Active MMP-8 point-of-care (PoC)/chairside enzyme-test as an adjunctive tool for early and real-time diagnosis of peri-implantitis

Hanna Lähteenmäki1 | Taina Tervahartiala1 | Ismo T. Räisänen1 | Pirjo Pärnänen1 | Matti Mauramo1,2 | Shipra Gupta3 | Victoria Sampson1 | Nilminie Rathnayake1 | Anna-Maria Heikkinen1 | Saeed Alassiri1,4 | Dirk-Rolf Gieselmann5 | Roland Frankenberger6 | Timo Sorsa1,7

1Department of Oral and Maxillofacial Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
2Department of Pathology, Haartman Institute and HUSLab, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
3Unit of Periodontics, Oral Health Sciences Centre, Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh, India
4Department of Periodontics and Community Dental Sciences, King Khalid University, Abha, Saudi Arabia
5Institute for Molecular Diagnostics IMOD, Solingen, Germany
6Department for Operative Dentistry, Endodontics, and Pediatric Dentistry, Philipps University Marburg and University Hospital Giessen and Marburg, Marburg, Germany
7Division of Periodontology, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden

Correspondence
Nilminie Rathnayake. Department of Oral and Maxillofacial Diseases, University of Helsinki and Helsinki University Hospital, Helsinki 00014, Finland.
Email: rathnayake.nilminie@helsinki.fi

Abstract

Objective: The aim of this study was to investigate the utility of the active matrix metalloproteinase (aMMP-8) point-of-care (PoC) test as a quantitative real-time chair-side diagnostic tool for peri-implant diagnosis, as well as to assess the potentially developing and ongoing risk relative to the traditional clinical methods.

Background: Current peri-implant and periodontal disease diagnoses rely on clinical and radiological examinations. This case-control study investigated the applicability of aMMP-8-PoC immunotest for quantitative real-time diagnosis and monitoring of dental implants in health and disease.

Methods: Sixty-eight patients visiting a specialist clinic for maintenance following dental implant placement underwent assessment of their peri-implant health. aMMP-8-PoC peri-implant sulcular fluid (PISF) lateral-flow immunotests were performed using ImplantSafe® technology quantitated by ORALyzer®. In addition, the PISF samples were analyzed for total MMP-8, calprotectin, and interleukin (IL)-6 by enzyme-linked immunosorbent assays (ELISA), aMMP-8 by western immunoblot, and MMP-2 and MMP-9 by gelatin zymography.

Results: The aMMP-8-PoC test promptly recorded and reflected peri-implant disease, differentiating it clearly from health. X-ray findings (bone loss > 2 mm), peri-implant pocket depth ≥ 3 mm, and bleeding on probing were significantly more prevalent among implants positive for the aMMP-8-PoC test. aMMP-8/ORALyzer analysis was more precise in recording disease than total MMP-8, calprotectin, IL-6, MMP-2, and MMP-9.

Conclusions: The aMMP-8-PoC test can be conveniently implemented to alert for and detect active collagenolysis affecting peri-implant tissues, both in the early and advanced stages of the disease. Active and fragmented MMP-8 exhibits a strong and

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2022 The Authors. Clinical and Experimental Dental Research published by John Wiley & Sons Ltd.
1 | INTRODUCTION

The popularity of dental implants as a treatment option for replacing missing teeth has substantially increased over the past 20 years (Elani et al., 2018). This development poses a challenge to oral health professionals because although in many cases dental implant treatments have been successful having a high survival rate, implant complications and failures have been frequent (PJetursson et al., 2012). Peri-implantitis, a pathological inflammatory condition around dental implants, is a major risk factor for late dental implant failures (Manor et al., 2009; Schwarz et al., 2018). Previous studies have estimated that the prevalence of peri-implantitis may be as high as 23% of dental implants, while peri-implant mucositis, which is considered a precursor for peri-implantitis, may affect more than 40% of implants (Derks & Tomasi, 2015; Heitz-Mayfield & Salvi, 2018).

Current peri-implant and periodontal disease diagnosis rely on clinical and radiological examinations, which have been commonly used by all oral health professionals for decades and are easy and practical to interpret. However, clinicians are only able to detect and measure peri-implant/periodontal diseases after clinical manifestations have occurred using the traditional methods. To overcome this limitation, biomarkers have been studied extensively (Alassy et al., 2019; Arias-Bujanda et al., 2019; Carinci et al., 2019; Gul et al., 2020). Key biomarkers of peri-implant and periodontal tissue destruction, once identified, could alert the clinician as to the onset of collagen breakdown, long before clinical manifestations set in. In adjunct with the traditional methods, they could increase the accuracy of early detection of peri-implant and periodontal diseases, prediction of disease progression, and monitoring of treatment effects (Gul et al., 2020).

Periodontal and peri-implant connective tissue consists mainly of Type I collagen. Results from previous studies support the concept of matrix metalloproteinase-8 (MMP-8), also known as neutrophil collagenase or collagenase-2, as a potential key biomarker responsible for connective tissue destruction or active degeneration of periodontal and peri-implant disease (Alassy et al., 2019; Al-Majid et al., 2018; Arias-Bujanda et al., 2019; Carinci et al., 2019; Gul et al., 2020; Sorsa et al., 2016). MMP-8 is a major host-derived collagenolytic proteinase and is regarded as primarily responsible for the irreversible destruction of periodontal and peri-implant tissues (Buduneli, 2020; Gul et al., 2020; Sorsa et al., 2006). MMP-8 is responsible for the disintegration and processing of collagens and bioactive inflammatory nonmatrix mediators, not only in various inflammatory and malignant tissue destructive diseases but also in wound healing, immune response and tissue remodeling (Buduneli, 2020; Dejonckheere et al., 2011). It can break down almost all the proteinaceous structural components of connective tissues and basement membranes, as well as process distinct bioactive nonmatrix inflammatory immune mediators. It can also act in a degradative manner upon serpins and insulin receptors (Lahtinen et al., 2016; Sorsa et al., 1993).

MMPs, such as MMP-8, are secreted from the cell as latent pro-MMPs (Nagase, 1997). The presence of a proteinase susceptible "bait" region in the propeptide allows reactive oxygen species, tissue and plasma proteinases, or opportunistic microbial proteinases (alone or in concert) to activate pro-MMPs. Once activated, the catalytically competent MMP, such as active MMP-8 (aMMP-8), acts as a potential initiator of interstitial collagenolysis at inflammatory sites. It is the pathologically elevated concentration of active MMP-8 and not the total or latent form, which has been demonstrated to distinguish healthy tissue from gingivitis, periodontitis, peri-implant mucositis, pre-implantitis, and peri-implantitis (Külli et al., 2002; Lähteenmäki et al., 2020; Lee et al., 1995; Ma et al., 2000; Mancini et al., 1999; Romanelli et al., 1999; Romero-Castro et al., 2020; Sorsa, Allassirî et al., 2020; Sorsa, Bacigalupo et al., 2020; Sorsa et al., 2006, 2010, 2016; Verhulst et al., 2019; Wang et al., 2016; Wohlfahrt et al., 2014). In healthy periodontal and peri-implant tissues, the concentration of the active form of MMP-8 is significantly lower or absent altogether, indicating a healthy status, compared to more severe inflammatory diseased conditions in these tissues (Gangbar et al., 1990; Golub et al., 2020; Keles Yucel et al., 2020; Kivelä-Rajamäki et al., 2003; Lee et al., 1995; Mancini et al., 1999; Räisänen et al., 2018; Romanelli et al., 1999; Sorsa, Bacigalupo, et al., 2020; Sorsa et al., 2006; Teronen et al., 1997; Xu et al., 2008). The aMMP-8 enzyme, unlike total MMP-8, shows consistent and sustained pathological elevation that can be assessed from the pocket/peri-implant pocket fluid and mouthwash by aMMP-8 analysis (Allassiri et al., 2018; Gangbar et al., 1990; Golub et al., 2020; Izadi Borujeni et al., 2015; Lee et al., 1995; Lorenz et al., 2017; Mancini et al., 1999; Öztürk et al., 2021; Romanelli et al., 1999; Schmalz et al., 2019; Sorsa, Allassirî et al., 2020).

The aim of this study was to investigate the utility of the aMMP-8-PoC/chairside enzyme (ImplantSafe/ORALyzer®)-test as a quantitative real-time chair-side diagnostic tool for peri-implant diagnosis, as well as assess the potentially developing and ongoing risk relative to peri-implantitis.

**KEYWORDS**

biomarkers, diagnosis, matrix metalloproteinase 8, peri-implantitis, preventive medicine
to the traditional clinical methods. Furthermore, the diseased and healthy peri-implant sulcular fluid (PISF) samples were analyzed by independent immunoassays, for total MMP-8, calprotectin, and interleukin (IL)-6 by enzyme-linked immunosorbent assays (ELISA) analysis and additionally by western immunoblotting for MMP-8, and MMP-2 and MMP-9 were evaluated by utilizing gelatin-zymography to compare them with the aMMP-8-PoC/chairside test.

2 | MATERIALS AND METHODS

2.1 | Study population

Following written informed consent, 68 patients visiting a private clinic "Hammasklinikka Kruunu" in Tampere, Finland, for dental implants were randomly selected for this case-control study. This study received approval from the local ethical committee of the Helsinki University Hospital, Finland (1065/26.06.2019; dnr HUS/1271/2019) and Regionala etikprövingsnämnden i Stockholm, (EPN) (2016-08-24/2016/1:8 and 2016-1:24; Dnr 2016/1410-31/1) in accordance with the Helsinki Declaration. The study was performed from July 2019 to January 2020. Patients who were males or females, smokers or nonsmokers, at least 45 years of age, and who had one or more dental implants were included in this study. If the patient had more than one dental implant, only one implant was randomly chosen and an aMMP-8 PoC test was performed in the buccal sulcus of that implant. Patients who had used anti-inflammatory medication and/or antibiotics or had received peri-implant treatment within the last 6 months were excluded from this study. Dental implants (Nobel Biocare® implants) had been surgically placed according to routine surgical procedures by an experienced dental implant specialist and maintenance therapy was advised at a gap of 6–12 months following implant placement. The surface types of dental implants placed varied between crowns and bridge structures. At the maintenance visit, oral fluid samples were collected and oral examination including bleeding on probing (BOP) was performed and pocket depths at six sites per implant (disto-buccal, mid-buccal, mesio-buccal, disto-palatal, mid-palatal, mesio-palatal) were recorded using a standard millimeter probe (Hu-Friedy Manufacturing Co., LLC). An X-ray of the implant area was also taken to assess alveolar bone destruction. Finally, maintenance therapy was provided at the end of the visit following the Swedish National Guidelines (https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/nationella-riktlinjer/2011-5-1.pdf). The treatment effect of anti-infective treatment on peri-implantitis was measured after 4–6 weeks after baseline by the aMMP-8 PoC test.

Previous studies demonstrate a large effect size for site-specific aMMP-8 measurements by immunofluorometric assay (IFMA) and POCT utilizing the same monoclonal antibodies in peri-implantitis diagnostics (Ala'asri et al., 2018; Golub et al., 2020; Lähteenmäki et al., 2020; Rathnayake et al., 2017; Sorsa, Bacigalupo, et al., 2020). Based on these studies, a minimum of 26 participants per group was calculated to identify the differences between healthy and peri-implantitis groups with a = .05 and power = 0.80. Two groups were enrolled: subjects with peri-implantitis (n = 26) and healthy subjects (n = 42). Peri-implantitis was defined as having the combination of X-ray findings (bone loss > 2 mm), BOP, and peri-implant pockets of ≥3 mm around the dental implant, while healthy controls were defined as the absence of these three clinical measurements and parameters. Clinical peri-implant measurements were performed by a trained and calibrated dental hygienist (H. L.).

2.2 | Analyses of PISF samples

2.2.1 | aMMP-8 analysis using ImplantSafe test kit and ORALyzer

aMMP-8-PoC lateral flow immunoassay test, ImplantSafe® Kit (Dentognostics) was performed by a trained dental hygienist before the maintenance therapy was initiated, in accordance with the manufacturer's instructions, as described by Golub et al. (2020), Lähteenmäki et al. (2020), Sorsa, Bacigalupo, et al. (2020). Briefly, the PISF strip of the test kit was placed apically into the peri-implant sulcus for 30 s, after which the strip was placed in the vial containing 0.6 ml of elution buffer for 5 min. Afterward, a dipstick from the test kit was dipped into the elution fluid for 15 s and then removed, ready for analysis with the ORALyzer® reader (Dentognostics GmbH). Five minutes later, the quantitative result was noted from the result window of the reader. The qualitative result was visible as blue lines on the dipstick; a single blue line indicating an aMMP-8 level less than 20 ng/ml (negative); and two blue lines as aMMP-8 level more than 20 ng/ml (positive). The visible result on the dipstick was documented with a photograph too. Among the positive cases with two lines there existed both weak or thin(ner) (a weak positive) or strong or thick(er) (a strong positive) second line (Sorsa et al., 2021). The remaining elution fluid of PISF was used for further analysis in the study and was analyzed for MMP-2 and MMP-9 as well as total MMP-8, IL-6, and calprotectin by Western immunoblotting, gelatin-zymography, and ELISA as described in Sections 2.2.2.2-2.2.4.

2.2.2 | Western immunoblotting for MMP-8

The molecular forms of MMP-8 were detected by a modified enhanced chemiluminescence Western blot analysis kit according to the protocol recommended by the manufacturer (GE Healthcare) and described by Gürsoy et al. (2018) and Hanemaaijer et al. (1997). Briefly, the PISF samples were mixed with Laemmli sample buffer without any reducing reagents and heated for 5 min, followed by protein separation with 11% sodium dodecyl sulfate (SDS)-polyacrylamide gels with prestained low range molecular weight sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) standards (Bio-Rad) as molecular weight marker and human neutrophil (PMN) MMP-8 (Protealmmun) as a positive control. Target detections were performed by using a primary antibody, polyclonal
anti-MMP-8, and a horseradish peroxidase-linked secondary antibody (GE Healthcare). The immunoblots were quantified by GS-700 Imaging Densitometer Scanner (Bio-Rad) using the Quantity One program (Bio-Rad).

2.2.3 | Gelatinolytic activity assay for MMP-2 and MMP-9

The gelatinolytic activity was assayed from PISF samples with zymographic technique, using 11% SDS-polyacrylamide gels impregnated with 1 mg/ml gelatin (Merck) as substrate, in line with previous descriptions by Paju et al. (2001) Sorsa et al. (1997). Before the electrophoresis, the samples were incubated with Laemmli sample buffer without any reducing reagents for 2 h at room temperature. Prestained low range molecular weight SDS-PAGE standards (Bio-Rad) were used as molecular weight markers and human MMP-9 (Merck) as a positive control. After electrophoresis, the gels were washed two times with 50 mM Tris-HCl buffer and then incubated in 50 mM Tris-HCl buffer, pH 7.5, containing 0.02% NaN3, 0.5 mM CaCl2, and 1 μM ZnCl2 overnight at 370 C. The gelatinolytic activity was then visualized with 1% Coomassie Brilliant Blue R 250 solution; clear bands against the blue background on stained gels. The intensities of gelatinolytic activities were evaluated with GS-700 Imaging Densitometer Scanner using Quantity One-program (Bio-Rad).

2.2.4 | ELISA for total MMP-8, calprotectin, and IL-6

Total MMP-8, IL-6, and calprotectin were measured in the collected PISF samples using ELISA (Quantikine ELISAs, R&D systems). ELISAs were performed according to the manufacturer’s protocols. The detection limits of R&D systems Quantikine kits for total MMP-8, calprotectin, and IL-6 were 0.013, 0.086, and 0.70 pg/ml, respectively (Lähteenmäki et al., 2020).

2.3 | Statistical analysis

General patient characteristics were summarized for study patients, those with dental peri-implantitis, and healthy controls. The differences between peri-implantitis and healthy controls were compared by Fisher’s exact test, Mann–Whitney U test, or t test. Levels of biomarkers (aMMP-8, total MMP-8, calprotectin, and IL-6) in peri-implantitis and healthy groups were compared with Mann–Whitney U test and t-test (unadjusted model) and logistic regression (adjusted model: biomarker, gender, age of dental implant and smoking). Based on the test of normality (Shapiro–Wilk) Mann–Whitney U test (non-normality) or t-test (normality) was used.

Diagnostic accuracy of the biomarker candidates to discriminate peri-implantitis from a healthy implant was studied by receiver operating characteristic (ROC) analysis and the area under the ROC curve (AUC). This was carried out using each biomarker and its adjusted logistic regression model (adjusted for gender, age of dental implant, and smoking). The Youden index was used to define the optimal cut-offs from the ROC curves for each biomarker (unadjusted and adjusted models). Diagnostic sensitivity (Se), specificity (Sp), the percentage of false negatives (FN) and false positives (FP), test accuracy (Acc), and Matthews correlation coefficient (MCC) were calculated for each biomarker by using the cut-off to assess the quality of classification.

There were two patients with peri-implantitis and four with a healthy implant that had missing values in the age of dental implant. Thus, the total sample size for unadjusted calculations was 68, and that for adjusted calculations, 62.

All statistical calculations were performed and figures were created using SPSS Statistics, version 27 (IBM Corp.), and JMP8 Pro, version 15 (SAS Institute Inc.). Statistical significance was set at 0.05 (two-tailed).

3 | RESULTS

3.1 | Patient characteristics

Sixty-eight adults with dental implants were assessed. There were 26 males and 42 females, and their ages ranged from 51 to 92 years. No significant differences were found between peri-implantitis and healthy groups in patients’ age, gender, smoking habits, or systemic diseases. Age of dental implant was significantly higher (mean difference 1.46 years) in the peri-implantitis group ($p = .030$). The demographic characteristics of the peri-implantitis patients and their healthy controls are shown in Table 1.

3.2 | Biomarker levels in PISF and peri-implant health

Analysis of biomarker levels in PISF was performed for aMMP-8, total MMP-8, calprotectin, and IL-6 to assess their potential association with peri-implantitis (Table 2). Levels of all biomarkers were higher in dental implants with peri-implantitis than in healthy controls (Figure 1a). Both quantitative and visual aMMP-8-PoC tests were positively associated with peri-implantitis ($p < .001$), even when adjusted for gender, age of dental implant, and smoking in a logistic regression model ($p < .001$). Similarly, total MMP-8 and calprotectin showed a positive association with peri-implantitis when unadjusted ($p < .001$ and $p < .001$, respectively) and adjusted ($p = .005$ and $p = .004$, respectively). The association with peri-implantitis was significant for IL-6 only in the adjusted model ($p = .044$).

Figure 1b illustrates the distribution of quantitative aMMP-8-PoC test results (aMMP-8 concentrations) in relation to visual aMMP-8-PoC test results (negative test, and weak/strong positive test). In that regard, the significant positive association between visual aMMP-8-PoC test and peri-implantitis ($p < .001$; Table 2) already showed that prevalence of peri-implantitis increased as negative test results changed to weak, and strong positives, that is, aMMP-8 concentration increased. The largest
agreement between the quantitative and visual aMMP-8-PoC tests was obtained by setting two thresholds: aMMP-8 = 20 and 80 ng/ml (Figure 1b). They can be used as estimates of cut-offs for peri-implant health and peri-implantitis risk: no/low risk (aMMP-8 < 20 ng/ml), elevated risk (aMMP-8 ≥ 20 and aMMP-8 < 80 ng/ml), and high risk (aMMP-8 ≥ 80 ng/ml) (Figure 1b).

Figure 2 demonstrates an example of an upper jaw dental implant. A deepened peri-implant pocket and BOP were observed on clinical evaluation. X-rays showed advanced horizontal alveolar bone destruction. The aMMP-8-PoC test assay was a strong positive (163.83 ng/ml).

## 3.3 Diagnostic accuracy of the studied biomarkers

ROC analysis for the studied biomarkers and their discriminatory ability to classify peri-implantitis and health is presented in Table 3 and Figure 3. Both univariable and adjusted (gender, age of dental implant, and smoking) models were assessed. ROC curves showed the highest diagnostic performance for quantitative and visual aMMP-8-PoC tests both in univariable (AUC = 0.833 and 0.773, respectively) and adjusted models (AUC = 0.880 and 0.883, respectively). They were followed by total MMP-8 (AUC = 0.750 for univariable model and AUC = 0.788 for adjusted model), calprotectin (AUC = 0.736 and 0.787) and IL-6 (AUC = 0.637 and 0.726).

Optimal cut-off points to classify healthy and peri-implantitis implants were defined for each biomarker (unadjusted and adjusted for gender, age of dental implant and smoking) by using Youden's index (Table 3). The best accuracy (unadjusted model) was obtained by calprotectin (accuracy = 77.9% and MCC = 0.57), quantitative aMMP-8-PoC test (accuracy = 77.9% and MCC = 0.56) and total MMP-8 (accuracy = 77.9% and MCC = 0.54). However, in adjusted models, the best accuracy was observed for quantitative aMMP-8-PoC test (accuracy = 80.6% and MCC = 0.59), visual aMMP-8-PoC test (accuracy = 79.0% and MCC = 0.58) and calprotectin (accuracy = 75.8% and MCC = 0.56).

### 3.4 MMP-8, MMP-2, and MMP-9 species versus peri-implant health and peri-implantitis

Western immunoblot analysis with independent polyclonal and specific MMP-8 antibody revealed that while consistently elevated MMP-8 species in activated and fragmented forms could be detected in the diseased PISF (Figure 4a, Lanes 1–6), MMP-8 was either hardly detectable or detectable in latent form only in the healthy PISF (Figure 4a, Lanes 7–12). Gelatin zymographic analysis of MMP-2 and MMP-9 using the same PISF samples could not differentiate between peri-implant health and disease (Figure 4b, Lanes 1–12).

Finally, Figure 4c demonstrates the successful effect of treatment of peri-implantitis according to the Swedish National Guidelines. (https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikeldatal og/nationella-riktlinjer/2011-5-1.pdf), monitored by aMMP-8-PoC test, evidencing changes from positive, ≥20 ng/ml (elevated risk), to negative, <20 ng/ml (no/low risk), of the visually recorded real-time PoC test outcomes.

## 4 DISCUSSION

In this study, we observed a significant association between the aMMP-8-PoC/chairside enzyme test results and the prevalence of peri-implantitis and clinical peri-implant risk factors. We found that peri-implant tissue damage involving active collagenolysis, indicated by the elevated aMMP-8 levels, was significantly more common in
dental implants that had peri-implantitis compared with healthy implants. Furthermore, low aMMP-8 levels (<20 ng/ml) in PISF were clearly linked to healthy implant defined as the absence of the combination of X-ray findings (bone loss > 2 mm), BOP, and peri-implant pockets of ≥3 mm around the dental implant. On the other hand, the increase in aMMP-8 levels was associated with the combination of these three clinical measurements and parameters (peri-implantitis): moderate aMMP-8 levels (≥20 ng/ml) indicated the elevated risk of peri-implantitis and high aMMP-8 levels (≥80 ng/ml) indicated an even higher risk for the three clinical peri-implantitis parameters that may suggest a more rapid disease progression. This was also confirmed with western immunoblot analysis utilizing an independent polyclonal and specific MMP-8 antibody demonstrating the elevation of activated and fragmented MMP-8 species in the diseased PISF, but not in the healthy PISF that contained only latent MMP-8 species. These findings support and further extend previous

### TABLE 2

Levels of biomarkers among healthy dental implants or with peri-implantitis (n = 68)

| Characteristic                  | Peri-implantitis (n = 26) | Healthy (n = 42) | Unadjusted p value<sup>a</sup> | Adjusted p value<sup>b</sup> |
|--------------------------------|---------------------------|------------------|-------------------------------|------------------------------|
| Quantitative aMMP-8 PoC test (ng/ml) | 142.32 ± 117.52           | 49.25 ± 33.45    | <.001                         | <.001                        |
| Visual aMMP-8 PoC test          |                           |                  |                               |                              |
| Negative –                     | 0                         | 6                |                               |                              |
| Weak positive +                | 8                         | 28               | <.001                         | <.001                        |
| Strong positive ++             | 18                        | 8                | <.001                         | <.001                        |
| Total MMP-8 (ng/ml)            | 4.62 ± 3.16               | 2.33 ± 3.17      | <.001                         | .005                         |
| Calprotectin (ng/ml)           | 7306.46 ± 5241.21         | 3999.62 ± 3149.57 | <.001                  | .004                         |
| IL-6 (pg/ml)                   | 2.14 ± 4.13               | 0.66 ± 1.15      | .052                          | .044                         |

Abbreviations: aMMP, active matrix metalloproteinase; IL, interleukin; PoC, point-of-care.

<sup>a</sup>Mann–Whitney U test and t test based on test of normality (Shapiro–Wilk).

<sup>b</sup>Logistic regression model (biomarker test for aMMP-8, total MMP-8, calprotectin or IL6 adjusted for gender, age of dental implant, and smoking.

**FIGURE 1** (a) Boxplots of biomarker concentrations per healthy implant and peri-implantitis groups; (b) quantitative versus visual active matrix metalloproteinase (aMMP-8) point-of-care test and estimates of cut-offs for peri-implant health and peri-implantitis maximizing the agreement between the two aMMP-8 tests (n = 68)
studies that have shown not only the direct role of collagenase activity (active MMP-8, not latent/total MMP-8) in the progression of attachment loss, (Köll et al., 2002; Kivelä-Rajamäki et al., 2003; Lee et al., 1995; Mancini et al., 1999; Romanelli et al., 1999; Sorsa et al., 2006) but also aMMP-8 levels in oral fluids correlating well with clinical periodontal/peri-implant parameters in adults and adolescents (Heikkinen et al., 2019; Izadi Borujeni et al., 2015; Lähteenmäki et al., 2015; Lorenz et al., 2017; Räisänen et al., 2019; Räisänen et al., 2020; Schmalz et al., 2019; Sorsa, Alassiri, et al., 2020; Sorsa, Bacigalupo, et al., 2020; Xu et al., 2008). Furthermore, previous studies indicate that microbial burden could act as an up-regulator in the aMMP-8 cascade (proteolytic activation of pro-MMP-8 to aMMP-8) (Gürsoy et al., 2018; Nieminen et al., 2018; Sorsa et al., 1992, 1995). The onset of disease is, hence, heralded by the elevation of periodontal/peri-implant tissue destruction associated biomarkers, indicating collagenolysis.

We extended our analysis to compare the aMMP-8-PoC test assay measuring aMMP-8 and its levels with other potential biomarkers (total MMP-8, calprotectin, and IL-6 ELISAs). Although their levels, in addition to aMMP-8, were elevated in peri-implantitis compared with healthy implants, aMMP-8 had the best diagnostic accuracy to discriminate peri-implant health and disease as judged from ROC-analysis. This supports and further extends our prior studies that have shown that aMMP-8 measured by the aMMP-8-PoC test assay is seemingly superior to other tested potential biomarkers, including neutrophil elastase, calprotectin, tissue inhibitor of MMPs (TIMP)-1, myeloperoxidase, BOP, and MMP-9 in classifying dental implants as healthy or diseased (Golub et al., 2020; Lähteenmäki et al., 2020; Sorsa, Bacigalupo, et al., 2020). Moreover, we found that successful peri-implantitis treatment converted the elevated (>20 ng/ml) aMMP-8 levels in the PISF samples to low/healthy (<20 ng/ml) aMMP-8 levels in PISF. Thus, our results support previous studies that have shown that the aMMP-8 levels can be used to monitor and assess periodontal/peri-implant treatment success and failure (Alassiri et al., 2020; Lee et al., 1995; Leppilahti et al., 2014; Lorenz et al., 2017; Räisänen et al., 2019; Räisänen et al., 2020; Schmalz et al., 2019; Sorsa, Alassiri, et al., 2020; Sorsa, Bacigalupo, et al., 2020; Xu et al., 2008).

### TABLE 3

| Biomarker/Univariable model (n = 68) | AUC (95% CI) | p value | Cut-off point | Se (%) | Sp (%) | FN (%) | FP (%) | Acc (%) | MCC |
|-------------------------------------|-------------|---------|--------------|--------|--------|--------|--------|---------|-----|
| Quantitative aMMP-8 PoC test (ng/ml) | 0.833 (0.728–0.938) | <.001 | 63.1 | 80.8 | 76.2 | 13.5 | 32.3 | 77.9 | 0.556 |
| Visual aMMP-8 PoC test* | 0.773 (0.657–0.888) | <.001 | 1.5 | 69.2 | 81.0 | 19.0 | 30.8 | 76.5 | 0.502 |
| Total MMP-8 (ng/ml) | 0.750 (0.627–0.872) | .001 | 2.68 | 73.1 | 81.0 | 17.1 | 29.6 | 77.9 | 0.537 |
| Calprotectin (ng/ml) | 0.736 (0.611–0.861) | .001 | 4772.0 | 84.6 | 73.8 | 11.4 | 33.3 | 77.9 | 0.568 |
| IL-6 (pg/ml) | 0.637 (0.498–0.776) | .059 | 1.46 | 38.5 | 90.5 | 29.6 | 28.6 | 70.6 | 0.348 |

| Biomarker/Adjusted model^ (n = 62) | AUC (95% CI) | p value | Cut-off point^ | Se (%) | Sp (%) | FN (%) | FP (%) | Acc (%) | MCC |
|------------------------------------|-------------|---------|---------------|--------|--------|--------|--------|---------|-----|
| Quantitative aMMP-8 PoC test (ng/ml) | 0.880 (0.798–0.963) | <.001 | 0.363 | 75.0 | 84.2 | 15.8 | 25.0 | 80.6 | 0.592 |
| Visual aMMP-8 PoC test* | 0.833 (0.731–0.935) | <.001 | 0.333 | 83.3 | 76.3 | 12.1 | 31.0 | 79.0 | 0.582 |
| Total MMP-8 (ng/ml) | 0.788 (0.677–0.900) | <.001 | 0.321 | 79.2 | 68.4 | 16.1 | 38.7 | 72.6 | 0.464 |
| Calprotectin (ng/ml) | 0.787 (0.672–0.903) | <.001 | 0.300 | 91.7 | 65.8 | 7.4 | 37.1 | 75.8 | 0.564 |
| IL-6 (pg/ml) | 0.726 (0.600–0.853) | .003 | 0.300 | 83.3 | 55.3 | 16.0 | 45.9 | 66.1 | 0.383 |

Note: The Youden index was used to define the optimal cut-offs for each biomarker from the ROC curves.

Abbreviations: Acc, accuracy; AUC, area under the ROC curve; CI, confidence interval; FN, false negatives; FP, false positives; MCC, Matthews correlation coefficient; PoC, point-of-care; Se, sensitivity; Sp, specificity.

*Visual aMMP-8 PoC test (0, negative; 1, weak positive; 2, strong positive).

*Adjusted for gender, age of dental implant and smoking.

^Cut-off points are the optimal predicted probabilities for the adjusted logistic regression model.

FIGURE 2 An example of an upper jaw dental implant with a visual aMMP-8 PoC test assay showing a significantly elevated, strong positive (++) test result.
et al., 2018; Golub et al., 2020; Lähteenmäki et al., 2020; Leppilahti et al., 2014, 2015; Sorsa, Bacigalupo, et al., 2020; Thierbach et al., 2016) However, the definition of healthy dental implant and peri-implantitis has eventually an impact on the balance between sensitivity and specificity and should be considered when comparing the results in this study with other studies. There is quite a variation in the case definitions of peri-implantitis in the literature, yet the main criteria for peri-implantitis in most of the studies have been based on BOP, PD ≥ 3 mm, and cases of crestal bone loss of ≥2 mm (Natto et al., 2019; Renvert et al., 2018). And in peri-implant health, PD should in general be ≤5 mm (Renvert et al., 2018). Thus, the definition used in this study is in line with what has been considered peri-implantitis in the literature. The rational and the reason for more strict definition regarding PD in this study was to make sure that the dental implants that were defined healthy (in terms of clinical signs) would really be healthy.

To ensure long-term periodontal/peri-implant health, it is imperative to maintain collagen stability and integrity. As discussed earlier, traditional tools like dental probes, X-rays, or clinical examination by inspection are unable to reliably detect active collagenolysis or the pivotal point between health and disease. Analyzing oral fluids for aMMP-8 levels offers a noninvasive and nonbacteremic method to make the invisible process of collagenolysis visible (Gul et al., 2020). Based on the result of the aMMP-8 analysis, it is possible to evaluate the current state and disease activity of the periodontal/peri-implant tissues and thus conveniently assess the risk of both developing and ongoing collagen degradation/collagenolysis, to predict possible future attachment tissue loss (Gul et al., 2020; Lee et al., 1995; Leppilahti et al., 2014, 2015). In other words, the beauty of the aMMP-8 POCT for clinicians is its ability to alarm collagenolytic tissue destruction prior appearance of clinical manifestations, that is, it makes invisible visible. This could help clinicians to personalize more precisely secondary prevention protocols based on breakdown intensity (collagenolytic activity) and, at the same time, improve patient compliance in terms of oral hygiene maintenance and adherence to recall appointments. At present, most implant patients are called in once a year, which may not be sufficient to capture disease initiation. Limitations of the study include the small sample size and lack of longitudinal follow-up data to confirm the ongoing progressing attachment loss. The clinical measurements and parameters used in this study to define peri-implantitis are not direct indicators of active progressing attachment loss, although they are associated with the disease. This may, for example, partly explain the number of false positives, as previous studies indicate that the active form of MMP-8 is an important biomarker for progressing periodontal and peri-implant attachment loss (Heikkinen et al., 2019; Izadi Borujeni et al., 2015; Kiili et al., 2002; Kivelä-Rajämäki et al., 2003; Lähteenmäki et al., 2020; Lee et al., 1995; Leppilahti et al., 2014; Lorenz et al., 2017; Mancini et al., 1999; Räisänen et al., 2019, 2020; Romanelli et al., 1999; Schmalz et al., 2019; Sorsa, Alassiri, et al., 2020; Sorsa, Bacigalupo, et al., 2020; Sorsa et al., 2006; Xu et al., 2008). Currently, there is no gold standard for disease activity and real-time progression of the disease. Thus, biomarker studies, like the present one, are forced to compare the measurements against the current clinical diagnostic parameters even if not necessarily perfectly accurate.

The 2018 classification system of periodontitis has the necessary framework to implement biomarkers (Tonetti et al., 2018). Similarly, biomarkers could be considered to be integrated for the assessment of peri-implant in the official classification system of peri-implant diseases (Berglundh et al., 2018). Recent studies regarding periodontitis as described by the new classification system of periodontitis have presented promising results of the use of aMMP-8 as an adjunctive diagnostic tool to traditional clinical methods (Chaparro et al., 2020; Keles Yucel et al., 2020; Öztürk et al., 2021; Sorsa, Alassiri, et al., 2020). Furthermore, the present study and our previous studies indicate similarly that aMMP-8 is a potential candidate to act as a biomarker in the disease

FIGURE 3  Receiver operating characteristic (ROC) analysis illustrating the diagnostic ability of the biomarker candidates to discriminate healthy implant from peri-implantitis: (a) unadjusted (n = 68) and (b) adjusted for gender, age of dental implant and smoking (n = 62)
classification for peri-implantitis (Allassiri et al., 2018; Golub et al., 2020; Lähteenmäki et al., 2020; Sorsa, Bacigalupo, et al., 2020; Thierbach et al., 2016). In addition to these diseases, elevated aMMP-8 concentrations in oral fluids are associated with (pre)diabetes and gestational diabetes, and the aMMP-8-PoC enzyme test can also identify the risk of diabetes (and eventually other serious systemic diseases) as well as the destructive oral side-effects of radiotherapy for head and neck cancer (Chaparro et al., 2020; Grigoriadis et al., 2019, 2021; Keskin et al., 2020; Räisänen et al., 2020). Overall, aMMP-8 has the potential to influence and improve not only the diagnostics of periodontal and peri-implant diseases but also the interdisciplinary collaboration between dental and healthcare professionals in their pursuit of attaining good oral and general health for their patients (Räisänen et al., 2020).

5 | CONCLUSION

aMMP-8-PoC/chair-side test can be conveniently implemented to alert for and detect active collagenolysis affecting peri-implant tissues, both in the early and advanced stages of the disease. Active and fragmented MMP-8 exhibits a strong and significant association with peri-implantitis as compared to total MMP-8 and other biomarkers. The data demonstrate that an aMMP-8 chairside assay can be used as a convenient and reliable adjunctive tool in the diagnosis and monitoring of peri-implantitis. These results need to be clarified in further studies.

ACKNOWLEDGMENT

This study was funded by the Helsinki and Uusimaa Hospital District (HUS) (grant numbers TYH2016251, TYH2017251, TYH2018229, TYH2019319, Y1014SL017, Y1014SL018, Y1014SULE1), Finland; Finnish Dental Association Apollonia, Finland; Karolinska Institutet, Sweden; P.P. has received a grant from the Minerva Foundation, Selma and Maja-Lisa Selander Foundation, The Finnish Dental Society Apollonia dissertation grant and Faculty of Biosciences (University of Helsinki) dissertation completion grant; and ITR has received dissertation grants from The Yrjö Jahnsson Foundation Sr, The Paulo Foundation, The Emil Aaltonen Foundation Sr, The Juhani Aho Foundation for Medical Research Sr, The Orion Research Foundation Sr, The Finnish Dental Society Apollonia and Helsingin Seudun Hammaslääkärit r.y. (Dentists of Helsinki Region Association), Finland.

CONFLICT OF INTERESTS

Prof. Timo Sorsa is the inventor of US 5,652,223, 5,736,341, 5,864,632, 6,143,476 and US 2017/0023571A1 (issued June 6, 2019), WO 2018/060553 A1 (issued May 31, 2018), 10,488,415 B2, and US 2017/0023671A1 and Japanese Patent 2016-554676. Dr. Pirjo Pärnänen has a patent EP 2585087B1. Dirk-Rolf Gieselmann is the inventor of US 20170023671A1 patent. Other authors report no conflicts of interest related to this study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

ETHICS STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Helsinki University Hospital, Finland (1068/26.06.2019; dnr HUS/1271/2019) and Regionala etikprövningsnämnden i Stockholm, (EPN) (2016-08-24/2016/1:8 and 2016-1-24; Dnr 2016/1410-31/1). Informed consent was obtained from all subjects involved in the study.

AUTHOR CONTRIBUTIONS

Collected the samples and did the clinical diagnosis, patient treatment: Hanna Lähteenmäki. Directed the lab work on biomarkers: Taina Tervahartiala. Statistical analysis, writing: Ismo T. Räisänen. Laboratory
work of samples, writing, clinical diagnosis, patient treatment: Pirjo Pärnänen. Patient clinical data evaluation, medical specialist: Matti Mauramo. Writing the paper, clinical planning, English language: Shipra Gupta. Writing paper, English language, clinical data evaluation: Victoria Sampson. Writing paper, revision planning and writing: Nilminie Rathnayake. Patient treatment, clinical data evaluation, and writing: Anna-Maria Heikkilä. Laboratory work/biomarker analysis of samples, writing the paper: Saeed Alassiri. Development of aMMP-8 poct test, writing of the paper: Dirk-Rolf Gieselmann. Writing of paper and clinical data evaluation: Roland Frankenberger. Discovery and development of the aMMP-8: Timo Sorsa.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

ORCID

Hanna Lähteenmäki https://orcid.org/0000-0002-7914-9726
Pirjo Pärnänen https://orcid.org/0000-0003-2994-8690
Matti Mauramo https://orcid.org/0000-0002-0107-4939
Shipra Gupta https://orcid.org/0000-0003-2097-2459
Nilminie Rathnayake https://orcid.org/0000-0002-7778-2835

REFERENCES

Alassiri, S., Parnanen, P., Rathnayake, N., Johannsen, G., Heikkilä, A. M., Lazzara, R., van der Schoor, P., van der Schoor, J. G., Tervahartia, T., Gieselmann, D., & Sorsa, T. (2018). The ability of quantitative, specific, and sensitive point-of-care/chair-side oral fluid immunotests for aMMP-8 to detect periodontal and peri-implant diseases, Disease Markers, 2018, 1306396.

Alassy, H., Parachuru, P., & Wolff, L. (2019). Peri-implantitis diagnosis and prognosis using biomarkers in peri-implant crevicular fluid: A narrative review. Diagnostics (Basel), 9, 214.

Al-Maidj, A., Alassiri, S., Rathnayake, N., Tervahartia, T., Gieselmann, D. R., & Sorsa, T. (2018). Matrix metalloproteinase-8 as an Inflammatory and prevention biomarker in periodontal and peri-implant diseases. International Journal of Dentistry, 2018, 7891323.

Arias-Bujanda, N., Requeira-Iglesias, A., Balca-Castro, C., Nibali, L., Donos, N., & Tomás, I. (2019). Accuracy of single molecular biomarkers in gingival crevicular fluid for the diagnosis of periodontitis: A systematic review and meta-analysis. Journal of Clinical Periodontology, 46, 1166–1182.

Berglundh, T., Armitage, G., Araujo, M. G., Avila-Ortiz, G., Blanco, J., Camargo, P. M., Chen, S., Cochrán, D., Derks, J., Figuero, E., Hämmerle, C., Heitz-Mayfield, L., Huynh-Ba, G., Iacono, V., Koo, K. T., Lambert, F., McCauley, L., Quirynen, M., Renvert, S., ... Zitzmann, N. (2018). Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of Clinical Periodontology, 45, 286–291.

Buduneli, N. (2020). Biomarkers for periodontal diseases. In N. Buduneli (Ed.), Biomarkers in Periodontal Health and Disease (p. 2020). Springer.

Carinci, F., Romans, G. E., & Scapoli, L. (2019). Molecular tools for preventing and improving diagnosis of peri-implant diseases. Periodontology 2000, 81, 41–47.

Chaparro, A., Realini, O., Hernández, M., Albers, D., Weber, L., Ramírez, V., Faro, F., Kusanovic, J. P., Sorsa, T., Rice, G. E., & Illanes, S. E. (2020). Early pregnancy levels of gingival crevicular fluid matrix metalloproteinases-8 and -9 are associated with the severity of periodontitis and the development of gestational diabetes mellitus. Journal of Periodontology. https://doi.org/10.1002/JPER.19-0743

Dejonckheere, E., Vandebroucke, R. E., & Libert, C. (2011). Matrix metalloproteinase-8 has a central role in inflammatory disorders and cancer progression. Cytokine and Growth Factor Reviews, 22, 73–81.

Derks, J., & Tomasi, C. (2015). Peri-implant health and disease. A systematic review of current epidemiology. Journal of Clinical Periodontology, 42, 158–171.

Elani, H. W., Starr, J. R., Da Silva, J. D., & Gallucci, G. O. (2018). Trends in dental implant use in the U.S., 1999-2016, and projections to 2026. Journal of Dental Research, 97, 1424–1430.

Gangbar, S., Overall, C. M., McCulloch, C. A., & Sodek, J. (1990). Identification of polymorphonuclear leukocyte collagenase and gelatinase activities in mouthrinse samples: Correlation with periodontal disease activity in adult and juvenile periodontitis. Journal of Periodontal Research, 25, 257–267.

Golub, L. M., Räisänen, I. T., Sorsa, T., & Preshaw, P. M. (2020). An unexplored pharmacologic/diagnostic strategy for peri-implantitis: A protocol proposal. Diagnostics (Basel), 10, 1050.

Grigoriadis, A., Räisänen, I. T., Pärnänen, P., Tervahartia, T., Sorsa, T., & Sakellari, D. (2021). Prediabetes/diabetes screening strategy at the periodontal clinic. Clin Exp Dent Res, 7, 85–92.

Grigoriadis, A., Sorsa, T., Räisänen, I., Pärnänen, P., Tervahartia, T., & Sakellari, D. (2019). Prediabetes/diabetes can be screened at the dental office by a low-cost and fast chair-side/point-of-care aMMP-8 immunotest. Diagnostics (Basel), 9, 151.

Gul, S. S., Abdulkareem, A. A., Sha, A. M., & Rawlinson, A. (2020). Diagnostic Accuracy of Oral fluids biomarker profile to determine the current and future status of periodontal and peri-implant diseases. Diagnostics (Basel), 10, 838.

Gürsoy, U. K., Könnönen, E., Tervahartia, T., Gürsoy, M., Pitkänen, J., Torvi, P., Suominen, A. L., Pussinen, P., & Sorsa, T. (2018). Molecular forms and fragments of salivary MMP-8 in relation to periodontitis. Journal of Clinical Periodontology, 45, 1421–1428.

Hanemaaijer, R., Sorsa, T., Konttinen, Y. T., Ding, Y., Sutinen, M., Visser, H., van Hinsbergh, V. W., Helaakoski, T., Kainulainen, T., Rönkä, H., Tschesche, H., & Salo, T. (1997). Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. Journal of Biological Chemistry, 272, 31504–31509.

Heikkinen, A. M., Räisänen, I. T., Tervahartia, T., & Sorsa, T. (2019). Cross-sectional analysis of risk factors for subclinical periodontitis; active matrix metalloproteinase-8 as a potential indicator in initial periodontitis in adolescents. Journal of Periodontal Research, 90, 484–492.

Heitz-Mayfield, L., & Salvi, G. E. (2018). Peri-implant mucositis. Journal of Clinical Periodontology, 45, 237–245.

Izadi Borujeni, S., Mayer, M., & Eickholz, P. (2015). Activated matrix metalloproteinase-8 in saliva as diagnostic test for periodontal disease? A case-control study. Medical Microbiology and Immunology, 204, 665–672.

Keles Yucel, Z. P., Afacan, B., Emingil, G., Tervahartia, T., Kose, T., & Sorsa, T. (2020). Local and systemic levels of aMMP-8 in gingivitis and stage 3 grade C periodontitis. Journal of Periodontal Research, 55, 887–894.

Keskin, M., Lähteenmäki, H., Rathnayake, N., Räisänen, I. T., Tervahartia, T., Pärnänen, P., Şensoy, A. M., Karaçetin, D., Yentek Balkanay, A., Heikkilä, P., Hagström, J., Rautava, J., Haglund, C., Gursoy, U. K., Silbereisen, A., Bostanci, N., & Sorsa, T. (2020). Active matrix metalloproteinase-8 and interleukin-6 detect periodontal degeneration caused by radiotherapy of head and neck cancer: A pilot study. Expert Review of Proteomics, 17, 777–784.

Kiili, M., Cox, S. W., Chen, H. Y., Wahlgren, J., Maisi, P., Eley, B. M., Salo, T., & Sorsa, T. (2002). Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: Molecular forms and levels in...
gingival crevicular fluid and immunolocalisation in gingival tissue. Journal of Clinical Periodontology, 29, 224–232.

Kivelä-Rajamäki, M., Maisi, P., Srinivas, R., Tervahartiala, T., Teronén, O., Husa, V., Salo, T., & Sorsa, T. (2003). Levels and molecular forms of MMP-7 (matrilysin-1) and MMP-8 (collagenase-2) in diseased human peri-implant sulcular fluid. Journal of Periodontal Research, 38, 583–590.

Kivelä-Rajamäki, M. J., Teronén, O. P., Maisi, P., Husa, V., Tervahartiala, T. I., Piriä, E. M., Salo, T. A., Mellanen, L., & Sorsa, T. A. (2003). Laminin-5 gamma2-chain and collagenase-2 (MMP-8) in human peri-implant sulcular fluid. Clinical Oral Implants Research, 14, 158–165.

Lähteenmäki, H., Umeizudike, K. A., Heikkinen, A. M., Rajamäki, M. J., Teronen, O. P., Siren, E., Tervahartiala, T., Rajamäki, M., Maisi, P., Srinivas, R., Tervahartiala, T., Sobey, J., & Sorsa, T. A. (2004). Association of MMP-8 with obesity, smoking and insulin resistance. European Journal of Clinical Investigation, 46, 757–765.

Lee, W., Aitken, S., Sodek, J., & McCulloch, C. A. (1995). Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: Role of active enzyme in human periodontitis. Journal of Periodontal Research, 30, 23–33.

Leppilähti, J. M., Hernández-Rios, P. A., Gamonal, J. A., Tervahartiala, T., Brignardello-Petersen, R., Mantyla, P., Sorsa, T., & Hernández, M. (2014). Matrix metalloproteinases and metalloproteinase-1 in gingival crevicular fluid provide site-specific diagnostic value for chronic periodontitis. Journal of Clinical Periodontology, 41, 348–356.

Leppilähti, J. M., Kallio, M. A., Tervahartiala, T., Sorsa, T., & Mäntylä, P. (2014). Gingival crevicular fluid matrix metalloproteinase-8 levels predict treatment outcome among smokers with chronic periodontitis. Journal of Periodontology, 85, 250–260.

Leppilähti, J. M., Sorsa, T., Kallio, M. A., Tervahartiala, T., Emingil, G., Han, B., & Mäntylä, P. (2015). The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns in prediction of site-level clinical treatment outcome. Journal of Periodontology, 86, 777–787.

Lorenz, K., Keller, T., Noack, B., Freitag, A., Netuschil, L., & Hoffmann, T. (2017). Evaluation of a novel point-of-care test for active matrix metalloproteinase-8: Agreement between qualitative and quantitative measurements and relation to periodontal inflammation. Journal of Periodontal Research, 52, 277–284.

Ma, J., Kitti, U., Teronen, O., Sorsa, T., Husa, V., Laine, P., Rönkä, H., Salo, T., Lindqvist, C., & Konttinen, Y. T. (2000). Collagenases in different categories of peri-implant vertical bone loss. Journal of Dental Research, 79, 1870–1875.

Mancini, S., Romanelli, R., Laschinger, C. A., Overall, C. M., Sodek, J., & McCulloch, C. A. (1999). Assessment of a novel screening test for neutrophil collagenase activity in the diagnosis of periodontal diseases. Journal of Periodontal Research, 70, 1292–1302.

Manor, Y., Oubaid, S., Martdinger, O., Chausu, G., & Nissam, J. (2009). Characteristics of early versus late implant failure: A retrospective study. Journal of Oral and Maxillofacial Surgery, 67, 2649–2652.

Nagase, H. (1997). Activation mechanisms of matrix metalloproteinases. Biological Chemistry, 378, 151–160.

Natto, Z. S., Almeganni, N., Alnaeeeb, E., Bukhari, Z., Jan, R., & laco, V. J. P. (2019). peri-implantitis and peri-implant mucositis case definitions in dental research: A systematic assessment. The Journal of Oral Implantology, 45, 127–131.

Nieminen, M. T., Listyariyah, D., Hagström, J., Haglund, C., Gnerien, D., Nordström, D., Uitto, V. J., Hernandez, M., Yucel-Lindberg, T., Tervahartiala, T., Ainoa, M., & Sorsa, T. (2018). Treponema denticola chymotrypsin-like proteinase may contribute to orodigestive carcinogenesis through immunomodulation. British Journal of Cancer, 118, 428–434.

ÖZTÜRK, V. Ö., Emingil, G., Umeizudike, K., Tervahartiala, T., Gieselmann, D. R., Maier, K., Köse, T., Sorsa, T., & Alassiri, S. (2021). Evaluation of active matrix metalloproteinase-8 (aMMP-8) chair-side test as a diagnostic biomarker in the staging of periodontal diseases. Archives of Oral Biology, 124, 104955.

Paju, A., Sorsa, T., Tervahartiala, T., Koivunen, E., Haglund, C., Lemenin, A., Wahlström, T., Salo, T., & Stenman, U. H. (2001). The levels of trypsinogen isoenzymes in ovarian tumour cyst fluids are associated with promatrix metalloproteinase-9 but not promatrix metalloproteinase-2 activation. British Journal of Cancer, 84, 1363–1371.

Pjetursson, B. E., Thoma, D., Jung, R., Zewehlen, M., & Zembic, A. (2012). A systematic review of the survival and complication rates of implant-supported fixed dental prostheses (FDPs) after a mean observation period of at least 5 years. Clinical Oral Implants Research, 23, 22–38.

Räisänen, I. T., Heikkinen, A. M., Pakbaznejad Esmaeil, A., Tervahartiala, T., Pajukanta, R., Silbereisen, A., Bostanci, N., & Sorsa, T. (2020). A point-of-care test of active matrix metalloproteinase-8 predicts triggering receptor expressed on myeloid cells-1 (TREM-1) levels in saliva. Journal of Periodontology, 91, 102–109.

Räisänen, I. T., Heikkinen, A. M., Siren, E., Tervahartiala, T., Gieselmann, D. R., van der Schoor, G. J., van der Schoor, P., & Sorsa, T. (2018). Point-of-care/chairside aMMP-8 analytics of periodontal diseases’ activity and episodic progression. Diagnostics (Basel), 8, 74.

Räisänen, I. T., Sorsa, T., van der Schoor, G. J., Tervahartiala, T., van der Schoor, P., Gieselmann, D. R., & Heikkinen, A. M. (2019). Active matrix metalloproteinase-8 point-of-care (PoC)/chairside mouthrinse test vs. bleeding on probing in diagnosing subclinical periodontitis in adolescents. Diagnostics (Basel), 9, 34.

Räisänen, I. T., Umeizudike, K. A., Pärämäen, P., Heikki, P., Tervahartiala, T., Wahlström, S. O., Grigoriadis, A., Sakellari, D., & Sorsa, T. (2020). Periodontal disease and targeted prevention using aMMP-8 point-of-care oral fluid analytics in the COVID-19 era. Medical Hypotheses, 144, 110276.

Rathnayake, N., Buhlin, K., Kjellstrom, B., Klinge, B., Lövbeere, C., Norhammer, A., Ryden, L., Sorsa, T., Tervahartiala, T., & Gustafsson, A. PAROKRANK Steering Committee. (2017). Saliva and plasma levels of cardiac-related biomarkers in post-myocardial infarction patients. Journal of Clinical Periodontology, 44, 692–699.

Renvet, S., Persson, G. R., Piini, F. Q., & Camargo, P. M. (2018). Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. Journal of Periodontology, 89, 304–312.

Romanelli, R., Mancini, S., Laschinger, C., Overall, C. M., Sodek, J., & McCulloch, C. A. (1999). Activation of neutrophil collagenase in periodontitis. Infection and Immunity, 67, 2319–2326.

Romero-Castro, N. S., Vázquez-Villamar, M., Muñoz-Morales, J. F., Reyes-Fernández, S., Serna-Radilla, V. O., García-Arellano, S., & Castro-Alarcón, N. (2020). Relationship between TNF-α, MMP-8, and MMP-9 levels in gingival crevicular fluid and the subgingival microbiota in periodontal disease. Odontology/the Society of the Nippon Dental University, 108, 25–33.

Schmalz, G., Hübscher, A. E., Angermann, H., Schmidt, J., Schmickler, J., Legler, T. J., & Ziebolz, D. (2019). Associations of chairside salivary aMMP-8 findings with periodontal parameters, potentially periodontal pathogenic bacteria and selected blood parameters in systemically healthy adults. Diagnostic Microbiology and Infectious Disease, 95, 179–184.

Schwarz, F., Derks, J., Monje, A., & Wang, H. L. (2018). Peri-implantitis. Journal of Clinical Periodontology, 45, 246–266.

Sorsa, T., Allassiri, S., Grigoriadis, A., Räisänen, I. T., Pärämäen, P., Wahlström, T., Sorsa, T., & Sorsa, T. (2018). Treponema denticola chymotrypsin-like proteinase may contribute to orodigestive carcinogenesis through immunomodulation. British Journal of Cancer, 118, 428–434.
Sorsa, T., Bacigalupo, J., Kõnnönen, M., Pärnänen, P., & Räisänen, I. T. (2020). Host-modulation therapy and chair-side diagnostics in the treatment of peri-implantitis. Biosensors (Basel), 10, 44.

Sorsa, T., Ding, Y. L., Ingman, T., Salo, T., Westerlund, U., Haapasalo, M., Tschesche, H., & Konttinen, Y. T. (1995). Cellular source, activation and inhibition of dental plaque collagenase. Journal of Clinical Periodontology, 22, 709–717.

Sorsa, T., Gursoy, U. K., Nwhator, S., Hernandez, M., Tervahartiala, T., Leppilähti, J., Gursoy, M., Kõnnönen, E., Emingil, G., Pussinen, P. J., & Mäntylä, P. (2016). Analysis of matrix metalloproteinases, especially MMP-8, in gingival crevicular fluid, mouthrinse and saliva for monitoring periodontal diseases. Periodontology 2000, 70, 142–163.

Sorsa, T., Hernández, M., Leppilähti, J., Munjal, S., Netuschil, L., & Mäntylä, P. (2010). Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. Oral Diseases, 16, 39–45.

Sorsa, T., Ingman, T., Suomalainen, K., Haapasalo, M., Konttinen, Y. T., Lindy, O., Saari, H., & Uitto, V. J. (1992). Identification of proteases from periodontopathogenic bacteria as activators of latent human neutrophil and fibroblast-type interstitial collagenases. Infection and Immunity, 60, 4491–4495.

Sorsa, T., Lindy, O., Konttinen, Y. T., Suomalainen, K., Ingman, T., Saari, H., Halinen, S., Lee, H. M., Golub, L. M., & Hall, J. (1993). Doxycycline in the protection of serum alpha-1-antitrypsin from human neutrophil collagenase and gelatinase. Antimicrobial Agents and Chemotherapy, 37, 592–594.

Sorsa, T., Sahni, V., Buduneli, N., Gupta, S., Räisänen, I. T., Golub, L. M., Lee, H. M., Päätäli, T., Bostanci, N., Meurman, J., Pärnänen, P., Nwhator, S. O., Singla, M., & Gauba, K. (2021). Active matrix metalloproteinase-8 (aMMP-8) point-of-care test (POCT) in the COVID-19 pandemic. Expert Review of Proteomics, 18, 1–11.

Sorsa, T., Salo, T., Koivunen, E., Tyynelä, J., Konttinen, Y. T., Bergmann, U., Tuuttila, A., Niemi, E., Teronen, O., Helkkilä, P., Tschesche, H., Leinonen, J., Osman, S., & Stenman, U. H. (1997). Activation of type IV procollagenases by human tumor-associated trypsin-2. Journal of Biological Chemistry, 272, 21067–21074.

Sorsa, T., Tjäderhane, L., Konttinen, Y. T., Lauhio, A., Salo, T., Lee, H. M., Golub, L. M., Brown, D. L., & Mäntylä, P. (2006). Matrix metalloproteinases: Contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Annals of Medicine, 38, 306–321.

Thierbach, R., Maier, K., Sorsa, T., & Mäntylä, P. (2016). Peri-implant sulcus fluid (PISF) matrix metalloproteinase (MMP)-8 levels in peri-implantitis. Journal of Clinical and Diagnostic Research, 10, 34–38.

Tonetti, M. S., Greenwell, H., & Kornman, K. S. (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of Clinical Periodontology, 45, 149–161.

Verhulst, M., Teeuw, W. J., Bizzarro, S., Muris, J., Su, N., Nicu, E. A., Nazmi, K., Bikker, F. J., & Loos, B. G. (2019). A rapid, non-invasive tool for periodontitis screening in a medical care setting. BMC Oral Health, 19, 87.

Wang, H. L., Garaicoa-Pazmino, C., Collins, A., Ong, H. S., Chudri, R., & Giannobile, W. V. (2016). Protein biomarkers and microbial profiles in peri-implantitis. Clinical Oral Implants Research, 27, 1129–1136.

Wohlfahrt, J. C., Aass, A. M., Granfeldt, F., Lyngstadaas, S. P., & Reseland, J. E. (2014). Sulcus fluid bone marker levels and the outcome of surgical treatment of peri-implantitis. Journal of Clinical Periodontology, 41, 424–431.

Xu, L., Yu, Z., Lee, H. M., Wolff, M. S., Golub, L. M., Sorsa, T., & Kuula, H. (2008). Characteristics of collagenase-2 from gingival crevicular fluid and peri-implant sulcular fluid in periodontitis and peri-implantitis patients: Pilot study. Acta Odontologica Scandinavica, 66, 219–224.

How to cite this article: Lähteenmäki, H., Tervahartiala, T., Räisänen, I. T., Pärnänen, P., Mauramo, M., Gupta, S., Sampson, V., Rathnayake, N., Heikkilä, A.-M., Alassiri, S., Gieselmann, D., R., Frankenberger, R., & Sorsa, T. (2022). Active MMP-8 point-of-care (PoC)/chairside enzyme-test as an adjunctive tool for early and real-time diagnosis of peri-implantitis. Clinical and Experimental Dental Research, 8, 485–496. https://doi.org/10.1002/cre2.537