LPS on two occasions separated by ≥3 weeks. However, none of these studies had observed tolerance towards subsequent LPS challenge(s) in their healthy human subjects at doses of LPS described that were higher than ours. It is possible that tolerance in healthy nonatopic human subjects only occurs in exposure to lower doses of inhaled endotoxin. In fact, existing literature indicates that exposure of 30–40 μg inhaled LPS is probably the clinical threshold to induce symptoms and lung function changes for healthy subjects [4].

More research is required to validate our preliminary observation.

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Pre-analytical conditions for the assessment of circulating MMP-9 and TIMP-1: consideration of pitfalls

To the Editors:

We read with interest the recent article of Higashimoto et al. [1], which reported an increased activity of tissue inhibitor of metalloproteinase (TIMP)-1 in patients with chronic obstructive pulmonary disease (COPD) and asthma. In contrast, the molar ratio between matrix metalloproteinase (MMP)-9 and TIMP-1 was significantly lower in COPD patients than in normal subjects.

FIGURE 1. Comparison of sputum neutrophilia post-inhaled lipopolysaccharide (LPS; 6th h) between two inhaled LPS challenges separated by 4 weeks. ■: baseline; ●: 6 h post-inhaled LPD; ◆: 1 week post-inhaled LPS. **: denotes p<0.01 between the mean differences.

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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 the 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].

A report of our own results of the effect of different blood specimens for the measurement of MMP and TIMP in blood was presented in a recent issue of Euros Respir J [1] and their comments upon the important issue of the analytical conditions for blood sampling. As M. John and colleagues pointed out, they 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].

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).

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 in peripheral blood [4].

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**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; ‡: from plasma-EDTA.