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 distinguish 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 preanalytical 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.

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REFERENCES


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Sir,


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, Kędź, 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-
cesses as was suggested by Jung et al. (6). Lindenmeyer et al. (7) were unable to demonstrate MMP-2 or MMP-9 activities in medium after incubation of thrombin-activated platelets at the concentration of $4 \times 10^8$ cells ml$^{-1}$, although Sawicki et al. (8) showed that MMP-2 is released from platelets during their activation. Using zymography we analyzed cell lysates of polymorphonuclear leukocytes (PMNL) and platelets and showed (Fig. 2) that whereas granulocytes are able to express MMP-9, the platelets express little MMP-2 only. So, we suppose that the platelets are not a source of MMP-9 in serum specimens. However, it has been shown (7) that activated platelets are able to stimulate breast cancer cells MDA-MB231 to secrete MMP-9, and it is not excluded that also some blood cells (e.g. PMNL) are stimulated by activated platelets to release MMP-9 during blood clotting. However, in the experiment performed by Lindenmeyer et al. (7) augmented release of MMP-9 by MDA-MB231 cells was preceded by augmented synthesis of this enzyme. Stimulation of synthesis of new particles of the enzyme via activated platelets followed by its release needs, of course, considerably more time than it is required for blood clotting. So, to date, the reasons for higher MMP-9 levels in serum than in plasma are unclear.

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REFERENCES


Universally low spirometry values in respiratory outpatients

An increasingly important part of a chest physician's workload is running outreach clinics. As formal lung function laboratories are often not available at these community hospitals, it is often the outpatient nurses who perform spirometry on patients prior to their consultation. The most common spirometer being used in these clinics is the bellows vitalograph.

At one of these clinics recently, we noticed that nurse-recorded spirometry in patients was consistently lower than previously recorded values, despite the fact that peak expiratory flow rates were similar and the patients were stable.

Inspecting the bellows vitalograph we found numerous cracks in the hosing (Fig. 1). This had been caused by repeated autoclaving of the hose to comply with infection control requirements. Cracking of the tubing secondary to autoclaving is an important cause of error, which may not be obvious unless the tubing is checked carefully.

Fig. 1

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