According to the latest findings that blood sampling markedly determines the concentration of circulating MMP-9 and TIMP-1, the authors were not aware that pre-analytical problems in analysing the MMP levels in serum may arise and, therefore, influence the results [2].

However, HIGASHIMOTO *et al*. [1] have rather inadequately taken into account the significance of blood collection as an important pre-analytical determinant of MMP and TIMP results. As there is rising evidence that blood sampling markedly determines the concentration of circulating MMP-9 and TIMP-1, we would point the readers' attention to these facts that have already been discussed in analytical journals [2, 3].

Studies from our own laboratory demonstrated the importance of a standard pre-analytic procedure for the collection of specimens for the measurement of MMP and TIMP in blood. A report of our own results of the effect of different blood sampling tubes on MMP-9 and TIMP-1 measurement is shown in figure 1. Briefly, venous blood samples from eight healthy volunteers were simultaneously collected in different devices for the preparation of serum samples. The tubes were centrifuged within 30 min after venipuncture at 1,600 × *g* for 15 min at room temperature. The MMP-9 and TIMP-1 concentration was measured in the supernatants using commercially available ELISA kits (Medac Diagnostika, Wedel, Germany).

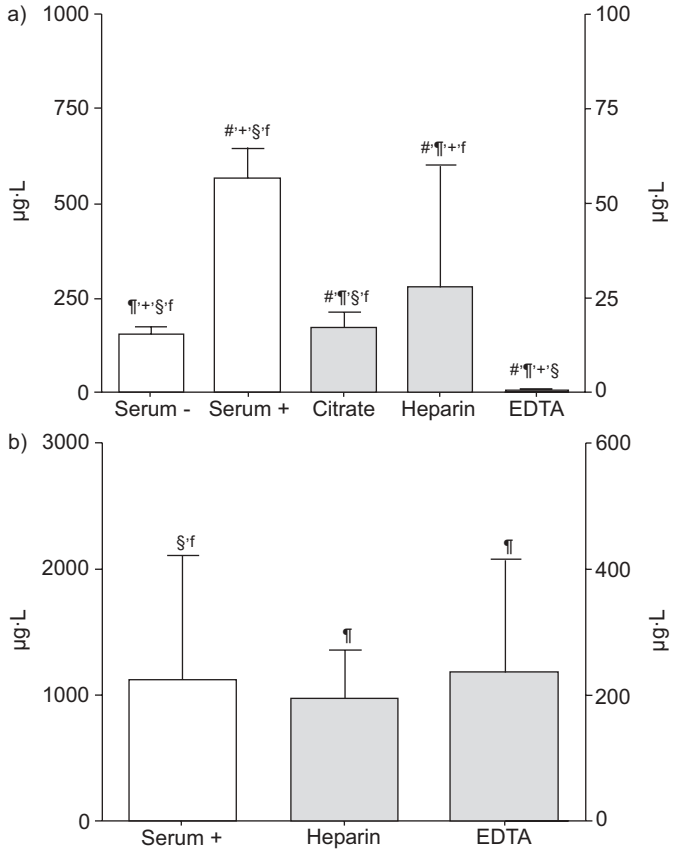

FIGURE 1. Effect of blood sampling on a) matrix metalloproteinase (MMP)-9 and b) tissue inhibitor of metalloproteinase (TIMP)-1 concentration in serum (□) and plasma (■). Median values and interquartile intervals are shown. MMP-9 and TIMP-1 were measured in samples prepared from the blood of eight and 10 healthy adults, respectively. Serum -: pure serum prepared in Monovette tubes without additive; serum +: serum prepared in tubes containing kaolin-coated granulate as clot activator. Plasma was prepared in tubes coated with sodium citrate, lithium heparin or K-EDTA. Significant differences of at least $p<0.05$ (Wilcoxon rank test) between the samples were indicated by the following symbols: $^{\#}$: from serum -; ¶: from serum +; $^{+}$: from plasma-citrate; §: from plasma-heparin; ^{f}: from plasma-EDTA.

MMP-9 concentrations in serum samples collected in tubes with clot activator were ~3-fold higher than in pure serum samples and essentially higher than the concentrations found in plasma samples (fig. 1a). The TIMP-1 concentrations were ~5–7-times higher in serum than in plasma (fig. 1b). Since platelets and leukocytes contain high concentrations of MMP-9 and TIMP-1, the varying release of these analytes from blood cells during the platelet activation or sampling process could cause these differences [4]. In addition, changes of white blood cell count are observed during COPD exacerbations and could subsequently lead to changed TIMP-1 concentrations when measurement was performed in serum. The MMP-9 concentration could be influenced by platelet activation or sampling process leading to MMP-9 release from platelets and leukocytes [3]. These important pre-analytical conditions should be considered in the interpretation of increased MMP-9 and TIMP-1 levels. HIGASHIMOTO *et al*. [1] did not clearly distinguish between serum or plasma samples, which may lead to misinterpretations.

Recently, the use of blood samples collected with sodium citrate was suggested to avoid the detrimental effect of other anticoagulants or serum, and to optimise the diagnostic validity of matrix metalloproteinase and tissue inhibitor of metalloproteinase in peripheral blood [4].

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From the authors:

We would like to thank M. John and K. Jung for their interest in our study [1] and their comments upon the important issue of the analytical conditions for blood sampling. As M. John and

K. Jung mentioned, we did not evaluate the influence of blood sampling and separation of serum from blood cells. We agree with M. John and K. Jung that we must be aware of pre-analytical problems when analysing the circulating matrix metalloproteinase (MMP)-9 and tissue inhibitor of metalloproteinase (TIMP)-1 levels. However, we used only serum samples in our study because we usually use serum samples for clinical examinations in our hospital. Therefore, we think that the comparison of the serum TIMP-1 and MMP-9 concentrations in each patient group is valid. Our results are consistent with serum TIMP-1 and MMP-9 concentrations reported elsewhere [2, 3].

We also agree with M. John and K. Jung that the changes of white blood cell count during chronic obstructive pulmonary disease (COPD) exacerbations could have influenced TIMP-1 concentrations, because the white blood cell count increased during most exacerbations. We are now planning to identify specific cell types that are involved in the production and secretion of circulating TIMP-1 and MMP-9 in COPD patients.

We thank M. John and K. Jung for their suggestions for our future studies.

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ECG for risk stratification in patients with pulmonary embolism

To the Editors:

We read with interest the paper by GEIBEL *et al.* [1] on the prognostic value of the ECG in patients with acute major pulmonary embolism (PE). In the last few years a number of studies have been published for risk stratification of patients with PE, with the suspicion that there could be a subgroup of haemodynamically stable patients (submassive PE), in whom thrombolysis could be beneficial [2–4].

The hypothesis of the study [1] was that ECG could be a simple baseline test (as compared with echocardiography) to identify risk of death in patients with PE. However, the population of the study included either patients with haemodynamically unstable PE, in whom thrombolysis is usually indicated, or patients with submassive PE, who are identified by echocardiographic findings. Therefore, the results may be of limited value in clinical practice.

We studied 302 consecutive normotensive patients with a diagnosis of PE in a 2-yr period. The mean age was 68 yrs (95% confidence interval (CI): 66–70) of whom 55% were female. We analysed the prognostic relevance of ECG with respect to early mortality (defined as those presented in the first 30 days). ECG was available in 278 patients, of which 116 (42%) were normal. ECG abnormalities were: 1) sinus tachycardia in 93 patients; 2) ST-T abnormalities in 29 patients; 3) complete right bundle branch block in 42 patients; 4) S1Q3T3 pattern in 32 patients; 5) atrial arrythmia in 22 patients; and 6) right axis pattern in two patients. Early death occurred in 16 patients (6%). The 12-lead ECG did not show differences between survivors and nonsurvivors during the first 30 days after admission. Univariate analysis revealed that ECG abnormalities were not significant independent predictors of outcome (odds ratio: 0.7; 95% CI: 0.2–2.5). Our results do not support the usefulness of ECG for risk stratification in haemodynamically stable patients with pulmonary embolism.

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