 7 *Association between MMP-8 and periodontal inflammatory burden*

8
9 MMP-8 levels increased along with periodontal inflammatory burden (Table 3). Enzyme
10 levels measured by dentoAnalyzer were significantly different among groups ($p = 0.018$) and
11 differences were more pronounced when MMP-8 levels were adjusted to the number of teeth for
12 both, dentoAnalyzer (Figure 1) and IFMA ($p = 0.007$ and $p = 0.032$, respectively).
13
14

15 16 17 *Correlations between MMP-8 detection methods*

18
19 Correlation between MMP-8 levels measured with IFMA and dentoAnalyzer were tested with
20 Spearman correlation test which gave a correlation coefficient 0.691 (statistically significant at $p <$
21 0.01 level). No correlation was found between MMP-8 analysis by dentoAnalyzer or IFMA and
22 Amersham ELISA. MMP-8 levels measured by Amersham ELISA test did not correlate with the
23 values of periodontal parameters.
24
25

26 27 28 *Statistical outliers*

29
30 Based on statistical outliers of MMP-8 levels adjusted to the number of teeth (Figure 1), we
31 determined the threshold for peak levels of MMP-8/number of teeth measured by both
32 dentoAnalyzer and IFMA. The thresholds were determined at 15 ng/ml level for dentoAnalyzer
33 results corresponding 80 ng/ml levels for IFMA. Percentages of MMP-8/number of teeth peak
34 levels in each study group are shown in Table 4. The higher the severity of periodontal disease
35 (heavier periodontal inflammatory burden) was, the higher the percentage of peak values of MMP-
36 8/number of teeth, except for group 3 (Table 4). The difference was statistically significant when
37 MMP-8 levels were measured by dentoAnalyzer ($p = 0.016$; Table 4). The Amersham ELISA
38 MMP-8 levels did not express statistical outliers.
39
40
41
42
43
44

45 46 47 *MMP-8/TIMP-1 ratio and TIMP-1 levels*

48
49 MMP-8/TIMP-1 molar ratios increased together with periodontal inflammatory burden
50 when measured by dentoAnalyzer ($p = 0.033$, Table 3).
51

52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
TIMP-1 levels decreased progressively with inflammatory burden and with age group, but

1
2 only the latter differences were significant ($p < 0.001$, Figure 2).
3
4
5

6 7 *Elastase activity*

8
9 A statistically significant difference ($p < 0.001$) in MMP-8/number of teeth levels between
10 groups with negative and positive elastase activity test results was detected when MMP-8 was
11 measured by dentoAnalyzer or IFMA but not when measured by Amersham ELISA. The difference
12 of dentoAnalyzer MMP-8/number of teeth median values between elastase activity groups was also
13 tested separately in each patient group and confirmed to be statistically significant (for groups with
14 mild, moderate and strong inflammatory burden, i.e. groups 2, 3, and 4 $p < 0.001$; for periodontally
15 healthy, i.e. group 1 $p = 0.029$; Figure 3). dentoAnalyzer MMP-8/number of teeth levels increased
16 in line with study groups (inflammatory burden), except for group 3 with moderate inflammatory
17 burden, as mentioned earlier in the Results and seen in Figure 1. However, the increase was
18 especially pronounced when the study groups were further categorized according to the elastase
19 activity (positive elastase activities: $p = 0.002$; negative elastase activity $p = 0.322$ ns; Figure 3).
20
21
22
23
24
25
26
27

Deleted: group 1

28 *ROC-curve analysis*

29
30 A Receiver Operating Characteristic (ROC) analysis was ran to evaluate the diagnostic
31 sensitivity and specificity of dentoAnalyzer MMP-8/number of teeth. The ability of dentoAnalyzer
32 MMP-8/number of teeth to distinguish between periodontally healthy subjects and subjects with
33 different levels of periodontal inflammatory burden (groups 2, 3 and 4 with mild, moderate and
34 strong inflammatory burden) was tested separately. The distinction was significant between
35 periodontally healthy (group 1) and subjects with strong periodontal inflammatory burden (group
36 4) (Figure 4). The area under the curve was 0.701 ($p = 0.002$; 95% confidence interval 0.59-0.81).
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Deleted: (group 1)

Deleted: most severe

Discussion

In this study the subjects were not selected based on a defined periodontal disease status. Instead, the randomly selected study subjects were categorised based on the level of periodontal inflammatory burden expressed as numbers and depths of deepened periodontal pockets and percentage of bleeding on probing.

MMP-8 levels in oral rinse samples were higher in subjects with the strongest periodontal inflammatory burden than in subjects with less inflammatory changes when oral rinse levels were adjusted to number of teeth, especially when measured with dentoAnalyzer appliance. This finding was confirmed with ROC analysis. Similarly, MMP-8/TIMP-1 ratios increased in subjects with strong periodontal inflammatory burden when MMP-8 was measured by dentoAnalyzer. MMP-8 increments were especially pronounced in subjects showing detectable elastase activity.

Deleted: rose

Deleted: highest

The measurement of host originating oral biomarker levels in periodontal diagnostics has faced the problem that it has been extremely difficult to define the “normal” levels of various biomarkers. Biomarkers which are expressed at high levels in periodontal inflammation can be detected at lower levels also in periodontally healthy oral cavity, obviously as an indication of the physiologic turnover of oral tissues (McCulloch, 1994; Uitto et al., 2003). Besides, the individual expression of biomarkers in healthy state as well as in disease may vary because of genetic variation (McCulloch, 1994; Uitto et al., 2003).

MMP-8 can be regarded as a central enzyme in periodontitis development and progression (Sorsa et al., 2004, 2006). It may be useful in site specific diagnostics to find out if individual sites or teeth are at risk of progression of periodontitis when analysed from GCF. Mäntylä et al. (2006) found that repeatedly high MMP-8 levels in GCF tested with a MMP-8 specific chair side dip-stick test identified sites/teeth which were at risk of progression of periodontal attachment loss. Site specific MMP-8 testing also identified the subjects with poor response to conventional periodontal treatment, i.e. scaling and root planing. These subjects had multiple sites with an unstable character, and these sites were repeatedly test positive during the 12 month maintenance phase of periodontal treatment. Repeated testing may thus give more reliable diagnostic information than single testing in site specific biomarker diagnostics.

Deleted: n

Smoking has been found in several studies to decrease MMP-8 levels in GCF because of the effects on local blood circulation and lowered signs of inflammation (Söder, 1999; Söder et al.,

2002). However, Mäntylä et al. (2006) detected the highest MMP-8 GCF levels in smoking subjects with poor response to conventional periodontal treatment (scaling and root planing, SRP). Thus it cannot be regarded categorically that all smoking periodontitis patients have lower levels of MMP-8 in GCF and further in oral rinse sample.

Periodontitis is regarded to be cyclic by nature, and part of the dentition may be in active and progressing phase of periodontitis while other teeth may be quiescent or even in the phase of recovery (Carranza & Camargo, 2006). Cyclic nature of periodontitis may be even more challenging for oral rinse than for GCF based diagnostics. In an oral rinse sample the GCF is pooled from all periodontal pockets, and the collected oral rinse sample represents GCFs from whole periodontium. For this reason the definition of the cut off level for the biomarker which would be indicative for the deterioration of periodontal health is demanding. For this reason an oral rinse sample biomarker analysis can be used only for a rough assessment of the periodontal inflammatory level. The activity of periodontitis can only be established by monitoring patients longitudinally, and the progression can only be assessed site specifically. In our current study we did not address the question of disease activity or progression. Instead, we wanted to find out, if the surface area for subgingival biofilm accumulation expressed as periodontal pocketing and the simultaneous bleeding on probing, which points out the possibility of an ongoing inflammation, are reflected in an oral rinse sample.

In this current study some subjects with clinically healthy periodontium (no deepened periodontal pockets and bleeding on probing < 10 %) as well as some subjects with mild to moderate inflammatory burden with limited numbers of deepened periodontal pockets indicating gingivitis or mild periodontitis, expressed high levels of oral rinse MMP-8 which was detected by IFMA and dentoAnalyzer. High oral rinse MMP-8 levels in subjects with no clinical periodontal disease and in subjects with mild to moderate periodontal inflammatory burden were statistical outliers or peak values and were expressed only by some individuals in groups of subjects with healthy periodontium or with mild or moderate inflammatory burden. The cut off level for "positive" MMP-8 finding in an oral rinse sample measured by dentoAnalyzer and adjusted to the number of teeth which indicates a person at risk, may be the level above which the statistical outliers or peak values in groups of subjects with mild to moderate periodontal inflammatory burden are situated in box and whiskers plots, i.e. 15 ng/ml. These peak values in periodontally healthy subjects' samples may indicate a proinflammatory condition in subjects which may have an

Deleted: et al.

Deleted: 1-3

Deleted: of

Deleted: 2 and 3 (

Deleted:)

Deleted: (group 1)

1 aggressive host response and can be susceptible to develop periodontitis, i.e. subjects with a high
2 “normal” MMP-8 level. In subjects with mild to strong inflammatory burden, oral fluid MMP-8 at
3
4
5 least partly originates from inflamed marginal gingival tissues but levels above the risk level (15
6
7 ng/ml) may also indicate the subjects who are at risk to progress from gingivitis to periodontitis, or
8
9 at risk of progression of periodontitis.

Deleted: (groups 2-4)

10 MMP-8 levels measured by IFMA and dentoAnalyzer, but not with Amersham ELISA, were
11
12 comparable. The obvious reason for the high correlation between IFMA and dentoAnalyzer MMP-8
13
14 levels is the same monoclonal antibody shared by both methods (Hanemaaijer et al., 1997; Mäntylä
15
16 et al., 2003, 2006; Sorsa et al., 2010), but based on its association with the severity of periodontal
17
18 inflammation, this antibody may also be applicable and promising in oral fluid periodontal
19
20 diagnostics. The antibody used in dentoAnalyzer and IFMA exert high sensitivity to target active
21
22 forms of MMP-8 (Sorsa et al., 2010; Hanemaaijer et al., 1997), whereas the ELISA might test
23
24 proform and thus target total MMP-8. IFMA is a method which can be used only in research
25
26 laboratories and for this reason it is not ideal for rapid chair-side use in a dentist’s office (Sorsa et
27
28 al., 2010). dentoAnalyzer has been designed for chair-side use and it gives numeric quantitative
29
30 information about the analysis in 15 minutes (Munjal et al., 2007; Sorsa et al., 2010).

31 It has been suggested that the simultaneous measurement of several oral fluid biomarkers
32
33 could be beneficial in periodontal diagnostics. In this current study oral rinse MMP-8 levels and
34
35 elastase activity, both enzymes which originate especially from PMN cells, associated with the level
36
37 of periodontal inflammatory burden. However, it is not necessarily useful to measure many
38
39 biomarkers with the same cellular origin. In this study we also analysed TIMP-1 levels, which is a
40
41 tissue inhibitor of MMPs, in oral rinse samples. MMP-8/TIMP-1 ratios had a trend to be higher in
42
43 the group of subjects with strong inflammatory burden, and the ratio was significantly higher when
44
45 MMP-8 levels were analysed by dentoAnalyzer.

Deleted: the

Deleted: est

Deleted: (group 4)

46 A novel finding was that oral rinse TIMP-1 levels decrease with increasing age which can be
47
48 regarded as an indication of age related changes in tissue homeostasis. Age as such has not been
49
50 regarded as a risk factor for periodontitis. In aging skin, expression of TIMP-1 is decreased with
51
52 fibroblast senescence (Hornebeck, 2003). Aging may have a similar effect in oral mucous
53
54 membranes and periodontal tissues. Relevant age related changes related to TIMP-1 decrease can
55
56 also be supported by the findings of Benatti et al. (2008) indicating that aging modulates important
57
58 biological properties of periodontal ligament cells and favours extracellular matrix remodelling.
59
60

1
2 Diagnostic tests are always somewhat unreliable and they should only be supplementary
3 when the likelihood of a disease is estimated (Pauletto et al., 2000; Uitto et al., 2003; Sorsa et al.,
4 2006). Very seldom a single biochemical test is diagnostically fully reliable but it may be helpful as
5 an adjunct to clinical examination (Mäntylä et al., 2003, 2006). No positive test finding in clinic
6 should lead to treatment unless clinical findings are in line, and vice versa, the negative finding
7 shouldn't lead to the neglect of health promoting measures if clinical findings indicate
8 inflammation. Based on our findings, the oral rinse sample analysis of MMP-8 could be clinically
9 useful in rough screening to identify individuals who are at risk to develop periodontitis or in
10 analysing the individual level of host response. Simultaneous analysis of MMP-8 and TIMP-1 could
11 be beneficial. Oral rinse sample analysis may be useful in defining the optimal period between
12 periodontal maintenance visits after active periodontal treatment. During the active treatment phase
13 it would be possible to monitor the decrease of oral rinse MMP-8 levels, and oral rinse MMP-8
14 levels at the end of the active treatment phase would show the individual level to keep the host
15 response in control. In this regard, the testing could give valuable information about the control of
16 inflammation which today is based on the clinical findings, i.e. shallower or eliminated periodontal
17 pockets and less bleeding on probing. At best the biomarker level could be monitored by a home
18 test to indicate when the cut off of for possibly unsafe biomarker level is reached. Also the effect of
19 MMP-8 inhibiting low-dose doxycycline (LDD) medication could be monitored by analysing the
20 oral rinse MMP-8 levels to find out when a possible brake in medication would be possible or when
21 the medication should be taken again (Golub et al., 2008; Reinhardt et al., 2010).

22
23 In conclusion, MMP-8 testing of oral rinse samples may be beneficial in periodontal
24 diagnostics, but should be applied at this phase of research with caution. Testing may be adjunctive
25 to other clinical screening methods if it is used correctly. Today it cannot be ruled out that there may
26 be false negative findings because of inadequate knowledge about the safe cut off levels, and the
27 levels may vary between different individuals depending on individual biomarker profiles.
28 Simultaneous analysis of TIMP-1 may be beneficial.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acknowledgements

This study was supported by grants from the Academy of Finland, the Research Foundation of Helsinki University Central Hospital, the Finnish Dental Society Apollonia, and the Fondecyt Foundation of Chile. Timo Sorsa is an inventor of US-patents 5652227, 5736341, 5866432 and 6143476. dentognostics GmbH has been the scientific developer of the dentoAnalyzer. However, dentognostics GmbH does not manufacture, sell or distribute this device.

References

- Armitage GC (1995). Clinical evaluation of periodontal diseases. *Periodontol 2000*. **7**: 39-53.
- Benatti BB, Silvério KG, Casati MZ, Sallum EA, Nociti FH Jr (2008). Influence of aging on biological properties of periodontal ligament cells. *Connect Tissue Res*. **49**: 401-408.
- Genco RJ (1996). Current view of risk factors for periodontal diseases. *J Periodontol*. **67**: 1041-1049.
- Golub LM, Lee HM, Stoner JA, Sorsa T, Reinhardt RA, Wolff MS, Ryan ME, Nummikoski PV, Payne JB (2008). Subantimicrobial-dose doxycycline modulates gingival crevicular fluid biomarkers of periodontitis in postmenopausal osteopenic women. *J Periodontol*. **79**: 1409-1418.
- Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, van Hinsbergh VW, Helaakoski T, Kainulainen T, Rönkä H, Tschesche H, Salo T (1997). T. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem*. **272**: 31504-31509.
- Herr AE, Hatch AV, Throckmorton DJ, Tran HM, Brennan JS, Giannobile WV, Singh AK (2007). Microfluidic immunoassays as rapid saliva-based clinical diagnostics. *Proc Natl Acad Sci U S A*. **104**: 5268-5273.
- Hornebeck W (2003). Down-regulation of tissue inhibitor of matrix metalloprotease-1 (TIMP-1) in aged human skin contributes to matrix degradation and impaired cell growth and survival. *Pathol Biol*. **51**: 569-573.
- Lindy O, Suomalainen K, Mäkelä M, Lindy S (2008). Statin use is associated with fewer periodontal lesions: A retrospective study. *BMC Oral Health*. **15**; 8: 16.
- Carranza FA, Camargo PM (2006). The periodontal pocket. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, eds., Carranza's clinical periodontology. 10th Edition. Saunders Elsevier: Louisville, USA, pp.434-451.

1 McCulloch CA (1994). Host enzymes in gingival crevicular fluid as diagnostic indicators of
2 periodontitis. *J Clin Periodontol.* **21**: 497-506.

3
4
5
6 Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV (2006). Salivary biomarkers of
7 existing periodontal disease: a cross-sectional study. *J Am Dent Assoc.* **137**: 322-329.

8
9
10
11 Munjal S, Miethe P, Netuschil L, Struck F, Maier K, Bauermeister C (2007). Immunoassay-based
12 diagnostic point-of-care technology for oral specimen. *Ann NY Acad Sci.* **1098**: 486-489.

13
14
15 Mäntylä P, Stenman M, Kinane DF, Tikanoja S, Luoto H, Salo T, Sorsa T (2003). Gingival
16 crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *J*
17 *Periodontal Res.* **38**:436-439.

18
19
20
21 Mäntylä P, Stenman M, Kinane D, Salo T, Suomalainen K, Tikanoja S, Sorsa T (2006). Monitoring
22 periodontal disease status in smokers and nonsmokers using a gingival crevicular fluid matrix
23 metalloproteinase-8-specific chair-side test. *J Periodontal Res.* **41**: 503-512.

24
25
26
27
28 Nieminen A, Nordlund L, Uitto VJ (1993). The effect of treatment on the activity of salivary
29 proteases and glycosidases in adults with advanced periodontitis. *J Periodontol.* **64**: 297-301.

30
31
32
33 Pauletto NC, Liede K, Nieminen A, Larjava H, Uitto VJ (2000). Effect of cigarette smoking on oral
34 elastase activity in adult periodontitis patients. *J Periodontol.* **71**: 58-62.

35
36
37
38 Ramseyer CA, Kinney JS, Herr AE, Braun T, Sugai JV, Shelburne CA, Rayburn LA, Tran HM,
39 Singh AK, Giannobile WV (2009). Identification of pathogen and host-response markers correlated
40 with periodontal disease. *J Periodontol.* **80**: 436-446.

41
42
43 Rautelin HI, Oksanen AM, Vejjola LI, Sipponen PI, Tervahartiala TI, Sorsa TA, Lauhio A (2009).
44 Enhanced systemic matrix metalloproteinase response in *Helicobacter pylori* gastritis. *Ann Med.* **41**:
45 208-215.

46
47
48
49 Reinhardt RA, Stoner JA, Golub LM, Lee HM, Nummikoski PV, Sorsa T, Payne JB (2010).
50 Association of gingival crevicular fluid biomarkers during periodontal maintenance with
51

1 subsequent progressive periodontitis. *J Periodontol.* in press 2010.

2
3
4 Socransky SS, Haffajee AD (2005). Periodontal microbial ecology. *Periodontol* 2000. **38**: 135-187.

5
6
7 Sorsa T, Suomalainen K, Uitto VJ (1990). The role of gingival crevicular fluid and salivary
8 interstitial collagenases in human periodontal diseases. *Arch Oral Biol.* **35 Suppl**: 193S-196S.

9
10
11 Sorsa T, Tjäderhane L, Salo T (2004). Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis.*
12 **10**: 311-318.

13
14
15
16 Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, Golub LM, Brown DL, Mäntylä P
17 (2006). Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of
18 periodontal inflammation. *Ann Med.* **38**: 306-321.

19
20
21 Sorsa T, Hernández M, Leppilähti J, Munjal S, Lutz Netuschil L, Mäntylä P (2010). Detection of
22 gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. *Oral Dis.*
23 **16**: 39-45

24
25
26
27 Söder B (1999). Neutrophil elastase activity, levels of prostaglandin E2, and matrix
28 metalloproteinase-8 in refractory periodontitis in smokers and non-smokers. *Acta Odontol Scand.*
29 **57**: 77—82.

30
31
32
33 Söder B, Jin LJ, Wickholm S (2002). Granulocyte elastase, matrix metalloproteinase-8 and
34 prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers
35 with persistent periodontitis. *J Clin Periodontol.* **29**: 384—391.

36
37
38
39 Tuomainen AM, Nyysönen K, Laukkanen JA, Tervahartala T, Tuomainen TP, Salonen JT, Sorsa T,
40 Pussinen PJ (2007). Serum matrix metalloproteinase-8 concentrations are associated with
41 cardiovascular outcome in men. *Arterioscler Thromb Vasc Biol.* **27**: 2722-2728.

42
43
44
45 Uitto VJ, Suomalainen K, Sorsa T (1990). Salivary collagenase. Origin, characteristics and
46 relationship to periodontal health. *J Periodontal Res.* **25**: 135-142.

47
48
49 Uitto VJ, Nieminen A, Coil J, Hurttia H, Larjava H (1996). Oral fluid elastase as an indicator of
50 periodontal health. *J Clin Periodontol.* **23**: 30-37.

1
2
3 Uitto VJ, Overall CM, McCulloch C (2003). Proteolytic host cell enzymes in gingival crevice fluid.
4 *Periodontol 2000*. **31**: 77-104.
5
6

7
8 Weidlich P, Lopes de Souza MA, Oppermann RV (2001). Evaluation of the dentogingival area
9 during early plaque formation. *J Periodontol*. **72**: 901-910.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Periodontal indices and demographic data of patient groups. Medians (IQR) indicated for each parameter.

Deleted: x

	Group 1 Periodontally healthy n = 36	Group 2 Mild inflammatory burden n = 21	Group 3 Moderate inflammatory burden n = 104	Group 4 Strong inflammatory burden n = 53	p-value
Teeth (n)	25 (22–27)	26 (22–28)	26 (23–28)	25 (18–27)	0.094
Age (years)	57 (52–64)	55 (50–57)	55 (52–61)	56 (51–63)	0.507
BOP (%)	4.2 (1.8–5.9)	13.7 (12.0–16.0)	6.9 (4.8–13.9)	19.7 (14.5–28.2)	<0.001
PIBI	0	0	2.5 (2–5)	15 (8–26)	<0.001
PIBI x BOP (%)	0	0	21.0 (10.9–46.1)	263.9 (166.6–487.2)	<0.001

Table 2. Association between periodontal indices and consensus risk factors. Medians (IQR) indicated for each parameter.

Deleted: x

		BOP%	PIBI	PIBI x BOP%
Gender	Males n = 68	11.6 (6.0–17.3)	5 (1–14)	40 (9–192)
	Females n = 146	9.0 (4.9–15.9)	2 (0–6)	15 (0–70)
p-value		0.323	0.001	0.002
Smoking (Cigarettes daily)	0 (no smoking)	8.3 (4.5–15.3)	2 (0–5)	14 (0–55)
	1–10	11.8 (7.0–19.3)	4 (1–10)	40 (5–203)
	11–20	14.5 (6.9–20.0)	9 (5–21)	112 (59–321)
	> 20	13.7 (9.7–47.6)	63 (32–102)	927 (317–5375)
p-value		0.03	< 0.001	< 0.001
Diabetes	No	10.5 (5.3–17.3)	2 (1–8)	23 (0–108)
	Yes	6.1 (4.2–10.1)	2 (0–4)	10 (0–25)
p-value		0.016	0.170	0.055

Table 3. MMP-8 and TIMP-1 levels among patient groups. Medians (IQR) indicated for each parameter. dA= dentoAnalyzer, AE = Amersham ELISA.

	Group 1 <u>Periodontally healthy</u> n = 36	Group 2 <u>Mild inflammatory burden</u> n = 21	Group 3 <u>Moderate inflammatory burden</u> n = 104	Group 4 <u>Strong inflammatory burden</u> n = 53	p- values
MMP-8 dA	39 (0–100)	44 (23–276)	68 (15–158)	131 (22–462)	0.018
MMP-8 dA/number of teeth	1.6 (0–3.9)	1.7 (0.8–9.9)	2.8 (0.6–7.0)	5.8 (0.9–22)	0.007
MMP-8 IFMA	398 (103–797)	376 (149–591)	341 (135–839)	628 (245–1131)	0.112
MMP-8 IFMA/number of teeth	16.4 (4.2–36.3)	13.2 (5.2–28.7)	13.7 (5.3–32.7)	30.9 (12–54.3)	0.032
MMP-8 AE	35.7 (14.4–82.9)	32.5 (19.8–69.3)	39.2 (17.9–80)	28.8 (15.1–62)	0.576
MMP-8 AE/number of teeth	1.55 (0.77–3.47)	1.31 (0.8–2.48)	1.49 (0.75–3.38)	1.31 (0.68–2.83)	0.921
dA MMP-8/TIMP-1	0.09 (0–0.37)	0.19 (0.03–0.97)	0.2 (0.03–0.59)	0.32 (0.04–2.61)	0.033
IFMA MMP-8/TIMP-1	1.2 (0.3–2.2)	0.9 (0.2–3.0)	1.1 (0.4–2.5)	1.6 (0.2–6.7)	0.298
AE MMP-8/TIMP-1	0.09 (0.04–0.21)	0.1 (0.04–0.21)	0.11 (0.05–0.21)	0.11 (0.06–0.23)	0.935
TIMP-1	172 (111–218)	170 (73–227)	147 (95.6–229)	143 (76.9–258)	0.770

Table 4. Percentages of subjects (%) representing peak levels of MMP-8 measured by dentoAnalyzer (dA) and IFMA adjusted to numbers of teeth.

	Peak threshold	Group 1 <u>Periodontally healthy</u> n = 36	Group 2 <u>Mild inflammatory burden</u> n = 21	Group 3 <u>Moderate inflammatory burden</u> n = 104	Group 4 <u>Strong inflammatory burden</u> n = 53	*p-value
IFMA MMP-8/number of teeth	>80 ng/ml	8.3% (n = 3)	9.5% (n = 2)	3.8% (n = 4)	15.1% (n = 8)	0.104
dA MMP-8/number of teeth	>15 ng/ml	13.9% (n = 5)	19.0% (n = 4)	13.5% (n = 14)	34.0% (n = 18)	0.016

Deleted:

*Contingency coefficient and Cramer V test was used for the statistical analyses.

1 Figure texts

2
3 Figure 1. Boxplots representing the distributions of MMP-8 levels detected by dentoAnalyzer and
4 IFMA adjusted to the number of teeth in study groups ($p = 0.007$). Threshold line for peak
5 dentoAnalyzer and IFMA MMP-8 levels is drawn at 15ng/ml and 80 ng/ml levels, respectively.
6

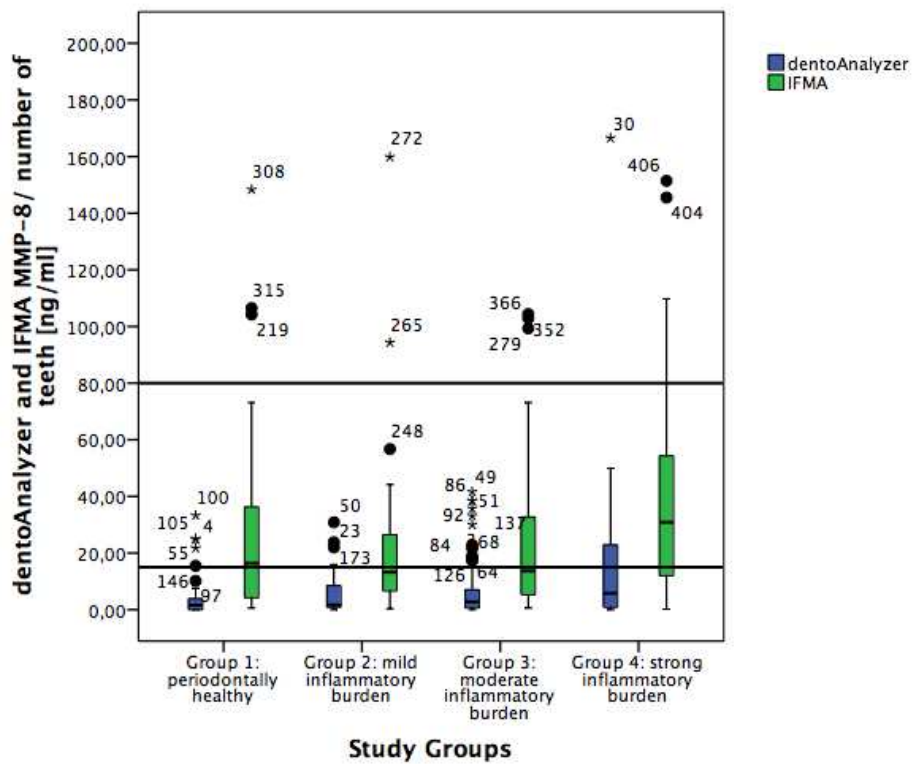
7
8 Figure 2. TIMP-1 values grouped according to age of studied subjects ($p < 0.001$).
9

10 Figure 3. Association between the detection of elastase activity and dentoAnalyzer MMP-8/number
11 of teeth levels in study groups. Green represents the category of positive elastase activity and blue
12 negative elastase activity, respectively. dentoAnalyzer MMP-8 levels v/s elastase activity in subject
13 groups 1-4 with healthy peridontium, and with mild, moderate and strong periodontal inflammatory
14 burden, respectively, $p < 0.05$; dentoAnalyzer MMP-8 levels v/s all study groups 1-4, in subjects
15 with positive elastase activity, $p = 0.002$. There were no statistically significant differences between
16 study groups with negative elastase activities.
17

Deleted: subject

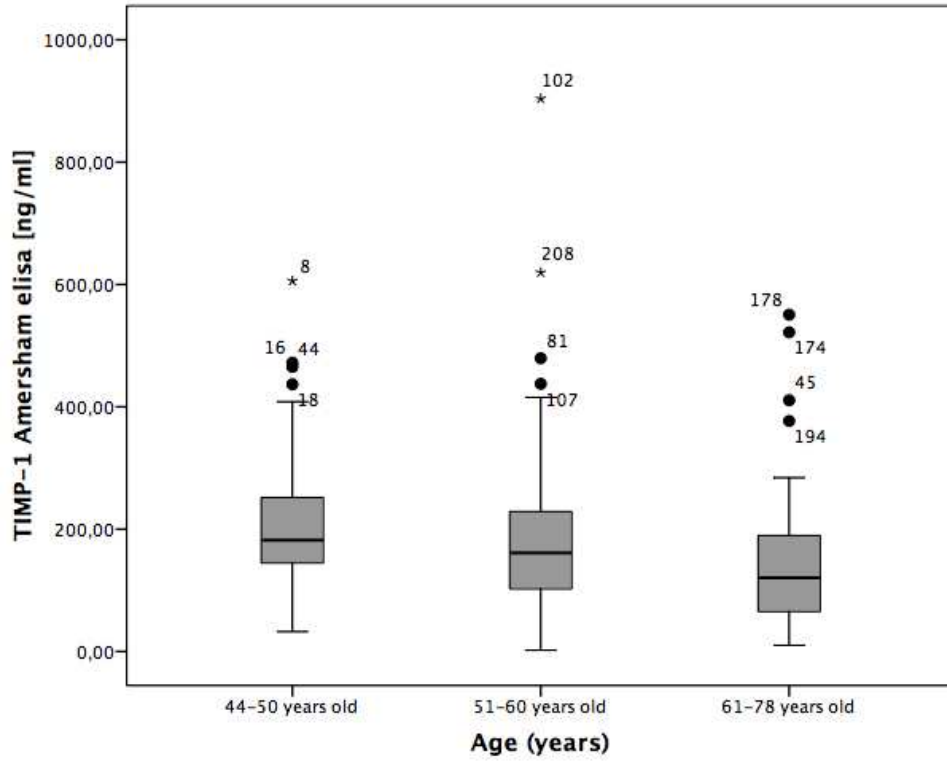
Deleted: having

18
19 Figure 4. ROC (receiver operating characteristic) curve analysis for the evaluation of diagnostic
20 sensitivity and specificity of dentoAnalyzer MMP-8/number of teeth levels in different levels of
21 periodontal inflammatory burden (study groups 1-4 with healthy peridontium, mild, moderate and
22 strong periodontal inflammatory burden, respectively). The area under the ROC curve, 95%
23 confidence interval, and p-values are given next to the figure.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



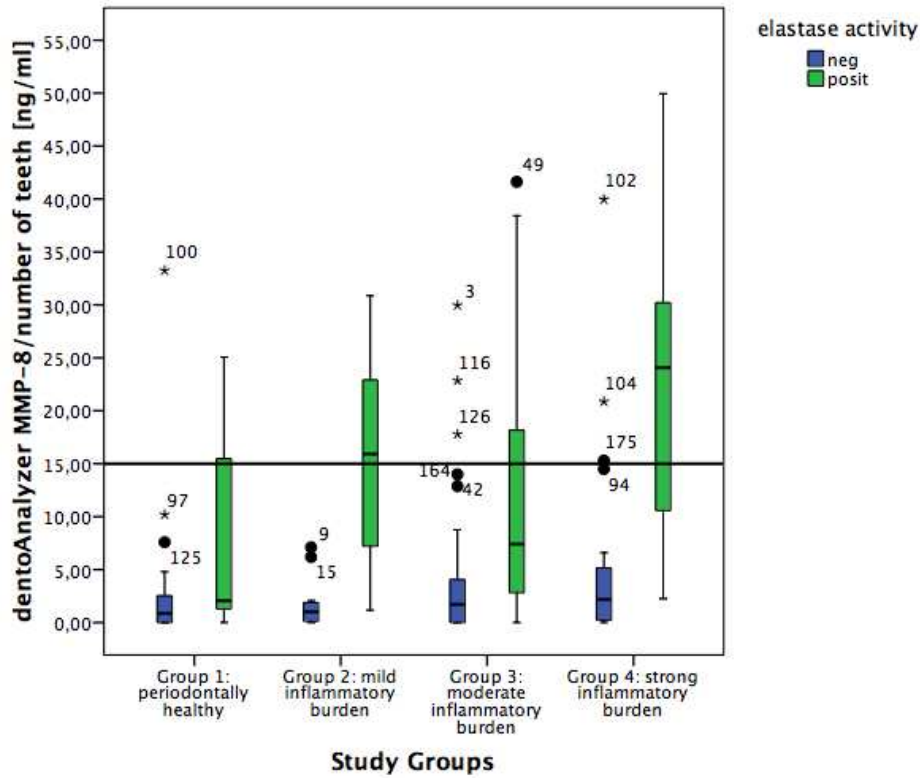
Boxplots representing the distributions of MMP-8 levels detected by dentoAnalyzer and IFMA adjusted to the number of teeth in study groups ($p = 0.007$). Threshold line for peak dentoAnalyzer and IFMA MMP-8 levels is drawn at 15ng/ml and 80 ng/ml levels, respectively.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



TIMP-1 values grouped according to age of studied subjects ($p < 0.001$).
166x133mm (96 x 96 DPI)

Manuscript Copy



Association between the detection of elastase activity and dentoAnalyzer MMP-8/number of teeth levels in study groups. Green represents the category of positive elastase activity and blue negative elastase activity, respectively. dentoAnalyzer MMP-8 levels v/s elastase activity in subject groups 1-4 with healthy peridontium, and with mild, moderate and strong periodontal inflammatory burden, respectively, $p < 0.05$; dentoAnalyzer MMP-8 levels v/s all study groups 1-4, in subjects with positive elastase activity, $p = 0.002$. There were no statistically significant differences between study groups having with negative elastase activities.

166x133mm (96 x 96 DPI)

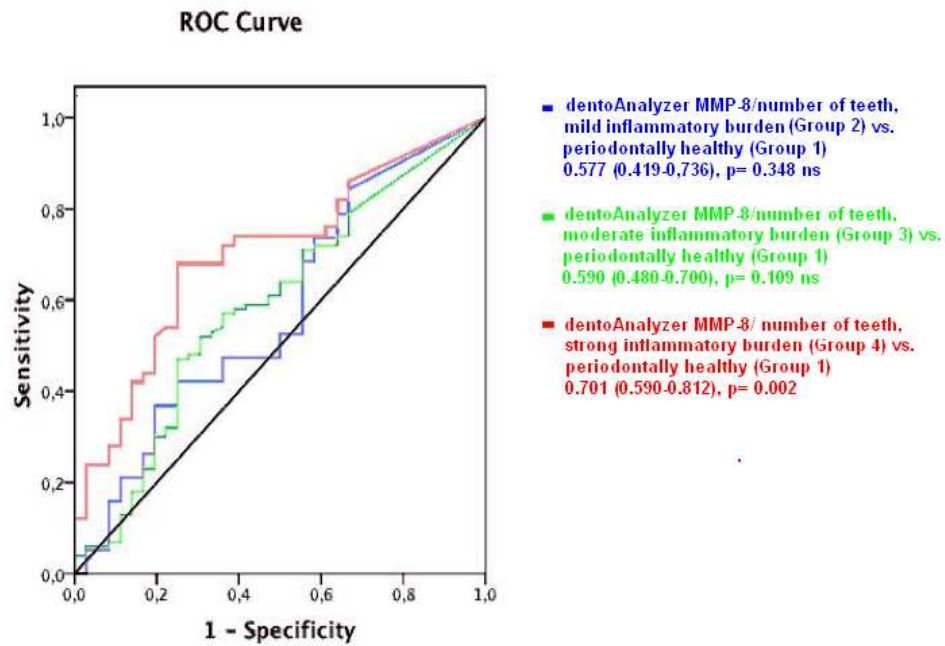


Figure 4. ROC (receiver operating characteristic) curve analysis for the evaluation of diagnostic sensitivity and specificity of dentoAnalyzer MMP-8/number of teeth levels in different levels of periodontal inflammatory burden (study groups 1-4 with healthy periodontium, mild, moderate and strong periodontal inflammatory burden, respectively). The area under the ROC curve, 95% confidence interval, and p-values are given next to the figure.

196x131mm (96 x 96 DPI)