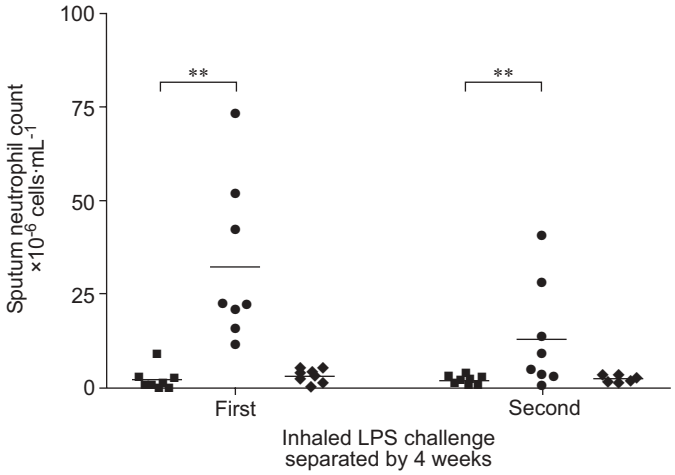

FIGURE 1. Comparison of sputum neutrophilia post-inhaled lipopolysaccharide (LPS; 6th h) between two inhaled LPS challenges separated by 4 weeks. ■: baseline; ●: 6 h post-inhaled LPD; ◆: 1 week post-inhaled LPS. **: denotes p=0.01 between the mean differences.

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 endoxin. 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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From the author:

The study that L.C. Loh describes in his letter above adds another interesting aspect that is critical for the response to inhaled endotoxin.

Lipopolysaccharide tolerance is a well-known feature of several host defence cells, although the mechanisms involved are not entirely clear [1]. Tolerance has also been shown to be associated with various cellular processes, such as decreased activity of Gi proteins, protein kinase C, mitogen-activated protein kinase, activator protein-1 and nuclear factor-κB (NF-κB). Inhibitory molecules such as IRAK-M, suppressor of cytokine-signaling-1 and inhibitor-κB are found activated. At the nuclear level, the NF-κB subunit p50 homodimer expression and peroxisome-proliferator-activated receptors-γ are increased. There is evidence from rodent studies that this phenomenon is also relevant for pulmonary innate immunity [2].

The preliminary results described in this letter support this view and it is likely that this mechanism is of biological relevance, because the lung is constantly exposed to small amounts of lipopolysaccharide. The pulmonary exposure with endotoxin probably has many consequences. At this time it is uncertain where lipopolysaccharide tolerance is functionally located in this scenario.

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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.

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).

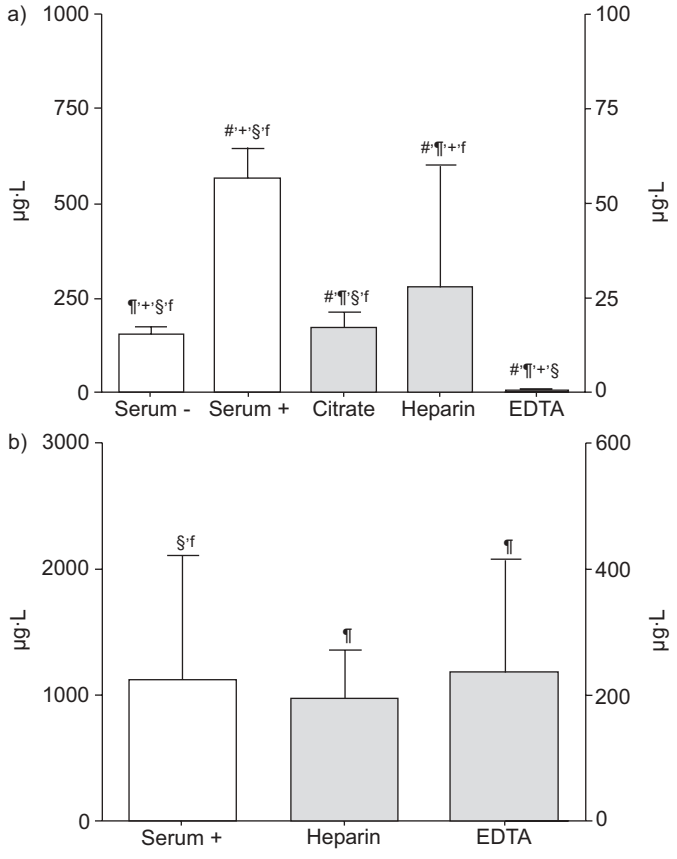

FIGURE 1. Effect of blood sampling on a) matrix metalloproteinase (MMP)-9 and b) tissue inhibitor of metalloproteinase (TIMP)-1 concentration in serum (□) and plasma (■). Median values and interquartile intervals are shown. MMP-9 and TIMP-1 were measured in samples prepared from the blood of eight and 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