Accuracy of aMMP-8 point-of-care test in indicating periodontal treatment outcomes in stage III/IV periodontitis : A 24-week follow-up study

Yilmaz, Mustafa

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ORIGINAL ARTICLE

Accuracy of aMMP-8 point-of-care test in indicating periodontal treatment outcomes in stage III/IV periodontitis: A 24-week follow-up study

Mustafa Yilmaz[1,2] | Timo Sorsa[3,4] | Esra Demir[5] | Mervi Gürsoy[1] | Auli Suominen[6] | Taina Tervahartiala[3] | Ismo T. Räisänen[3] | Ulvi K. Gürsoy[1]

[1]Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland

[2]Department of Periodontology, Faculty of Dentistry, Biruni University, Istanbul, Turkey

[3]Department of Oral and Maxillofacial Diseases, Head and Neck Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[4]Division of Periodontology, Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden

[5]Department of Periodontology, Faculty of Dentistry, Bezmialem Vakif University, Istanbul, Turkey

[6]Department of Community Dentistry, Institute of Dentistry, University of Turku, Turku, Finland

Correspondence
Mustafa Yilmaz, Department of Periodontology, Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, Turku FI-20520, Finland.
Email: myilmaz@biruni.edu.tr and muyilm@utu.fi

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Abstract

Objective: To analyse the correspondence between aMMP-8 PoC test results and the clinical endpoints of non-surgical periodontal treatment in stage III/IV periodontitis.

Background: The diagnostic success of the active-matrix metalloproteinase-8 (aMMP-8) point-of-care (PoC) test has been demonstrated in various studies, but the evidence of its accuracy following periodontal treatment is limited.

Materials and methods: Altogether 42 stage III/IV grade C periodontitis patients were included in this prospective diagnostic study. Clinical periodontal indices were recorded, aMMP-8 PoC test was applied and mouthrinse was collected before and at 6, 12 and 24 weeks after non-surgical periodontal treatment. Quantitative aMMP-8 levels were determined with immunofluorometric assay (IFMA) for the verification of the PoC test results. The accuracy of the aMMP-8 PoC test was assessed using previously established clinical endpoints as references.

Results: Sensitivity and specificity of aMMP-8 PoC test to indicate clinical endpoints were ranged as follows: Sensitivity 71.4% at baseline, 39.3%–42.4% at week 6, 28.6%–32.4% at week 12 and 35.3%–42.9% at week 24; specificity 64.3%–80% at week 6, 40%–57.1% at week 12 and 56%–64.3% at week 24.

Conclusions: The accuracy of aMMP-8 PoC test in identifying clinical endpoints after non-surgical periodontal treatment is reduced in relation to baseline. Individual healing patterns of each diseased pocket eventually limit the accuracy of the dichotomous aMMP-8 oral rinse test during the post-treatment period.

KEYWORDS
periodontitis, non-surgical therapy, collagenase

1 | INTRODUCTION

Point-of-care (PoC) tests are used to obtain fast and easy diagnostic results without requiring any additional laboratory processing.[1] Ideally, diagnostic biomarkers should be able to detect the existence or absence of a disease, but they can also be used to identify disease severity, to monitor treatment response and to predict prognosis.[2] Proposed biomarkers for periodontitis exist within a wide spectrum; markers of infection (i.e. *Porphyromonas gingivalis* gingipains),[3,4] inflammation (i.e. interleukin (IL)-1β and -6),[5,6] and tissue degradation (i.e. matrix metalloproteinase (MMP)-8, -9 and -13)[7] are widely studied.

Diagnostic PoC tests that use active MMP-8 (aMMP-8),[8–10] MMP-9[11] or IL-1β[5] as target biomarkers are promising tools with regard to their accuracy in discriminating periodontal health and disease. Independent studies demonstrated high sensitivity and specificity of commercially available aMMP-8 PoC tests in diagnosing periodontitis,[9,12,13] while the test's accuracy can vary depending on periodontal status,[14–16] smoking habits[17–19] and systemic conditions of the evaluated individuals.[20,21]

MMP-8 (neutrophil collagenase or collagenase-2) is a host-derived endopeptidase that is primarily expressed and released by human polymorphonuclear leukocytes, although it can also be produced by a wide range of other cells.[22] MMP-8 is one of the main enzymes responsible for connective tissue and bone destruction in periodontitis.[7] A variety of laboratory or chair-side methods can be used for detecting MMP-8. However, there are only a few methods to detect its active forms of MMP-8 such as lateral flow immunoassay and immunofluorometric assay (IFMA).[23,24] aMMP-8 is frequently detected in elevated levels in oral fluids of patients with periodontitis and peri-implantitis,[25–27] while salivary[28,29] and crevicular fluid[19,30] MMP-8 levels decline following non-surgical treatment.

The goals of non-surgical periodontal treatment are to reduce inflammation, eliminate pockets and gain clinical attachment. Various clinical endpoints have been proposed for successful non-surgical periodontal treatment, such as having at most four residual sites with PPD ≥5 mm,[31] having no residual pockets with PPD ≥6 mm,[32,33] having no pockets with PPD ≤4 mm, which do not bleed[34] and full pocket closure.[32] Clinical recordings are, therefore, the main method for assessing and predicting short- and long-term treatment results. Although there are some promising studies on candidate biomarkers to indicate treatment success or failure,[35,36] there remains a need for validated PoC tests that can monitor the healing outcomes of periodontal treatment.[37]

The accuracy of aMMP-8 PoC test in the diagnosis of periodontitis has been widely studied, but there is no information on the associations between aMMP-8 PoC test results and non-surgical treatment endpoints.[14,25,38,39] We hypothesised that aMMP-8 PoC test outcomes relate to clinical endpoints of non-surgical periodontal treatment. Therefore, this study aimed to reveal the accuracy of aMMP-8 PoC test in indicating various clinical endpoints of non-surgical periodontal treatment. In addition, the PoC test results were verified using quantitative aMMP-8 levels.

2 | MATERIALS AND METHODS

2.1 | Ethics and sample size

This prospective, single-gate diagnostic test accuracy study was approved by the Clinical Research Ethics Committee of Biruni University's Medical Faculty, Istanbul, Türkiye (2015-KAEK-44-20). The protocol was explained and written informed consent was obtained from all participants. The study was planned as part of the project registered on clinicaltrials.gov (NCT04792372), in which the sample size was decided based on the assumption of the overall project's primary outcomes. However, an additional sample size calculation was conducted for the present study, which was based on the ability of a negative aMMP-8 PoC test result to indicate disease remission (defined by having ≤4 residual pockets with PPD ≥5 mm). Prior research demonstrated that the aMMP-8 PoC test shows a specificity of 96.7%[40] and that approximately 68.6% of periodontitis patients have ≤4 residual pockets with PPD ≥5 mm at the end of treatment.[33] The calculations estimating a specificity of 96.7%, residual disease prevalence of 31.4%, marginal error of 0.1 and alpha of .05 resulted in a minimum required number of 18 participants.[41]

2.2 | Study population and eligibility criteria

Systemically healthy patients, who applied to the Biruni University, Faculty of Dentistry, Istanbul, Türkiye, were invited to the study. Individuals who used antibiotics or anti-inflammatory drugs during the 3 months prior to the study, were pregnant or in lactation, had fewer than 15 teeth or were not willing to participate in the study were excluded. Smokers were chosen among people who consumed ≥5 cigarettes/day for at least one year, while non-smokers were individuals who had never smoked before.

2.3 | Clinical assessments and periodontal treatment

Full-mouth plaque index (PI), probing pocket depth (PPD), indirect clinical attachment level (CAL) and bleeding on probing scores (BoP) were measured with the help of a UNC-15 periodontal probe (54B XSI™, LM-Dental) at six sites from each tooth. Gingival recession was assessed by measuring the distance between the cemento-enamel junction and the free gingival margin. If the gingival margin was located more coronally than the cemento-enamel junction, a negative value was recorded. CAL was calculated by summing PPD with gingival recession. The clinical parameters were recorded by the same examiner (ED). The examiner was previously calibrated for reproducibility of

site-level PPD of ten individuals, who were not participants in the present study (intraclass correlation coefficient = .87 to .90).

Patients who were diagnosed with generalised stage III or IV, grade C periodontitis were recruited to the study.[42] The stage was determined based on the greatest clinical attachment loss and the number of lost teeth due to periodontitis. The extent and distribution of periodontitis were determined according to the percentage of affected teeth (≥30%). The grade was determined based on indirect evidence of disease progression (% bone loss/age >1.0) and the potential impact of smoking (≥10 cigarettes daily) on clinical manifestation. All clinical variables were recorded before (T0) and at 2, 6 (T1), 12 (T2) and 24 (T3) weeks after periodontal treatment.

Non-surgical periodontal treatment consisted of full-mouth scaling and subgingival debridement under local anaesthesia, combining a piezoelectric scaler (Variosurg™, NSK) and hand instruments (American Eagle Instruments). The treatment was completed in two consecutive sessions on the same day with a brief break (30–60 min) in between to allow the operator (MY) and the patient to rest, followed by the delivery of detailed oral hygiene instructions to the patient. In the follow-up sessions, patients received supragingival prophylaxis when necessary, and oral hygiene was reinforced.

2.4 | aMMP-8 PoC test and aMMP-8 IFMA levels in relation to clinical endpoints

At all time points, the aMMP-8 PoC test (PerioSafe®, Dentognostics GmHb) was performed before the clinical measurements. Participants were asked to avoid brushing, rinsing with commercial mouthwash products, or food and liquid consumption for at least two hours prior to the session. The test was conducted according to the manufacturer's instructions. Patients rinsed with tap water for 30 s, and after a one-minute gap, they rinsed with the test solution for 30 s. The solution was then transferred to the syringe, the filter was attached, and three droplets were dispensed onto the test cassette. The result was read after 5 min by a trained dental assistant, who was blinded to the periodontal status of the participants. A double stripe was accepted as a positive result, while a single stripe was accepted as negative.

The mouthrinses were immediately transferred to cryotubes, stored at −80°C and then sent to Helsinki University, Finland in dry ice for the biochemical analysis. aMMP-8 levels were determined with IFMA as described previously.[23] Mouthrinse aMMP-8 IFMA levels at each time point were categorised as >20 ng/ml or ≤20 ng/ml.

The clinical endpoints that were used to test the accuracy of the tests are as follows: having ≤4 sites with PPD ≥5 mm, having ≤1 site with bleeding pocket (PPD ≥6 mm) and having ≤1 site with bleeding pocket (PPD ≥4 mm).

2.5 | Data analysis

The normality of the data was evaluated with the Shapiro–Wilk test. A chi-square test was used to observe the distribution of gender and age according to smoking status. Wilcoxon signed-rank test was applied to evaluate the differences in the clinical variables and aMMP-8 IFMA levels (linear) between T0 and T3. The changes in frequencies of positive aMMP-8 PoC test and aMMP-8 IFMA > 20 ng/ml (nominal) were assessed between T0 and T3 with the McNemar test. The difference in aMMP-8 PoC test results and aMMP-8 IFMA > 20 ng/ml (nominal) between smokers and non-smokers was evaluated with square crosstabs and Fisher's exact test. The agreement of the PoC test (test method) and aMMP-8 IFMA with 20 ng/ml threshold (reference method) was calculated as follows: positive percent agreement (PPA) = number of simultaneously positive PoC test and IFMA >20 ng/ml divided by the total number of IFMA >20 ng/ml; negative percent agreement (NPA) = number of simultaneously negative PoC test and IFMA ≤20 ng/ml divided by the total number of IFMA ≤20 ng/ml.[9,43]

Sensitivity, specificity, positive and negative predictive values, likelihood ratios and accuracy of the test were calculated according to the clinical endpoints. At all time points, the participants who did not meet the criteria of a clinical endpoint were defined as positive cases, that is 'with ongoing disease'. The participants who met the criteria were defined as negative cases, that is 'healed'. aMMP-8 PoC test results with a double stripe or aMMP-8 IFMA levels >20 ng/ml were accepted as positive test results individually. The sensitivity and specificity of the test were then calculated as follows: Sensitivity = (number of positive cases with a positive PoC test result)/(number of positive cases with positive test result + number of positive cases with a negative test result). Specificity = (number of negative cases with a negative test result)/(number of negative cases with a negative test result + number of negative cases with a positive test result). Namely, the test's ability to predict a favourable clinical outcome was based on its specificity, while its ability to predict 'ongoing disease' was based on its sensitivity.

The associations of the aMMP-8 PoC test, aMMP-8 IFMA >20 ng/ml and aMMP-8 IFMA levels with the clinical endpoints were analysed with binomial logistic regression analysis adjusted for age, gender, smoking and number of teeth. Statistical analyses were conducted using IBM SPSS Statistics Version 27.0. $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Study population

Following drop-outs for various reasons, 42 participants (25 non-smokers, 20 male) out of 52 eligible periodontitis patients were included to the statistical analysis (Figure 1). The missing data of the two participants, one smoker and one non-smoker, who did not attend to the 24-week control were replaced with their corresponding values in T2. The mean age of the participants was 42.3 ± 9.8. There was no statistically significant difference in age ($p = .196$) and gender ($p = .067$) distribution between smokers and non-smokers.
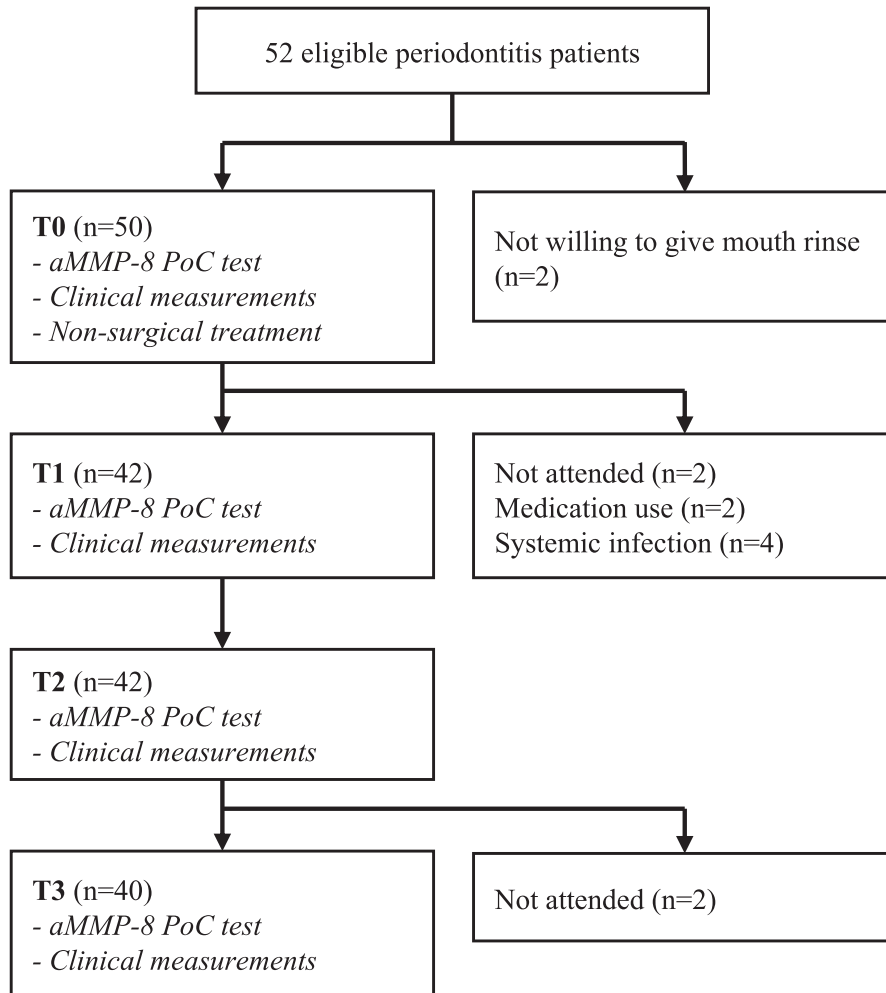
FIGURE 1 Study flow chart.

TABLE 1 Clinical variables, aMMP-8 PoC test results, aMMP-8 IFMA >20 ng/ml and aMMP-8 IFMA levels

	T0	T1	T2	T3
PI%, median (min–max)	86.1 (31.4–100)	38.2 (6.8–100)***	40.4 (6.2–89.9)***	52.6 (5.3–98.2)***
BoP%, median (min–max)	70.3 (22.2–100)	28.6 (2.5–69.1) ***	19.6 (0–48.2) ***	8.7 (0–54.9)***
PPD, median (min–max)	3.7 (2.6–5.9)	2.8 (1.8–5.1)***	2.6 (2.0–5.2)***	2.5 (1.7–4.8)***
CAL, median (min–max)	4.0 (3.1–6.1)	3.2 (2.0–5.5)***	3.1 (2.0–5.7)***	3.1 (1.7–5.4)***
No. pockets PPD ≥4 mm, median (min–max)	74 (21–126)	29 (5–77)***	20 (4–77)***	18 (2–81)***
No. bleeding pockets PPD ≥4 mm median (min–max)	58.5 (13–126)	14 (0–46)***	10 (0–43)***	3 (0–38)***
No. pockets PPD ≥6 mm, median (min–max)	21 (4–67)	5 (0–40)***	3 (0–44)***	2 (0–39)***
No. bleeding pockets PPD ≥6 mm median (min–max)	18 (2–53)	3 (0–32)***	2 (0–27)***	0 (0–25)***
Positive aMMP-8 PoC test, n (%)	30 (71.4)	16 (38.1%)***	15 (35.7%)***	17 (40.5%)**
aMMP-8 IFMA, median (min–max)	35.8 (1.2–106.8)	29.6 (2.8–107.6)	28.8 (2–93.2)	19.2 (1.2–61.2)**
aMMP-8 >20 ng/ml, n (%)	30 (71.4)	31 (73.8%)	29 (69%)	20 (47.6%)*

Note: *$p<.05$, **$p<.01$, ***$p<.001$ Significantly lower when compared to baseline (T0).

Abbreviations: BoP, bleeding on probing; CAL, clinical attachment level; PI, plaque index; PPD, probing pocket depth; T0, baseline; T1, 6 weeks; T2, 12 weeks; T3, 24 weeks.

3.2 | Clinical outcomes

Full-mouth PI%, BoP%, PPD and CAL scores, number of pockets with PPD ≥4 mm or ≥6 mm and bleeding pockets with PPD ≥4 mm or ≥6 mm significantly improved following treatment (Table 1,

Figures S1 and S2). The number of participants meeting the clinical endpoint criteria were as follows: ≤4 sites with PPD ≥5 mm: 9 (T1), 10 (T2) and 17 (T3); ≤1 site with bleeding pocket (PPD ≥6 mm): 14 (T1), 21 (T2) and 25 (T3); ≤1 site with bleeding pocket (PPD ≥4 mm): 5 (T1), 5 (T2) and 14 (T3).

FIGURE 2 The scatter-plot graph of the aMMP-8 IFMA levels at baseline (T0), 6 weeks (T1), 12 weeks (T2) and 24 weeks (T3). The lines represent the median levels at the corresponding time points. Connector lines and p values show the significant differences in the entire population across time points.

FIGURE 3 The scatter-plot graph of the aMMP-8 PoC test results at baseline (T0), 6 weeks (T1), 12 weeks (T2) and 24 weeks (T3). The lines represent the median levels at the corresponding time points. Connector lines and p values show the significant differences in the entire population across time points.
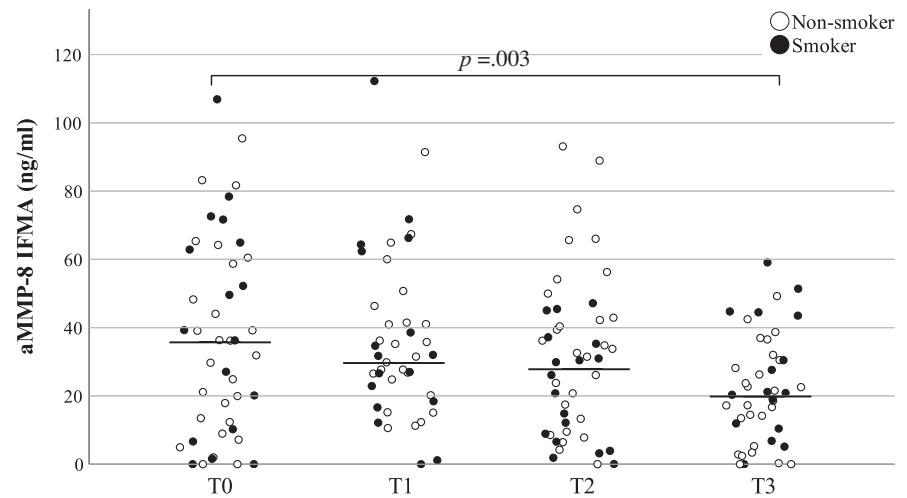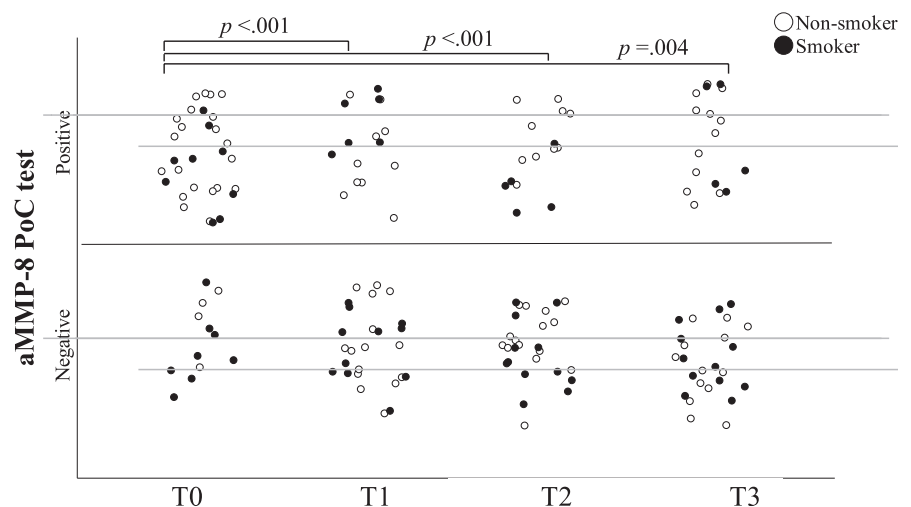

3.3 | The relation between aMMP-8 PoC test results, aMMP-8 IFMA levels and clinical endpoints

Because of the non-existence of false positive and true negative cases at baseline for the clinical endpoint definitions, only the sensitivity of the aMMP-8 PoC test was calculated at T0 (71.4%). The sensitivity of aMMP-8 IFMA >20 ng/ml was also 71.4% at T0. aMMP-8 IFMA levels (Figure 2) significantly decreased at T3 ($p = .003$). The number of positive aMMP-8 PoC test results at T1 ($p < .001$), T2 ($p < .001$) and T3 ($p = .004$) (Figure 3) and the number of dichotomous aMMP-8 IFMA >20 ng/ml results at T3 ($p = .021$) were significantly lower compared to baseline. Smokers had a lower number of positive aMMP-8 PoC test results when compared to non-smokers at baseline ($p = .041$).

The accuracies of the aMMP-8 PoC test and aMMP-8 IFMA >20 ng/ml according to the clinical endpoints are presented in Tables 2 and 3, respectively. The sensitivity of the test was decreased at all post-treatment sessions compared to T0, varying between 28.6% and 47.9%. The specificity of the test was 40%–80% at post-treatment sessions. Logistic regression models revealed no association between negative aMMP-8 PoC test results and the established

clinical endpoints (Table 4). IFMA >20 ng/ml had comparably higher sensitivity at T1–T3 varying between 46.4% and 81.8%, while its specificity varied between 0% and 64.7%. At T1, aMMP-8 <20 ng/ml was found to be associated with having ≤4 sites with PPD ≥5 mm ($p = .033$, unadjusted and $p = .042$, adjusted) and with having ≤1 site with bleeding pocket (PPD ≥4 mm) ($p = .048$, adjusted).

The agreement between aMMP-8 PoC test with aMMP-8 IFMA (20 ng/ml threshold) were as follows: PPA 70%, NPA 25% (measure of agreement [κ] = −.050, $p = .746$) at T0; PPA 48.4%, NPA 90.9% (κ = .273, $p = .021$) at T1; PPA 44.8%, NPA = 84.6% (κ = .227, $p = .066$) at T2; PPA 40%, NPA 59.1% (κ = −.009, $p = .952$) at T3.

4 | DISCUSSION

Here we show that aMMP8-PoC test results of stage III or IV grade C periodontitis patients are not necessarily associated with clinical endpoints of non-surgical treatment following an average to late healing period.[44] While there were prior reports on the decline of positive aMMP-8 PoC test results following periodontal treatment,[9,27] our study is the first to investigate the associations of

TABLE 2 Accuracy of aMMP-8 PoC test to indicate treatment outcomes

		aMMP-8								
	n	Neg	Pos	Sens (%)	Spec (%)	PLR	NLR	PPV (%)	NPV (%)	Acc (%)
≤4 sites with PPD ≥5 mm										
T0										
No	42	12	30	71.4%						
Yes	0	0	0							
T1										
No	33	19	14	42.4%	77.8%	1.91	0.74	87.5%	26.9%	50.0%
Yes	9	7	2							
T2										
No	32	22	10	31.3%	50.0%	0.63	1.38	66.7%	18.5%	35.7%
Yes	10	5	5							
T3										
No	25	15	10	40.0%	58.8%	0.97	1.02	58.8%	40.0%	47.6%
Yes	17	10	7							
≤1 site with bleeding pocket (PPD ≥6 mm)										
T0										
No	42	12	30	71.4%						
Yes	0	0	0							
T1										
No	28	17	11	39.3%	64.3%	1.10	0.94	68.8%	34.6%	47.6%
Yes	14	9	5							
T2										
No	21	15	6	28.6%	57.1%	0.67	1.25	40.0%	44.4%	42.9%
Yes	21	12	9							
T3										
No	17	11	6	35.3%	56.0%	0.80	1.16	35.3%	56.0%	47.6%
Yes	25	14	11							
≤1 site with bleeding pocket (PPD ≥4 mm)										
T0										
No	42	12	30	71.4%						
Yes	0	0	0							
T1										
No	37	22	15	40.5%	80.0%	2.03	0.74	93.8%	15.4%	45.2%
Yes	5	4	1							
T2										
No	37	25	12	32.4%	40.0%	0.54	1.69	80.0%	7.4%	33.3%
Yes	5	2	3							
T3										
No	28	16	12	42.9%	64.3%	1.20	0.89	70.6%	36.0%	50.0%
Yes	14	9	5							

Abbreviations: Acc, accuracy; BoP, bleeding on probing; Neg, negative; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; Pos, positive; PPD, probing pocket depth; PPV, positive predictive value; Sens, sensitivity; Spec, Specificity; T0, baseline; T1, 6 weeks; T2, 12 weeks; T3, 24 weeks.

post-treatment aMMP-8 results with previously established clinical endpoints.

Although the diagnostic criteria for periodontitis are well characterised, a universal definition of a successful treatment endpoint is still missing. Therefore, in the present study, a variety of definitions

for successful treatment were chosen as clinical endpoints of non-surgical periodontal treatment. The scientific rationale of the selected endpoints are as follows: (1) ≤4 sites with PPD ≥5 mm: Having ≤4 sites with PPD ≥5 mm was proposed as a low risk for disease progression priorly.[45,46] Thus, the endpoint of ≤4 sites with PPD

TABLE 3 Accuracy of aMMP-8 levels with 20 ng/ml threshold to indicate treatment outcomes

| | | aMMP-8 IFMA | | | | | | | | |
	n	≤20 ng/ml	>20 ng/ml	Sens (%)	Spec (%)	PLR	NLR	PPV (%)	NPV (%)	Acc (%)
≤4 sites with PPD ≥5 mm										
T0										
No	42	12	30	71.4%						
Yes	0	0	0							
T1										
No	33	6	27	81.8%	55.6%	1.84	0.33	87.1%	45.5%	76.2%
Yes	9	5	4							
T2										
No	32	9	23	71.9%	40.0%	1.20	0.70	79.3%	30.8%	64.3%
Yes	10	4	6							
T3										
No	25	11	14	56.0%	64.7%	1.59	0.68	70.0%	50.0%	59.5%
Yes	17	11	6							
≤1 site with bleeding pocket (PPD ≥6 mm)										
T0										
No	42	12	30	71.4%						
Yes	0	0	0							
T1										
No	28	6	22	78.6%	35.7%	1.22	0.60	71.0%	45.5%	64.3%
Yes	14	5	9							
T2										
No	21	6	15	71.4%	33.3%	1.07	0.86	51.7%	53.8%	52.4%
Yes	21	7	14							
T3										
No	17	8	9	52.9%	56.0%	1.20	0.84	45.0%	63.6%	54.8%
Yes	25	14	11							
≤1 site with bleeding pocket (PPD ≥4 mm)										
T0										
No	42	12	30	71.4%						
Yes	0	0	0							
T1										
No	37	8	29	78.4%	60.0%	1.96	0.36	93.5%	27.3%	76.2%
Yes	5	3	2							
T2										
No	37	13	24	64.9%	0%	0.65		82.8%	0%	57.1%
Yes	5	0	5							
T3										
No	28	15	13	46.4%	50%	0.93	1.07	65%	31.8%	47.6%
Yes	14	7	7							

Abbreviations: Acc, accuracy; BoP, bleeding on probing; Neg, negative; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; Pos, positive; PPD, probing pocket depth; PPV, positive predictive value; Sens, sensitivity; Spec, Specificity; T0, baseline; T1, 6 weeks; T2, 12 weeks; T3, 24 weeks.

≥5 mm was considered effective in distinguishing remission from uncontrolled disease. (2) ≤1 site with bleeding pocket (PPD ≥6 mm): A residual pocket with PPD ≥6 mm was accepted as an incomplete treatment outcome since it is a risk factor for disease progression.[33] (3) ≤1 site with bleeding pocket (PPD ≥4 mm): Bleeding pockets with PPD ≥4 mm are considered unstable sites.[32]

A limitation of our study is that the caries findings of the participants were not recorded, although all patients received full-mouth dental examinations by specialists. In fact, the studies regarding the association between aMMP-8 levels and carious lesions are contradictory.[47,48] Nevertheless, the participants in our study were referred for their restorative treatments as part of the initial treatment

TABLE 4 Associations of aMMP-8 PoC test, aMMP-8 IFMA ≤20 ng/ml and quantitative aMMP-8 IFMA results with clinical endpoints

	Negative aMMP-8 PoC test				aMMP-8 IFMA ≤20 ng/ml				Quantitative aMMP-8 IFMA levels			
	OR	95% CI	p	R²	OR	95% CI	p	R²	OR	95% CI	p	R²
≤4 sites with PPD ≥5mm												
T1												
Unadjusted	2.579	0.463–14.35	.279	.047	5.625	1.153–27.44	**.033**	.162	1.024	0.983–1.065	.254	.056
Adjusted[a]	4.77	0.567–40.10	.15	.406	9.08	1.083–76.16	**.042**	.468	1.026	0.977–1.978	.307	.376
T2												
Unadjusted	0.455	0.107–1.933	.286	.04	1.704	0.387–7.495	.481	.017	1.017	0.981–1.056	.36	.033
Adjusted[a]	0.358	0.069–1.854	.221	.2	1.514	0.308–7.446	.61	.16	1.021	0.984–1.059	.276	.193
T3												
Unadjusted	0.952	0.272–3.338	.939	0	2.333	0.655–8.309	.191	.055	1.021	0.977–1.067	.354	.029
Adjusted[a]	1.294	0.313–5.339	.722	.174	3.38	0.664–17.20	.142	.233	1.027	0.973–1.085	.335	.198
≤1 site with bleeding pocket (PPD ≥6mm)												
T1												
Unadjusted	1.165	0.308–4.406	.822	.002	2.037	0.493–8.408	.325	.031	0.998	0.971–1.026	.899	.001
Adjusted[a]	1.548	0.323–7.412	.584	.249	2.111	0.408–10.92	.373	.263	0.996	0.966–1.027	.809	.243
T2												
Unadjusted	0.533	0.148–1.922	.336	.029	1.25	0.337–4.639	.739	.004	0.992	0.965–1.021	.597	.009
Adjusted[a]	0.549	0.131–2.303	.549	.183	1.679	0.370–7.623	.502	.177	1.001	0.971–1.033	.934	.165
T3												
Unadjusted	0.694	0.195–2.472	.573	.01	1.432	0.416–4.934	.57	.01	1	0.953–1.043	.999	0
Adjusted[a]	0.886	0.207–3.791	.871	.209	0.837	0.180–3.892	.82	.209	0.982	0.934–1.033	.982	.222
≤1 site with bleeding pocket (PPD ≤4mm)												
T1												
Unadjusted	2.727	0.277–26.86	.39	.039	5.437	0.771–38.33	.089	.13	1.054	0.982–1.130	.145	.139
Adjusted[a]	9.091	0.442–187.2	.153	.413	136.7	1.033–18099	**.048**	.571	1.238	0.969–1.582	.088	.577
T2												
Unadjusted	0.32	0.047–2.176	.244	.063	0	0	.999	.175	0.992	0.952–1.033	.689	.007
Adjusted[a]	0.264	0.021–3.387	.306	.359	0	0	.998	.442	1.003	0.958–1.049	.914	.315
T3												
Unadjusted	1.35	0.359–5.078	.657	.007	0.867	0.240–3.130	.867	.002	1.009	0.965–1.055	.681	.006
Adjusted[a]	1.392	0.340–5.699	.646	.06	0.633	0.140–2.873	.554	.065	1.002	0.954–1.052	.94	.053

Note: Logistic regression models on associations of aMMP-8 PoC test, aMMP-8 IFMA ≤20 ng/ml and quantitative aMMP-8 IFMA results with clinical endpoints at 6 (T1), 12 (T2) and 24 (T3) weeks, presenting odds ratios (OR) with 95% confidence intervals (CI). *p*-values in bold indicate statistically significant difference (<.05). R^2 Pseudo-R-squared (Nagelkerke).

Abbreviations: BoP, bleeding on probing; PPD, probing pocket depth; T1, 6 weeks; T2, 12 weeks; T3, 24 weeks.

[a]Adjusted for age, gender, smoking and number of teeth.

when necessary. Hence, the study population in the follow-up period is considered caries-free and the potential impact of carious lesions on aMMP-8 PoC test results after treatment is mitigated. None of the participants were diagnosed with autoimmune mucosal lesions or pericoronitis. It should be noted that this study was conducted in a single university hospital with a relatively small sample size and only in severe periodontitis cases, limiting the generalisability of our findings. Indeed, since only stage III/IV periodontitis cases were included in our study, full pocket closure was not achieved in most cases. It is possible that a negative PoC test can indicate favourable treatment outcomes in stage I or II periodontitis cases or if full pocket closure and a stable periodontium are attained. Thus, validation of our results with multiple-centre trials including cases with varying periodontitis severity may be beneficial.

Our baseline aMMP-8 PoC test results are in accordance with prior research revealing the test's high diagnostic sensitivity, particularly in patients with advanced periodontal tissue loss.[12,14,38,49,50] These results should also be interpreted in light of the fact that smoking reduces the accuracy of the aMMP-8 PoC test since smokers were included in the present study.[5,18] The low number of positive test results in smoking periodontitis patients can be an indication of suppressed host response,[51] considering that decreased aMMP-8 levels in saliva[17] and in GCF,[19] and impaired granulocyte functions[52] have been demonstrated in smokers previously.

In line with the previous studies,[9,12] the number of positive aMMP-8 PoC test results in our study declined following periodontal treatment, which reflects the decreasing levels of aMMP-8 during the healing period.[19] The accuracy of the test was reduced after non-surgical periodontal treatment and the aMMP-8 PoC test results were not associated with the chosen clinical endpoints, which are the primary findings of the present study. Schmalz et al.[16] similarly reported that salivary aMMP-8 PoC test results were unrelated to periodontal clinical parameters and risk factors during the maintenance therapy. The difference between the pre-treatment and post-treatment accuracy of the aMMP-8 PoC test results can be related to the complex nature of pocket healing. Non-treated periodontal pockets are easily detectable with the aid of biomarkers as they have an ongoing infection, inflammation and tissue degradation.[53] Healing of periodontal pockets, on the other hand, requires re-establishment of a symbiotic flora, suppression of inflammation and cellular attachment and tissue maturation.[32,54,55] A failure in the accomplishment of any of these components eventually creates an inconsistency between the biomarker levels and the clinical outcomes.

In order to verify the results of the aMMP-8 PoC test, aMMP-8 levels were also quantitatively evaluated with IFMA. The aMMP-8 levels significantly decreased following treatment, as is observed in positive PoC test results. The agreement between the aMMP-8 PoC test and aMMP-8 IFMA levels, however, was relatively lower than the results of a prior study where the researchers compared the PoC test results with aMMP-8 levels (ELISA) and reported high agreement between the two methods, particularly between negative results.[9] A significant amount of healthy individuals and

gingivitis patients were included in that study, which can be one explanation for the consistency of negative test results they reported, since aMMP-8 is less frequently detected in gingivitis patients and healthy participants when compared to periodontitis.[14,26] The inconsistency between the PoC test and IFMA results in our study can be attributed additionally to differences between the laboratory-based IFMA and chair-side lateral flow test in detecting aMMP-8 levels. It is also worth noting that the chair-side aMMP-8 PoC test is prone to user-related errors: particularly borderline cases with a weak second stripe that can be difficult to distinguish in a clinical setting. In the present study, aMMP-8 IFMA >20 ng/ml demonstrated a higher sensitivity than the PoC test in detecting patients who did not fulfil the criteria of the selected clinical endpoints, especially at 6- and 12-week-control sessions. Interestingly, a statistically significant association was found between aMMP-8 IFMA ≤20 ng/ml with disease remission and having at most one bleeding pocket with ≥4 mm, but it should be noted that a limited number of participants demonstrated favourable clinical outcomes at 6 weeks.

Our study shows that the decrease in the collagenolytic periodontal disease activity, reflected and monitored by the aMMP-8 PoC test, is reduced following non-surgical periodontal treatment with varying efficiencies among severe periodontitis patients. It can be clearly observed from the aMMP-8 IFMA levels that, despite a significant decrease following treatment, aMMP-8 levels do not necessarily drop below the threshold of 20 ng/ml, the detection limit of the PoC test.[25] In such cases, that is if the aMMP-8 levels are still in the 'red zone', it is possible to consider adjunctive treatment such as subantimicrobial dose doxycycline to inhibit the collagenolytic activity.[56] Likewise, although aMMP-8 levels of the participants with slightly higher initial aMMP-8 levels than the threshold may decrease easily below the detection limit, they may not meet the criteria of defined clinical endpoints at that particular time point due to ongoing healing. However, at present, there are no other tools to monitor collagenolytic disease activity or establishment of clinical health.

5 | CONCLUSION

Within the limitations of our study, the aMMP-8 PoC oral rinse test's accuracy is reduced after non-surgical periodontal treatment and PoC test results are not associated with post-treatment clinical endpoints. Variations in the healing patterns of each diseased pocket may limit the accuracy of the dichotomous aMMP-8 oral rinse test during the post-treatment period.

ORCID
Mustafa Yilmaz https://orcid.org/0000-0002-2005-1417
Esra Demir https://orcid.org/0000-0002-7442-1778
Ismo T. Räisänen https://orcid.org/0000-0001-5821-5299

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.