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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

Klaus Jung and Michael Lein

Department of Urology and Berlin Institute for Urologic Research, University Hospital Charité, Berlin, Germany

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Dear Editor,

The general interest in the measurement of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in body fluids directed our attention to the recently published article by Emingil et al. (2008) in this journal. The authors determined MMP-8 and TIMP-1 in gingival crevicular fluid and in serum of renal transplant patients receiving either cyclosporine-A or tacrolimus therapy. The alterations of both analytes found in gingival crevicular fluid were mainly interpreted as effect of the gingival inflammation but not by the immunosuppressive therapy. In serum, MMP-8 was elevated in patients with gingivitis, in patients with gingival outgrowth and treated with cyclopsorine-A, and in patients treated with tacrolimus but without gingival outgrowth, while TIMP-1 was increased in all the immunosuppressively treated patients but not in the gingivitis patients. The authors concluded that the changes in serum of patients treated by tacrolimus could be partially explained by a systemic effect. Without going into further detail, we would like to advise the readership of this journal of the simple, but important, aspect of blood sampling as critical determinant to quantify circulating MMPs and TIMPs. Several current reports discussing the issue of measuring TIMPs and MMPs concluded that serum is a rather inappropriate sample material for the determination of TIMP-1 and MMP-8 as well (Holten-Andersen et al. 2003, Makowski & Ramsby 2003, Gerlach et al. 2005, Jung et al. 2005, Mannello & Tonti 2007, Mannello et al. 2007, Jung et al. 2008). The determination of true concentrations of circulating MMPs and TIMPs in serum is impaired by the release of both components from blood cells during the sampling process. MMPs and TIMPs deriving from blood cells cause a highly unspecific background not related to the actual pathological process of interest. The critical pre-analytical aspect of a study (Tworoger & Hankinson 2006) was obviously overlooked by Emingil et al. (2008).

To illustrate this problem, we summarized from our own experiments that MMP-8 and TIMP-1 values determined both in serum and plasma samples (Fig. 1a and b). Blood samples were simultaneously collected from 10 healthy adults for MMP-8 and from eight other adults for TIMP-1 in plastic tubes (Monovette Systems; Sarstedt AG, Nümbrecht, Germany) without additives or with kaolin-coated granulate as clot activator to prepare native serum (serum$^{(-)}$) and serum after enhanced coagulation (serum$^{(+)}$), respectively, or using tubes coated with lithium heparin, sodium citrate or dipotassium EDTA to prepare plasma samples. The blood samples were centrifuged at 1,600 *g* at 4°C for 15 min., and within 30 min. after venipuncture, the supernatants were stored at −80°C until analysis. MMP-8 was measured with the Fluorokine MultiAnalyte Profiling assays (R&D Systems, Minneapolis, MN, USA) detecting pro-, mature and TIMP complexed MMPs, while TIMP-1 was assayed using an Amersham ELISA kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Figure 1 summarizes the results and allows the following conclusions: (I) both MMP-8 and TIMP-1 concentrations were several times higher in serum

Conflict of interest and source of funding statement

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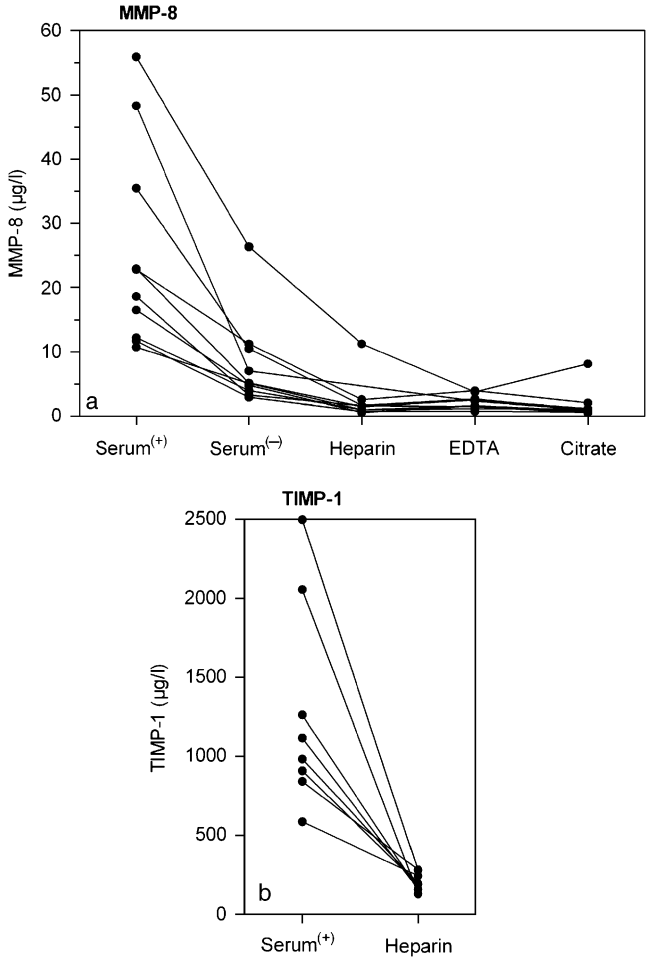

Fig. 1. Influence of blood sampling on the determination of matrix metalloproteinase-8 (MMP-8) and tissue inhibitor of matrix metallorpteinase-1 (TIMP-1) concentrations. Values are data from 10 healthy adults for MMP-8 and from other eight healthy adults for TIMP-1, respectively. Symbols of serum$^{(+)}$ and serum$^{(-)}$ correspond to serum samples collected with or without kaolin-coated granulate as coagulation accelerator.

than in plasma samples and (II) distinctly higher concentrations were found in serum$^{(+)}$ samples that were conventionally collected with clot activator for routine use than in serum$^{(-)}$ samples collected without clot activator. These data clearly prove that MMP-8 and TIMP-1 measured in serum samples do not reflect the true concentrations of both components circulating in blood. Because platelets and leukocytes abundantly contain MMPs and TIMPs, high analyte concentrations occur in serum due to their release from platelets and leukocytes depending on the blood collection and processing procedures (Mannello & Tonti 2007). It is obvious that misinterpretation of data could therefore result and the conclusions of Emingil et al. (2008) based on the serum data of MMP-8 and TIMP-1 should take that issue into account.

In conclusion, the inclusion of MMPs and TIMPs as markers in clinical studies demands knowledge and consideration of the blood sampling process as a critical pre-analytical factor that decides on the validity of the study.

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Address:
Klaus Jung
Department of Urology and Berlin Institute for Urologic Research
University Hospital Charité
Schumannstrasse 20/21
10117 Berlin
Germany
E-mail: klaus.jung@charite.de