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Letter to the Editor

By mistakes we learn: determination of matrix metalloproteinase-8 and tissue inhibitor of matrix metalloproteinase-1 in serum yields doubtful results

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In response,

We appreciate the opportunity to respond to the letter from Dr. Jung. He raises an important issue concerning the methods of collecting blood samples for assay determinations and points out the differences between serum and plasma matrix metalloproteinase (MMP)-8 measurements. The serum samples used in our study were collected in vacuum glass tubes as previously described in detail (Salonen et al. 1991, Tuomainen et al. 2007). Clotting in glass tubes is faster and more effective than in plastic tubes, and thus no clot activator was used (Salonen et al. 1991). Instead Dr. Jung described (2008) the use of plastic tubes without additives or kaolin-coated granulate as a clot activator to prepare native serum and serum after enhanced coagulation, respectively. By using tubes coated with lithium heparin, sodium sitrate, or dipotassium EDTA plasma samples were prepared (Jung & Lein 2008). However, despite these described numerical differences in MMP-8 concentrations in serum and plasma (Jung & Lein 2008), a recently published report using our methods (Tuomainen et al. 2008) to prepare serum samples (Salonen et al. 1991) and serum MMP-8 analysis (Tuomainen et al. 2007, Emingil et al. 2008) showed significant correlations between total MMP-8 levels in serum and plasma. Unfortunately, no plasma samples were available for our studies (Emingil et al. 2008).

Briefly, we are aware of the potential differences in the MMP-8 concentrations between serum and plasma samples. In this regard, Tuomainen et al. (2008) recently studied the MMP-8 concentrations of 15 serum and plasma samples from patients with chronic periodontitis. For determinations, we use both time-resolved immunofluorometric assay (IFMA) with monoclonal catching and tracer antibodies (Medix Biochemica, Kauniainen, Finland) as in the original article (Emingil et al. 2008), and Amersham ELISA were used (Tuomainen et al. 2008). Both methods gave significantly higher serum MMP-8 values compared with plasma samples from the same patients (Tuomainen et al. 2008). The differences between serum IFMA and ELISA results may, at least in part, be explained by the high sensitivity of IFMA and by the obviously different specificities of the antibodies used in the assays. The differences were most notable with high serum MMP-8 concentrations as measured by IFMA (>20 ng/ml), whereas smaller concentrations gave similar results with both procedures (Tuomainen et al. 2008). Most importantly, there were significant positive correlations between serum and plasma IFMA and ELISA measurements (Tuomainen et al. 2008).

Moreover, in recent studies Aquilante et al. (2007, 2008) showed that total concentrations of MMP-8 in serum prepared as described by Tuomainen et al. (2007, 2008) and Emingil et al. (2008), but measured by different analytical techniques (Aquilante et al. 2007), were consistent yielding similar findings with respect to cardiovascular risk and the effect of smoking.

We agree with Dr. Jung that it is crucial to know what kind of method is used in collecting blood samples before determinations. In addition, we emphasize the importance of the selection of assay. However, the aim of our study was to investigate gingival crevicular fluid (GCF) and serum MMP-8

and TIMP-1 (tissue inhibitor of matrix metalloproteinase-1) levels from immunosuppressive patients receiving either cyclosporine-A or tacrolimus with or without gingival overgrowth (GO) and compare these levels with those from patients with gingivitis as also with those from healthy subjects, irrespective of the origin of the protein. We used methods developed and successfully used in our laboratories for GCF and serum MMP-8 analysis (Mäntylä et al. 2003, 2006, Emingil et al. 2004, 2008, Sorsa et al. 2004, 2006, Tuomainen et al. 2007, 2008, Vihinen et al. 2008). Serum and GCF concentrations may reflect the overall homeostasis of MMP-8 and TIMP-1, and therefore we consider our conclusion of their prognostic and diagnostic value concerning periodontal disease to be still valid.

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