Matrix Metalloproteinase-8 and Tissue Inhibitor of Metalloproteinase-1 in Serum Do Not Reflect the Analytes Circulating in Blood

To the Editor:

It was with great interest that I read the article by Tuomainen et al.\(^1\) recently published in this journal on the association of matrix metalloproteinase (MMP)-8 and tissue inhibitor of MMP-1 (TIMP-1) with cardiovascular diseases. The authors measured both analytes in serum and concluded from their results that serum MMP-8 and the ratio of MMP-8 to TIMP-1 were useful biomarkers with prognostic and diagnostic signiﬁcances in men with cardiovascular disease, especially in those with prevalent or subclinical arteriosclerosis.\(^1\) Although I am not experienced in this clinical ﬁeld, I believe it might be meaningful to make the readership of this journal aware of an important issue that was obviously not considered by Tuomainen et al. in their study design. It refers to the inﬂuence of blood sample processing as essential precondition for the correct determination of circulating MMPs and TIMPs in peripheral blood. The effect of the type of blood sample, either collected as serum with or without clot activator or as plasma with the anticoagulants heparin, citrate, or EDTA, on the measurement of pro- and antiﬁns of platelets and leukocytes during coagulation or blood collection.\(^2,8-9\) Tuomainen et al. used serum samples for their own experiments.

Serum and plasma samples from 10 healthy adults for MMP-8 and from 8 adults for TIMP-1 were prepared in plastic tubes (Monovette, Sarstedt AG) by centrifugation of the collected blood samples at 1600g for 15 minutes within 30 minutes after venipuncture. Tubes without additives or with kaolin-coated plastic granulate as coagulation accelerator were used to prepare native serum. Tubes coated with lithium heparin, sodium citrate, or dextan, potassium EDTA were used to prepare corresponding plasma samples. The supernatants were carefully removed and stored at −80°C until analysis. MMP-8 measurements were performed with the Fluorokine MultiAnalyte Profiling assay (R&D Systems) on a Luminex 100 Bioanalyzer (Luminex Corp). The assay detects pro-, mature, and TIMP-1 complexed MMPs with less than 0.5% cross-reactivity between the different MMPs. TIMP-1 was determined using the Luminex 1ELISA from Amersham. GraphPad Prism 5.01 for Windows (GraphPad Software) was used for statistical analyses using ANOVA and Student paired t-test. Statistical signiﬁcances were considered with P<0.05.

Data are summarized in the Figure with the following conclusions: (1) MMP-8 and TIMP-1 concentrations are several times higher in serum than in plasma samples; (2) MMP-8 showed distinctly higher values in serum(\(^1\)) samples collected with clot activator as the conventional way to prepare serum for routine use compared with those in serum(\(^1\)) samples collected without clot activator.

Our results show that the measurement of MMP-8 and TIMP-1 in serum does not only detect these components as they are primarily circulating in blood but to an extremely high degree as released from platelets and leukocytes depending on the different blood collection procedures. MMP-8 and TIMP-1 are abundantly expressed in platelets and leukocytes,\(^1,11\) The data conﬁrm the importance of measuring true concentrations of circulating MMPs and TIMPs in peripheral blood. The problem is not only restricted to MMP-9, which was recently studied in detail.\(^9\) Concerns have been raised that this high unspeciﬁc background of MMPs and TIMPs released from blood cells in serum could hamper the diagnostic truth or might be the reason for a misinterpretation of data.\(^12\)

In conclusion, clinicians should be aware of these preanalytical pitfalls of data if MMPs and TIMPs are used as diagnostic or prognostic biomarkers for clinical purposes. Therefore, it might be reasonable to reconsider the conclusions of Tuomainen et al. under this aspect.

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Disclosures

None.

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**Figure.** Effect of blood sampling on the MMP-8 and TIMP-1 concentrations in serum and plasma samples. Values are given as arithmetic means with 95% confidence intervals from 10 (for MMP-8) and 8 (for TIMP-1) healthy adults. Further details are described in the text. Signiﬁcances between the samples are indicated by probability values. Letters on the bars of the plasma samples indicate signiﬁcant differences to MMP values determined in serum(\(^1\)) samples: a, P<0.05; b, P<0.01; c, P<0.001. Symbols in the ﬁgure: serum(\(^1\)) and serum(\(^2\)) correspond to serum prepared in plastic tubes with and without clot activator, respectively.


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