From the authors:

We would like to thank S. Greco and coworkers for their comments on our paper about the predictive value of bronchoalveolar lavage (BAL) cell differentials in the diagnosis of interstitial lung disease (ILD) [1]. In their study, which they discuss in their letter to the Editors, they tested the feasibility of our data for the differential diagnosis of pulmonary tuberculosis (TB) and sarcoidosis. In contrast to our study, they retrospectively analysed 88 patients with biopsy-proven sarcoidosis and 76 patients with culture-positive pulmonary TB.

First of all, we agree with the major points of their study. The high grade of lymphocytosis (>50%) is the best predictor for sarcoidosis, and the presence of elevated neutrophils rendered the diagnosis of sarcoidosis very unlikely. In our experience and clinical practise, however, the proportion of TB with a comparable ILD pattern seems to be extremely low. In our own hospital (Hospital Grosshansdorf, Center of Pneumology and Thoracic Surgery, Grosshandorf, Germany), >100 patients per year are treated for TB. Within the study interval of 7 yrs (~700 TB patients), only seven of these patients showed a clinical and/or radiological pattern of ILD [1].

Although many clinicians use BAL fluid to confirm the diagnosis of TB microbiologically, it does not seem to be the only diagnostic tool that can be used to confirm TB. In addition, other diseases with an ILD pattern (nonsarcoid ILD) are much more frequent than TB with an ILD pattern. In our study [1], nonsarcoid ILD is not equal to TB with an ILD pattern. Therefore, the lower predictive value seen in the study by S. Greco and coworkers is not unexpected.

Our analysis was carried out in a manner similar to that adopted by most clinicians in the diagnostic process, with a special emphasis on interstitial lung disease. The use of cut-off values in the interpretation of bronchoalveolar lavage cellular results seems to be more practical compared with the use of a discriminant score.

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DOI: 10.1183/09031936.05.00048705

The prevalence of ΔF508 in primary osteoporotic patients

To the Editors:

We read with great interest the paper by KING *et al*. [1] in a recent issue of the *European Respiratory Journal*. The authors found a strong association between reduced bone mineral density (BMD) and carrier state of ΔF508 cystic fibrosis (CF) allele (the most common cystic fibrosis transmembrane regulator (CFTR) gene variant) in an adult CF population. Their results suggest that reduced BMD in CF appears to have a genetic component, independent of the disease severity and nutritional deficits. This fascinating observation is in line with the results found by our group [2]. It was found that healthy mothers of CF children (who are obligate heterozygous carriers of a CFTR mutation) have lower than normal BMD values. Furthermore, a correlation with the BMD values of their CF children was demonstrated. Although there are no data on the role of the CFTR gene in bone, it has been reported that not only BMD but also the bone structure of patients with CF was altered compared with healthy individuals [3].

These data further support a possible genetic component in the development of a CF-associated bone deficit. If so, one can speculate that the presence of a single diseased CFTR gene may contribute to the development of osteoporosis in the otherwise healthy adult population.

In a pilot study, we tested the prevalence of the ΔF508 CFTR gene mutation in subjects with severe primary osteoporosis. A total of 137 Caucasian post-menopausal females (aged 46–80 yrs) with osteoporosis were enrolled. Osteoporosis was defined by a T-score of ⩽-2.5 at the lumbar spine and/or hip sites. Individuals with secondary causes of osteoporosis or bone loss were excluded. All study participants gave informed consent. The prevalence of the ΔF508 variant of CFTR gene was screened by capillary electrophoresis [4].

Three patients with a single ΔF508 allele were detected, which corresponds to the prevalence observed previously in the general Hungarian population [5]. These patients did not suffer from a more severe form of osteoporosis than those without a CFTR gene mutation.

These results do not support the hypothesis that the ΔF508 mutation is more common among females with primary osteoporosis. However, a serious limitation of this study is that the number of patients was too low to establish any association between the heterozygosity for the ΔF508 allele and the epidemiology of primary osteoporosis. Given the frequency of the ΔF508 allele in Hungary (3.5%) [5], the number of patients in this study would have been enough to reveal a four-fold difference when the expected allele frequency is 14%, with a power of 85%, among patients with osteoporosis in the

overall population. In order to reveal a lower, but still significant difference in the cystic fibrosis transmembrane regulator ΔF508 allele prevalence, the number of patients should be increased dramatically. Hopefully, the worldwide existing large collections of DNA specimens from osteoporotic patients will provide an opportunity to enlighten the possible implication of a cystic fibrosis transmembrane regulator mutation in the development of osteoporosis.

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SUPPORT STATEMENT
This study was supported by grants OTKA D048351-T046086 and NKFP 1A/002/2004 from the Hungarian Government (Budapest).

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DOI: 10.1183/09031936.05.00044605

Tolerance to repeat exposure of inhaled endotoxin: an observation in healthy humans

To the Editors:

We read with interest the articles on endotoxin research in the May issue of the *European Respiratory Journal*. The editorial by BALS [1] aptly raised the yet unanswered questions concerning the timing (acute *versus* chronic) and doses of inhaled endotoxin relevant to health and disease, and the questions of whether the outcome of such exposure is always detrimental.

To this end, we wish to add our own preliminary observation of the possibility of tolerance to repeat exposure of inhaled lipopolysaccharide (LPS) in healthy nonatopic humans at 4 weeks. In a double-blind, crossover study, eight healthy human subjects were randomised to receiving either a single inhaled dose of 50 μg salmeterol or placebo prior to being challenged with a 15-μg dose of *Escherichia coli* serotype 026:B6 (Sigma, Poole, UK), in two visits separated by 4 weeks. Using 1 week prior as a baseline, sputum induced at the 6th h after LPS challenge showed no significant differences in the increase of total cell counts in the two treatment periods (mean difference (95% confidence interval) salmeterol *versus* placebo: 10.6×10^6 cells·mL^{-1} (-9.71–30.9); p=0.25) or neutrophils (11.7×10^6 cells·mL^{-1} (-8.33–31.92); p=0.20; unpublished data). The assertion that salmeterol does not protect against airway neutrophilic inflammation was subsequently supported in a more robust study, where subjects were randomised to receiving either daily salmeterol for 3 weeks or placebo, prior to inhaled LPS challenge, in a crossover study [2].

Retrospective power analysis of our results first alerted us to the possibility of intrinsic biological phenomena in a study design of sequential inhaled LPS challenges. Data were then re-analysed with the purpose of looking into the reproducibility of sputum neutrophilia between the two inhaled challenges, treating the effects of the single-dose salmeterol as no more than placebo [2]. Our findings showed that following the first LPS challenge, the mean total sputum cell counts increased by 31.23×10^6 cells·mL^{-1} (95% CI: 13.27–49.20) and the mean sputum neutrophil counts rose by 30.3×10^6 cells·mL^{-1} (12.59–48.11). However, following the second LPS challenge, the mean total sputum cell counts only increased by 11.3×10^6 cells·mL^{-1} (2.14–24.89) and mean sputum neutrophil counts by 10.9×10^6 cells·mL^{-1} (1.02–22.9). The difference between the means was statistically significant (p=0.01; mean difference: 19.8×10^6 cells·mL^{-1} (6.16–33.56) for total sputum cell counts; 19.4×10^6 cells·mL^{-1} (4.73–34.08) for sputum neutrophil counts; fig. 1).

Using such a human experimental model of airway neutrophilia to understand the inhaled effects of endotoxin [3], and to examine for potential anti-inflammatory properties of therapeutic agents [2] appears to be a validated approach. MICHEL *et al.* [3] employed a model of weekly inhaled challenges of incremental LPS doses (0.5 μg, 5 μg and 50 μg) to provide evidence for dose responsiveness of LPS in airway inflammation and systemic effects in healthy human subjects. WALLIN *et al.* [2] tested for possible anti-inflammatory effect of salmeterol *versus* placebo, *via* findings from bronchoscopy, based on a study design of inhaling 50 μg