Vol. **96** (2002) 126–133

Available online at http://www.idealibrary.com on IDEAL®

respiratoryMEDICINE

LETTERS TO THE EDITOR

doi:10.1053/rmed.2001.1222

Sir,

Re: elevated level of circulating matrix metalloproteinase-9 in patients with lung cancer (*Respir Med* 2001; 95: 1–4)

We read with interest the paper by Hrabec *et al.* (1) on the concentrations of matrix metalloproteinase 2 and 9 (MMP-2, MMP-9) in blood of patients with lung cancer. They reported the observation that the levels of MMP-9 measured in serum or plasma of lung cancer patients were significantly increased compared to normal controls while MMP-2 concentrations were unchanged. The authors concluded that this phenomenon is not simply due to an over-expression of this enzyme in cancer cells.

From our point of view the authors have not taken into account that analytical problems in analysing the MMP levels in serum may arise and therefore influence the results (2). Our previous studies showed distinct differences in MMP-9 levels between serum and EDTA or heparin plasma (3). In serum samples higher MMP-9 were detected compared to plasma samples.

These studies demonstrated the importance of a standard preanalytic procedure for the collection of specimen and the measurement of MMPs and TIMPs in blood. We inferred from these data that heparin plasma should be used as sample to avoid pre-analytical mistakes for MMP-9 measurements. This is generally in line with other reports that have recommended plasma samples for MMP-9 determinations in blood (4). We have extended our study on the effect of sample preparation on the measurement of MMP-9 and MMP-2 in blood to investigate the influence of different blood sampling tubes on MMP levels. To investigate the effect of blood collection on MMP-9, venous blood samples from eight healthy volunteers were simultaneously collected in various devices for preparation of serum samples, namely: (1) S-Monovette Neutral tubes 01·1728 (plastic tubes with no additive for preparation of native serum, indicated in the following as serum-negative; (2) S-Monovette Serum tubes 01·1601 (plastic tubes with kaolin-coated plastic granulate coagulation accelerator for preparation of serum, indicated in the following as serum-positive; (3) S-Monovette plastic tubes coated either with lithium heparin, potassium EDTA or sodium citrate for preparation of plasma samples (all the devices were from Sarstedt GmbH, Nürnberg, Germany). The tubes stored at room temperature were centrifuged within 30–60 min after venipuncture at 1600 ***g*** for 15 min. The supernatants were carefully removed, centrifuged again at 10000 ***g***, and stored at −80°C until analysis was performed. We used ELISAs for MMP-2 and MMP-9 supplied by Amersham Pharmacia Biotech, Little Chalfont, U.K. Oncogene, Boston MA, U.S.A and Medac Diagnostika, Wedel Germany.

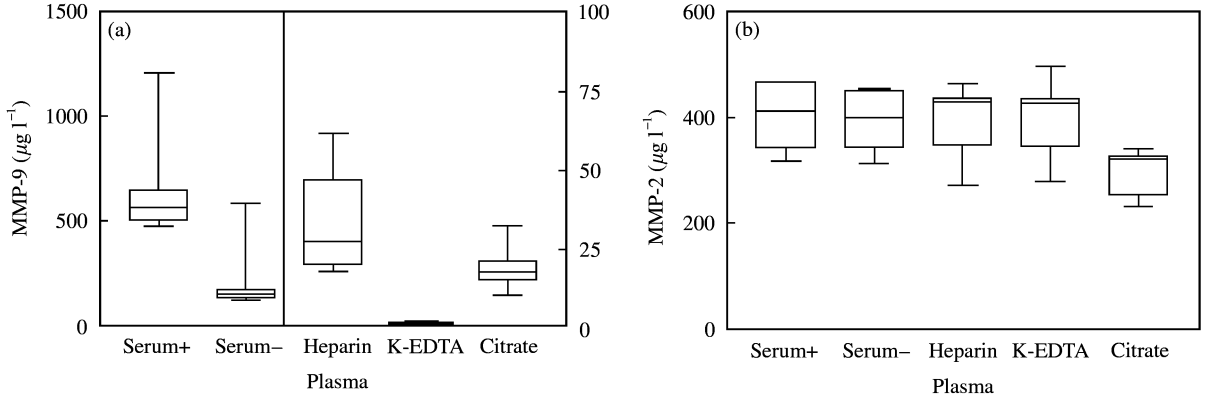

Fig. 1. MMP-9 and MMP-2 concentrations in dependence on sample processing. MMP-9 (a) and MMP-2 (b) were measured in serum and plasma derived from blood samples of eight healthy persons collected in Monovette systems from Sarstedt (Germany) (serum-negative tubes with no additive; serum-positive: tubes with kaolin-coated granulate as clot activator; heparin: heparin-coated tubes, K-EDTA: EDTA coated tubes, citrate: citrate coated tubes). The measurements were performed with the Medac MMP-9 and MMP-2 assay. The results are expressed as boxes and whiskers (boxes with the horizontal line at the median extend from the 25th to the 75th percentiles; whiskers show the range of the data). Data between groups were compared using the *t*-test for paired data. Significant differences (at least $P < 0 \cdot 05$) were between MMP-9 values in both kinds of serum samples and plasma samples and among all plasma samples, respectively. In MMP-2 measurements, no differences ($P > 0 \cdot 05$) were found between both serum preparations, heparin and EDTA plasma samples but all these samples showed higher analyte concentrations than citrate plasma.

Our results showed increased MMP-9 levels in serum depending on the clotting surface of the tubes used for sample collection compared to heparin and EDTA plasma (Fig. 1). Increased values in serum are supposedly caused by the release of MMP-9 during platelet activation and clotting process. These important preanalytical conditions should be considered in the interpretation of increased MMP levels. Hrabec *et al*. did not clearly distinct between serum or plasma samples that may lead to misinterpretations. This point would fit in their conclusion which assumed that mechanisms additional to enzyme over-expression in lung cancer may be responsible for the enhanced MMP levels.

The pre-analytical sampling is therefore an important feature for the MMP measurement in the blood regardless of the method which is finally used for the enzyme quantification. We believe that it is necessary to consider this fact in future studies on the clinical validity of MMP measurements in blood.

M. JOHN, U. OLTMANNS, C. WITT and K. JUNG

Department of Pneumology and
Clinic of Urology, University Hospital Charité,
Humboldt University,
Berlin, Germany

REFERENCES

1. Hrabec E, Strek M, Nowak D, Hrabec Z. Elevated level of circulating matrix metalloproteinase-9 in patients with lung cancer. *Respir Med* 2001; **95**: 1–4.
2. Lein M, Nowak L, Jung K, *et al*. Analytical aspects regarding the measurement of metalloproteinases and their inhibitors in blood. *Clin Biochem* 1997; **30**: 491–496.
3. Jung K, Laube C, Lein M, *et al*. Kind of sample as pre-analytical determinant of matrix metalloproteinases 2 and 9 (MMP2; MMP9) and tissue inhibitor of metalloproteinase 2 (TIMP2) in blood. *Clin Chem* 1998; **44**: 1060–1062.
4. Zucker S, Lysik RM, DiMassimo BI, *et al*. Plasma assay of gelatinase B: tissue inhibitor of metalloproteinase complexes in cancer. *Cancer* 1995; **76**: 700–708.

doi:10.1053/rmed.2001.1223

Sir,

Re: Elevated level of circulating matrix metalloproteinase-9 in patients with lung cancer (*Respir Med* 2001; 95: 1–4): a reply

We are aware of the letter by Dr M. John and colleagues to the Editor of *Respiratory Medicine* as well as the results of their study on the role of specimen collection in the measurement of matrix metalloproteinases (MMPs) and matrix metalloproteinase inhibitors (TIMPs) published earlier (1). We of course agree with Dr John that appropriate selection of specimens is very important for correct evaluation of MMPs activities in blood.

The studies conducted in other laboratories in the 1990s showed that serum or plasma type IV collagenase levels are significantly elevated in many neoplastic diseases and it has been suggested that the rise in circulating MMP-2 and/or MMP-9 levels in cancer patients reflects enhanced secretion of these enzymes in tumour area (2,3). Using a zymographic test we analysed the type IV collagenases levels in serum samples of lung cancer patients and healthy volunteers, hoping that MMP-2 activity would be elevated in lung cancer sera, since our previous study showed that this enzyme is predominantly over-expressed in lung cancer tissue as compared to normal lung parenchyma (4). However, the serum levels of MMP-2 in lung cancer patients were similar to those of healthy donors, whereas MMP-9 levels were 3·59-fold higher in lung cancer. We presented the results of this study in our article (5). Since the differences between normal and cancer sera concerned MMP-9, but not MMP-2, we took into consideration the results of the study reported by Dr John and colleagues (6) and tested these activities also in plasma samples. In the zymographic test we compared the serum and plasma levels of type IV collagenases from the same individuals (lung cancer patients and healthy volunteers). For plasma preparation venous blood samples were collected into lithium heparin-coated plastic tubes (Vacuette-Greiner Labortechnik, Kódź, Poland). These results were also presented in our article (5). We showed that MMP-2 levels tested in serum and plasma specimens were similar in the range of analytical precision and did not differ between both groups tested (normal and cancer), whereas MMP-9 levels tested in serum were about two fold higher than in plasma samples. However, the MMP-9 activities tested in plasma of lung cancer patients were 3·42-fold higher than in plasma from normal individuals. Thus, the differences in MMP-9 levels between cancer and normal samples tested in plasma were similar to those obtained for serum. We wish to emphasize that in each case comparisons were made between consistent specimens (i.e. MMP-9 activities in cancer plasma samples were compared with normal plasma samples and MMP-9 activities in cancer serum samples were compared with normal serum). In the first version of our manuscript submitted to *Respiratory Medicine* we inserted a figure illustrating the differences between serum and plasma levels of these enzymes evaluated by zymography. However, by request from the Editor, we shortened our article and this figure was not published. Now, for a better illustration of the results of the study we present this figure (Fig. 1).

We do not believe that increased MMP-9 values observed in serum are a result of releasing of this enzyme from platelets during their activation and clotting pro-