bound iron. We now use the Unimate method as our routine method. With this method, serum iron is determined linearly up to 180 μmol/L and the detection limit is 0.9 μmol/L. Within-run CVs are ≤0.4% (iron concentration, 7.7–38.0 μmol/L) and the analytical recoveries of added iron are 99.2–101.2%. We think that this colorimetric method, which does not involve sample deproteinization or heating, would be an ideal reference method for measuring serum iron.

References

Role of Specimen Collection in Preanalytical Variation of Metalloproteinases and Their Inhibitors in Blood, Klaus Jung,* Lars Nowak, Michael Lein, Wolfgang Henke, Dietmar Schnorr, and Stefan A. Loening (Dept. of Urol., Univ. Hosp. Charité, Humboldt Univ., Schumannstr. 20/21, D-10098 Berlin, Germany; *author for correspondence: fax +4930 28021402; e-mail jung@rz.charite.hu-berlin.de)

Matrix metalloproteinases (MMPs) are important enzymes involved in all physiological processes occurring during tissue remodeling and repair [1]. Their catalytic activities are controlled by various mechanisms including the enzyme synthesis, secretion as zymogens, which undergo extracellular activation, and inhibition of the activated enzymes by specific inhibitors, the so-called tissue inhibitors of metalloproteinases (TIMPs). Thus, the balance between MMPs and TIMPs has been suggested to play a crucial role in such pathological conditions as tissue fibrosis, arthritis, and tumor invasion and metastasis [2, 3]. Since these changes may be reflected in body fluids, the determinations of MMPs, TIMPs, and their complexes in blood have been recommended as useful diagnostic tools [4, 5].

The components have been measured both in serum and in plasma samples [4–9]. The data have been described by few groups, with in-house tests. Commercial test kits are only now available (e.g., Amersham). However, until now, there has been no clear information on how the specimen collection affects the preanalytical variation of these components. In this study we representatively examined the effect of the sampling process on MMPs and TIMPs by measuring MMP-1, TIMP-1, and the MMP-1/TIMP-1 complex in serum and plasma samples. We draw the clinician's attention to the appropriate sampling process for determining these analytes in blood to avoid misinterpretations of these indicators.

We used Biotrak™ ELISAs for MMP-1, TIMP-1, and the MMP-1/TIMP-1 complex (Amersham Int., Bucks, UK). The assays are based on the two-sided sandwich principle. All tests were performed in duplicate according to the manufacturer's instructions. The measurements were made on the microplate reader Anthos HTIII (Anthos Labtec Instruments, Salzburg, Austria) with the cubic-spline method for calculation of concentrations (EIA/KIN-Star software, version 7.0; Wepah-Med, Berlin, Germany). Average values were used for further calculations. Venous blood samples from healthy men were simultaneously collected into plastic tubes for preparation of serum samples, and into EDTA- as well as into lithium heparin-coated plastic tubes for preparation of plasma samples (Sarstedt, Nümbrecht, Germany; Monovette systems 03.1528, 05.1167, 03.1589). The tubes stored at room temperature were centrifuged within 30 min after venipuncture at 1600g for 15 min at 4 ºC. The supernatants were carefully removed and stored at −80 ºC until analysis within 10 days after collection. The procedures followed were in accordance with the ethical standards of the responsible committee at the Charité hospital. Figure 1 shows results from the experiments, expressed as the mean and individual values of MMP-1, TIMP-1, and MMP-1/TIMP-1 complex vs the kind of sample used for determination. Comparisons of mean values showed that MMP-1,TIMP-1, and MMP-1/TIMP-1 complex concentrations determined in EDTA plasma and serum were −2.6 and 5, 2.7 and 7, 1.3 and 7.3 higher than the respective mean values determined in heparin plasma (16.2 and 31.2 μg/L vs 6.2 μg/L; 462 and 1379 μg/L vs 201 μg/L; 73.3 and 415 μg/L vs 56.9 μg/L). To exclude the probability that the lower values in heparin plasma were caused by the inhibitory effect of lithium heparin on the determination of the analytes, we did additional experiments. Known amounts of MMP-1, TIMP-1, and MMP-1/TIMP-1 complex were added to serum and heparin plasma samples (n = 4), and the concentrations measured before and after were compared. Taking into account the increases of concentrations of MMP-1, TIMP-1, and MMP-1/TIMP-1 complex in serum samples after the addition as 100%, the average increases in heparin plasma samples amounted to 111% for MMP-1, 97% for TIMP-1, and 87% for the complex. These differences were in the range of analytical precision; however, an interference effect of lithium heparin on the determinations could be clearly excluded. Therefore, we concluded from all these data that serum and EDTA plasma are not appropriate samples to achieve reliable data of MMPs and TIMPs because increases during the sampling process are unspecific.

There are only a few reports and no systematic studies on the influence of sampling process on various MMP and TIMP concentrations. For example, concentrations of MMP-2 [8] and MMP-8 [10] have been found to be higher in serum than in plasma, but no further details have been given [8, 10]. On the other hand, the mean value of MMP-1 in serum of healthy persons found by Maeda et al. [7] corresponds to our results of MMP-1 in serum. Plumpton et al. [6] found that TIMP-1 concentrations were ~30–40% higher in serum than in plasma. Other authors reported a serum-to-plasma TIMP ratio of ~2 [11] and 2.7 [12]. One reason for these discrepancies perhaps results from the different storage temperature when the blood was allowed to clot. For example, Plumpton et al. [6] kept blood samples at 4 ºC after venipuncture, whereas our samples were stored at room temperature for 30 min. However, we found that MMP-1, TIMP-1, and MMP-1/TIMP-1 complex in serum did not increase when the time between venipuncture and centrifugation of blood samples was prolonged from 30 min to 3 h. Increased values in serum are supposedly caused by the release of these components during platelet activation [11, 12]. As the
clotting process is generally finished within 30 min, the release of MMP and TIMP is also confined to that period. However, the possible release of these analytes from platelets made it necessary to test the centrifugation conditions to assure the removal of platelets before collecting and freezing the samples. For that purpose, we collected supernatants from five different heparin plasma samples after centrifugation at 1600g at 15 min as usual and at 23 000g, and measured the analytes after the above-mentioned freeze–thaw process. Considering the concentrations measured in the supernatant at the conventional centrifugation procedure of 1600g as 100%, the concentrations in the plasma samples collected at 23 000g were between 89% and 109%. We concluded from these results that the concentrations measured represented the true circulating concentrations and did not result from platelets incompletely removed and released during the freeze–thaw process.

We believe the effect of sampling process is important because combined measurements of TIMP-1 and metalloproteinases are generally necessary for diagnostic purposes [4, 9]. Thus, heparin plasma was selected as the specimen of choice. Consequently, for establishing reference limits we measured these analytes in heparin plasma samples of 40 healthy females (mean age 46.9 years) and 40 males (mean age 44.9 years). Reference values did not depend on age and sex and were combined for further calculations according to the IFCC recommendations [13]. Our results of the Kolmogorov–Smirnov test for the goodness of fit to the normal distribution showed that the data were not normally distributed. Thus, we calculated preliminary 95% central reference intervals on the basis of the nonparametric approach [13]. The corresponding medians (and lower and upper limits) were 5.63 μg/L (1.25 and 29.1 μg/L) for MMP-1, 441 μg/L (121 and 1669 μg/L) for TIMP-1, and 30.3 μg/L (4.81 and 136 μg/L) for the MMP-1/TIMP-1 complex.

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References

Fig. 1. (A) MMP-1, (B) TIMP-1, and (C) MMP-1/TIMP-1 concentrations and dependence on sample processing.

The analytes were measured in serum and plasma derived from blood samples of 10 healthy men collected either into EDTA- or lithium heparin-coated tubes. Arithmetic means ± SD including the individual values are given. Significance levels calculated by the Wilcoxon ranks test for paired data (at least P <0.05): a, different between heparin plasma and EDTA plasma; b, different between heparin plasma and serum; c, different between EDTA plasma and serum.


