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Letter to the Editor

MMP activation in diagnostics of periodontitis and systemic inflammation

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Dear Editor:

We read with great interest the recent extensive and comprehensive review on host-derived oral fluid biomarkers by Buduneli and Kinane (2011). Regarding MMP studies in oral fluid [gingival crevicular fluid (GCF), peri-implant sulcular fluid (PISF), mouthrinse and saliva], the authors describe mainly investigations addressing total levels and activities of MMPs, i.e. collagenases, gelatinases, etc. (Buduneli & Kinane 2011). In this context, the pathophysiological significance of increased MMP levels in periodontitis finally depends on the interplay among activating factors and endogenous inhibitors, which determine MMP activity. As a result, all human MMPs are known to exist in multiple forms, i.e. latent proforms, active or activated forms, fragmented species, complexed species and cell-bound forms (Sorsa et al. 2011). Commonly used MMP immunoassays do not differentiate these forms (Buduneli & Kinane 2011, Sorsa et al. 2011). Regarding MMPs, especially MMP-8, -9 and -13, it is worthy of note that clinical progression of periodontitis in active sites *versus* inactive sites and/or patients has been repeatedly demonstrated to be reflected as pathologically excessive elevation of active MMP forms, i.e. conversion of latent proform to active form, or as activity, i.e. quantification of substrate hydrolysis in GCF/PISF, mouthrinse and saliva samples collected from periodontitis/peri-implantitis sites and patients (Mäntylä et al. 2006, Hernández Ríos et al. 2009, Hernández et al. 2010, Sorsa et al. 2010, 2011). Regarding periodontitis/peri-implantitis progression in disease-active sites, pro-MMP-8, -9 and -13 have been demonstrated to be activated by independent and/or co-operative cascades involving other host proteinases (MMPs, serine proteases), reactive oxygen species and/or microbial proteases (Hernández Ríos et al. 2009, Hernández et al. 2010, Sorsa et al. 2011). GCF collagenase activity and MMP-8 activation are found to correlate with the levels of type I collagen breakdown fragments overcoming the protective shield provided by TIMP-1 (Reinhardt et al. 2010, Sorsa et al. 2011). Similarly, MMP-13 activity and C-telopeptide pyridinoline cross-links have shown to increase in active sites compared with inactive sites from progressive patients and healthy subjects (Hernández Ríos et al. 2009). Clinical trials testing sub-antimicrobial dose doxycycline (synthetic MMP-inhibitor) medication have repeatedly reported an association between improvement of clinical parameters and reduction in GCF and serum MMP-8, -13 and -9 activation and levels (Reinhardt et al. 2010, Sorsa et al. 2011). It is possible to monitor the effect of treatment and adjunctive medication by point-of-care MMP-8 immunoassays (Sorsa et al. 2011).

Furthermore, the immunoassays and antibodies used for the detection of MMPs and their regulators affect the measurement outcome (Gursoy et al. 2010, 2011, Sorsa et al. 2010, 2011, Leppilahti et al. 2011). Nevertheless, especially MMP-8 immunoassays and activity assays targeting neutrophil (PMN)-type MMP-8 isoenzyme species in oral fluids have been found to be useful to differentiate periodontitis/peri-implantitis and gingivitis sites/patients as well as healthy sites/subjects (Hernández et al. 2010, Sorsa et al. 2010, 2011). Although clinical exami-

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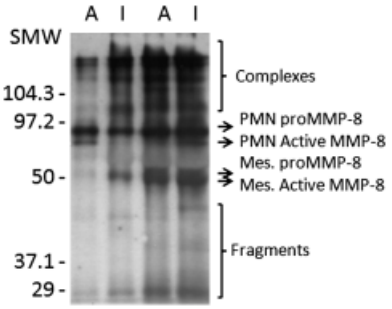

Fig. 1. This is a representative western-immunoblot data of molecular forms of MMP-8 in gingival crevicular fluid from active (A) and inactive (I) sites from progressive periodontitis subjects. MMP-8 proenzyme, active forms and fragments can be identified in all samples, but the levels of active forms and ratio of active enzyme showed a tendency to be higher for active sites ($p > 0.05$). The quantitative outcome in active and inactive sites has been reported in Hernández et al. (2010). A, active sites; I, inactive sites; PMN, neutrophil isoenzyme; Mes., fibroblastic/mesenchymal isoenzyme.

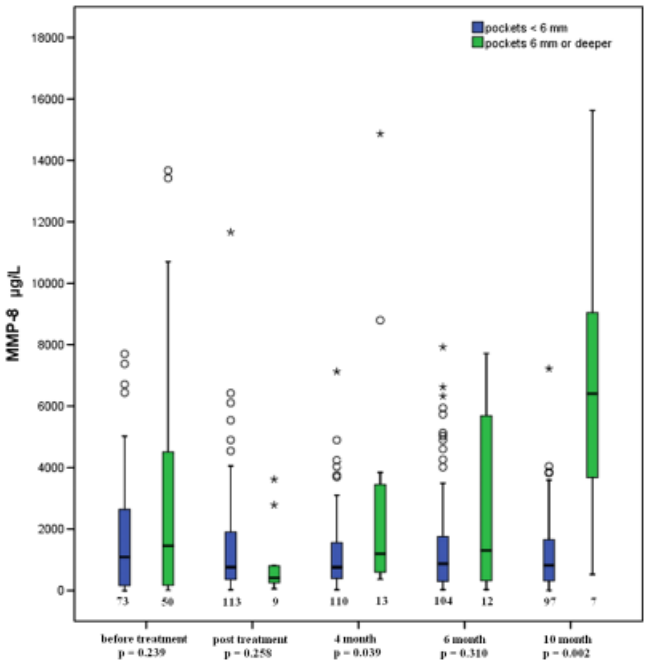

Fig. 2. Boxplots indicating medians, quartiles and extreme values of gingival crevicular fluid MMP-8 levels measured with an immunofluorometric assay (IFMA; µg/l) from individual sites of 15 chronic periodontitis patients. Pockets are recategorized at each point in time into probing depth (PD) categories < 6 mm (blue) and $\geqslant 6$ mm (green) before any periodontal treatment (baseline), 1 month after completion of scaling and root planning (post-treatment), and at 4, 6 and 10 month maintenance visits (Mann–Whitney test). Number of tested sites belonging to one or the other PD category is indicated below each boxblot.

nation is necessary and cannot be replaced in periodontal diagnostics, biomarker testing could give relevant clinical adjunctive information. Figure 1 demonstrates the molecular forms of GCF MMP-8 detected in active and inactive periodontitis sites. MMP-8 active forms and the ratio of active MMP-8 from total enzyme predominated in active sites compared with inactive sites, although results were non-significant (Hernández et al. 2010). Antibodies selective for active MMP-8 detection in oral fluids have been utilized as adjunctive diagnostic point-of-care/chair-side tests identifying sites susceptible for periodontitis progression (Fig. 2) and patients susceptible for periodontitis (Mäntylä et al. 2006, Leppilahti et al. 2011, Sorsa et al. 2011).

We agree with the conclusion by Buduneli and Kinane that more work, especially on the biomarkers' prognostic impact, is required within the promising area of oral fluid point-of-care diagnostic development, of which technologies may also well be applied to other systemic inflammatory and infectious conditions (Tuomainen et al. 2007, Buduneli et al. 2011, Kinane et al. 2011, Lauhio et al. 2011, Sorsa et al. 2011). Host biomarker testing from saliva/oral rinse could be especially useful and cost effective in periodontitis patients' maintenance care interval determination (Leppilahti et al. 2011). Furthermore, we suggest that antibodies or catalytic activity assays being as selective and/or specific as possible for activated forms of MMP-8, -13 and/or -9 should be developed to be utilized in oral fluid and serum/plasma point-of-care technologies.

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