Our aim was to demonstrate the effect of blood sampling on the concentrations of collagenase-1 (EC 3.4.24.7; MMP-1), stromelysin-1 (EC 3.4.24.17; MMP-3), matrilysin (EC 3.4.24.33; MMP-7), and collagenase-2 (EC 3.4.24.34; MMP-8) circulating in blood. Because the preanalytical impact of blood sampling is well known for gelatinase A (EC 3.4.24.24; MMP-2) and MMP-9 (4), we measured these substances for use as comparisons. The study was performed in accordance with the ethical standards of the Helsinki Declaration and was approved by the ethical board of the hospital. All sample donors recruited on a voluntary basis and informed about the objectives of the study provided written informed consent.

During single venipunctures performed in 10 healthy adults [4 female, 6 male; mean (SD) age, 42 (14) years] venous blood samples were collected in plastic tubes (Sarstedt) without additives or with kaolin-coated granulate as coagulation accelerator (serum(+) and serum(−), respectively) and in tubes coated with lithium heparin, disodium citrate, or dipotassium EDTA in that specified sequence and centrifuged within 30 min after venipuncture (1600g; 15 min; 4 °C). The supernatants were removed and stored at −80 °C until analysis. MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, and MMP-13 were simultaneously measured using the Fluorokine MultiAnalyte Profiling assays (R&D Systems) on a Luminex 100 Bioanalyzer (Luminex). The assays measure pro-, mature, and tissue inhibitor of metalloproteinase (TIMP)-1–complexed MMPs with <0.5% cross-reactivity between the MMPs.

Fig. 1 summarizes the different MMP concentrations in all samples examined. MMP-12 and MMP-13 could not be determined because the measurements gave signals below the detection limit. ANOVA analyses showed significant differences for all MMPs between the serum(+) and serum(−) samples and among the plasma samples (P < 0.0001). Posthoc paired Student t-tests were used in further statistical analysis of the differences between serum(+) and serum(−), among the plasma samples, and between the plasma and serum(−) samples. We additionally related the MMP values in serum and plasma to the results obtained in citrate-plasma taken as 100%, because citrate was previously recommended as anticoagulant of choice to prepare plasma samples for MMP-9 measurements (5).

The main novel findings of this study were: (a) MMP-1, MMP-8, and MMP-9 showed significantly higher values in serum(+) than in serum(−), but there were no differences for MMP-2, MMP-3, and MMP-7; (b) with a few exceptions, all MMPs were characterized by higher concentrations in serum than in plasma samples, particularly for MMP-1, MMP-8, and MMP-9, which also showed 2- to 4-fold higher concentrations in serum(+) than in serum(−) samples; (c) MMP-2, MMP-3, and MMP-7 measured in citrate-plasma were roughly comparable to the 2 serum-type samples and heparin-plasma, with differences of only 15% for MMP-2 and about 25% for MMP-3 and MMP-7; (d) MMP measurement results in EDTA-plasma samples were more highly variable than those obtained in citrate- or heparin-plasma samples.

Our data demonstrate that in blood the measured concentrations of not only the gelatinases but also of other MMPs are strongly influenced by the sampling procedure. Our study is the first detailed investigation of simultaneous measurements of multiple MMPs in serum with and without clot activator, and in citrate-, EDTA-, or heparin-plasma. Our findings highlight the

**Impact of Blood Sampling on the Circulating Matrix Metalloproteinases 1, 2, 3, 7, 8, and 9**

To the Editor:

Matrix metalloproteinases (MMP) play a crucial role in numerous pathological processes. Because cellular changes may be reflected in body fluids, measurements of MMP in blood have been recommended as noninvasive tools in diagnosis and monitoring of diseases (1). MMP measurements can be affected by the blood sampling procedures, as shown for gelatinase B (EC 3.4.24.25; MMP-9), for which higher concentrations have been noted in serum than in plasma samples (2, 3). However, the impact of the blood sampling process on measurements of many other MMPs has not yet been studied in detail.
important differences between the MMP concentrations in serum collected with and without clot activator as well as the differences among both serum types and plasma samples. It is noteworthy that MMPs such as MMP-1, MMP-8, and MMP-9 are abundantly expressed in platelets and leukocytes. Increased MMP concentrations in serum may be due both to their release during coagulation and to their secretion, which is induced by the clot activator itself, as has been shown for MMP-9 (3, 4).

In conclusion, each MMP to be analyzed as a circulating biomarker in blood requires careful consideration of the preanalytical effects of blood collection methods. Despite the currently available knowledge base, some studies performed on gelatinases and collagenases in serum do not address the concerns raised by reports of pitfalls and misinterpretations linked to preanalytical aspects of blood processing. Our results indicate that the use of serum should be avoided for measurement of MMP-1, MMP-8, and MMP-9. In contrast, MMP-2, MMP-3, and MMP-7 measurements showed comparable values among citrate- and heparin-plasma and the 2 serum types. Citrate-plasma seems to be the sample of choice for the measurement of all MMPs.

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