COPD sputum eosinophils: relationship to blood eosinophils and the effect of inhaled PDE4 inhibition

To the Editor:

Patients with COPD who have higher eosinophil numbers in the airways and peripheral blood demonstrate a greater clinical response to inhaled corticosteroids (ICS) [1–3]. Furthermore, the effect of the oral phosphodiesterase-4 (PDE4) inhibitor roflumilast on exacerbations in severe COPD patients with chronic bronchitis, who are treated with ICS and long-acting bronchodilators, also appears to be greater at higher blood eosinophil counts [4]. The mechanisms responsible for these differential drug effects remain to be defined, but may relate to increased type-2 inflammation and/or decreased presence of colonising airway bacteria in COPD patients with more eosinophils [5, 6], leading to different responses to anti-inflammatory drugs. An association between blood and sputum eosinophils has been observed in some, but not all studies [7–12]. Accurate sputum eosinophil count measurement requires good quality samples to make cytospins where eosinophils can be clearly counted; variable quality of sputum samples, particularly in multicentre studies, will affect the ability to show a relationship with blood eosinophil counts.

CHF6001 is an inhaled PDE4 inhibitor which showed anti-inflammatory effects in the airways after 32 days of treatment in COPD patients with chronic bronchitis on top of triple therapy [13]. In sputum, CHF6001 reduced the levels of certain cytokines and down-regulated inflammatory genes associated with eosinophil activation [14]. Previous studies with roflumilast showed inhibition of the total number of inflammatory cells in sputum, and inhibition of sputum eosinophil counts accompanied by a reduction in bronchial mucosal eosinophil numbers [8, 15]. We performed a *post hoc* analysis of the CHF6001 biomarker study [13] with two aims: 1) to investigate whether CHF6001 suppressed sputum eosinophil counts in those COPD patients with greater eosinophilic inflammation; and 2) to investigate the relationship between blood and sputum eosinophils, as divergent results have been published on this topic.

Samples were collected from a multicentre, three-way, placebo-controlled, double-blind crossover study which has been previously reported [13]. Patients received 32 days of treatment with CHF6001, 800 or 1600 µg twice daily, or matching placebo *via* a dry powder inhaler (NEXThaler). Eligibility criteria were post-bronchodilator ratio of forced expiratory volume in 1 s (FEV_1) to forced vital capacity ratio <0.70 and FEV_1 30–70% predicted, a history of chronic bronchitis and treatment with inhaled ICS/long-acting β-agonist/long acting muscarinic antagonist therapy for at least 2 months prior to enrolment. The study was approved by independent ethics committees for each institution. All patients provided written informed consent prior to study start. Induced sputum samples had to have a viability factor of at least 70% and epithelial cells lower than 30%. In this *post hoc* analysis, patients were stratified into two subgroups using a baseline (screening visit) sputum eosinophil threshold of 3%, *i.e.* "eosinophil^{high}" (⩾3%) and "eosinophil^{low}" (<3%). The differential treatment response in these subgroups, measured as change from baseline of % sputum eosinophils to the end of the treatment period (with the latter expressed as an average of days 20, 26 and 32 assessed at 2 h after the morning dose) was evaluated using an ANCOVA model with subgroup, subject within subgroup, period, treatment, treatment-by-subgroup interaction and baseline value as independent variables. The same model was used to evaluate a potential effect of other relevant subgroups (gender or smoking status) on sputum eosinophils per cent change from baseline. In the receiver operating characteristic (ROC) curves analysis, the sensitivity was plotted as a function of

100-specificity to test the performances of per cent and absolute blood eosinophils to predict the two groups of patients. The maximum value of the Youden-index (J = sensitivity + specificity-1) was used to identify the optimum cut-off point for the diagnostic tests.

All randomised patients (n=61; mean age 66 years; 43 males; 34 current smokers) were included in the analysis. Mean±SD post-bronchodilator predicted FEV_1 was 50.2±11.8% and COPD Assessment Test score was 20.7±5.8. The mean±SD baseline blood eosinophil count was 252±144 cells·μL^{-1} or 3.3±2.0%. Mean±SD and median (interquartile range) sputum count was 3.6±4.3% and 2% (2.9%) for eosinophils and 82.7±9.5% and 82.8% (16.9%) for neutrophils, respectively. 20 patients (33%) were eosinophil^{high}; sputum mean±SD eosinophils was 0.292±0.336×10^6 g^{-1} or 8.4±4.5%. 41 patients (67%) were eosinophil^{low}; sputum mean±SD eosinophils was 0.069±0.093×10^6 g^{-1} or 1.3±0.8%. Eosinophil^{high} in comparison to eosinophil^{low} patients were characterised by a higher proportion of males (80% *versus* 66%) and ex-smokers (70% *versus* 32%).

A significant effect of the eosinophil$^{high/low}$ groups (p<0.01) but not of gender or smoking status was observed on sputum eosinophils per cent change from baseline. In the eosinophil^{high} group, both CHF6001 doses significantly reduced the percentage of sputum eosinophils over placebo: 95% CI −3.09 to −0.58 for CHF6001 800 µg twice daily, and −2.71 to −0.25 for CHF6001 1600 µg twice daily (change from baseline estimates for placebo, CHF6001 800 µg twice daily and 1600 µg twice daily were 1.69, −0.15 and 0.21, respectively). In the eosinophil^{low} group, the effect of CHF6001 on the percentage of eosinophils was less pronounced, and hence not statistically significant: 95% CI −1.41 to 0.47 for CHF6001 800 µg twice daily, and −1.27 to 0.60 for CHF6001 1600 µg twice daily) (figure 1a).
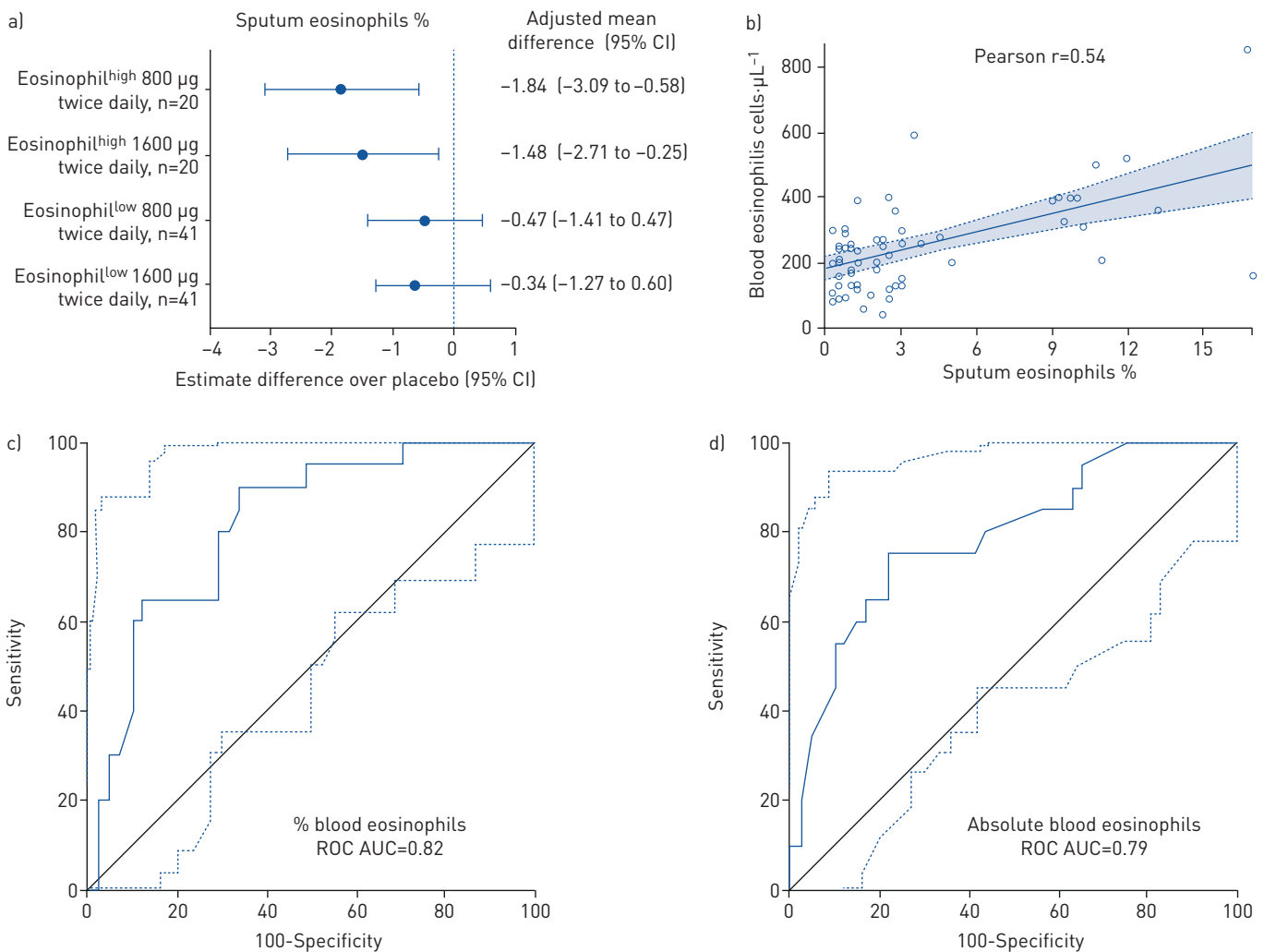

FIGURE 1 a) Effect of CHF6001 (800 ug and 1600 ug twice daily) over placebo on sputum eosinophil per cent levels in eosinophil^{high} and eosinophil^{low} patients. b) Correlation between blood absolute and percentage sputum eosinophil values with regression line and 95% confidence intervals. Receiver operating characteristic (ROC) area under the curve (AUC) analysis of sensitivity and specificity of c) per cent and d) absolute blood eosinophil count to predict eosinophil^{high} and eosinophil^{low} patients, with the corresponding 95% confidence intervals.

Eosinophil^{high} patients had higher mean±SD absolute and per cent count in blood compared to eosinophil^{low}; 350±172 cells·μL^{-1} or 4.7±2.0% *versus* 204±101 cells·μL^{-1} or 2.6±1.6% (t-test p⩽0.0001). Furthermore, there was a moderate correlation between per cent sputum and per cent blood eosinophils (Pearson r=0.46, p=0.0002) and between per cent sputum and absolute blood eosinophils (Pearson r=0.54, p<0.0001) (figure 1b). ROC curves analysis (figure 1c and d) showed that both per cent and absolute blood eosinophils are good predictors of per cent eosinophils levels in sputum. Specifically, per cent and absolute blood eosinophils were able to predict eosinophil^{high} and eosinophil^{low} patients with ROC areas under the curve (AUCs) of 0.82 and 0.79, respectively (p<0.001). For per cent blood eosinophils, a threshold of 2.8% showed a sensitivity of 90% and a specificity of 66%. For absolute blood eosinophils, the optimal threshold was identified at 257 cells·μL^{-1}, showing a sensitivity of 75% and a specificity of 78%.

This clinical trial employed highly standardised conditions of sputum collection, processing and centralised reading, leading to a median viability of 92% and very low contamination by epithelial squamous cells (median 1.3%). Additionally, all the patients had chronic bronchitis, making the acquisition of sputum samples easier. In these circumstances, we were able to demonstrate an association between blood and sputum eosinophil counts, and good predictive performance for blood eosinophil counts to predict sputum eosinophilia. Larger multicentre studies can suffer with practical difficulties in obtaining sufficient evaluable samples [9], likely due to a combination of patient factors and variations in laboratory expertise. Nevertheless, it has been reported that blood absolute and per cent eosinophil counts can identify sputum counts ⩾3% with ROC-AUCs ranging from 0.75 to 0.8 [7, 12]. In our study, thresholds in blood of 257 cells·μL^{-1} and 2.8% predicted sputum eosinophils ⩾3% in approximately 75% and 90% of patients, respectively, with diagnostic performances (ROC-AUC) of 0.79–0.82.

The ability of CHF6001 to reduce sputum eosinophil counts appears most relevant to individuals with higher eosinophils. Although this effect could be driven by the higher eosinophil levels at baseline, our data are compatible with the reduction of sputum and bronchial mucosal eosinophils by roflumilast [8]. A *post hoc* analysis showed that higher blood eosinophil counts predict a greater effect of roflumilast on exacerbations [4]. These previous results, coupled with our current data, indicate an effect of PDE4 inhibitors on eosinophilic inflammation in COPD patients. The good predictivity of blood eosinophils to identify sputum eosinophilia suggests promise for this blood biomarker as a predictive marker of response to PDE4 inhibitors in COPD patients with chronic bronchitis already being treated with triple therapy.

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