Current smoking reduces small airway eosinophil counts in COPD

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To the Editor:

Inhaled corticosteroids (ICS) prevent COPD exacerbations [1]. Randomised controlled trials (RCTs) have shown that higher blood eosinophil counts (BECs) are associated with better clinical responses to ICS treatment in COPD patients with a history of exacerbations [1]. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) report recommends using BEC to identify individuals most likely to benefit from ICS treatment [1]. Higher BECs are associated with increased type 2 pulmonary inflammation [2–4], potentially explaining the differential response to ICS.

RCT analyses have demonstrated that ICS have a greater effect on exacerbation prevention in ex- *versus* current smokers [5, 6]. A *post hoc* analysis of the SUMMIT study reported a blunted response to ICS-containing therapies with regards to improvement in forced expiratory volume in 1 s (FEV_1) and exacerbation reduction in current *versus* ex-smokers [5], with similar observations in pooled and meta-analysis of COPD RCTs [6, 7].

The small airways (<2 mm diameter) show enhanced immune cell infiltration in COPD *versus* controls [8], associated with airway remodelling causing airflow obstruction and gas trapping [9]. Few COPD studies investigating small airway inflammation have quantified eosinophil counts. Hogg *et al.* [8] reported that the proportion of small airways with positive eosinophil staining were similar in COPD *versus* controls, without quantifying the number of cells per airway. This is relevant for small airway eosinophils in COPD, which exist in discrete microenvironments characterised by type 2 inflammation [10]. Quantitative analysis reported increased small airway eosinophil counts in very severe COPD [10]. Current smoking may be an uncontrolled confounder in these studies [8, 10, 11]. We hypothesised that current smoking in COPD alters the pattern of type 2 inflammation in the small airways, thereby influencing responses to ICS. To address this hypothesis, we investigated small airway eosinophil counts in COPD patients and controls, focusing on the effects of current smoking.

Tissue blocks were obtained from 27 controls and 48 COPD patients undergoing surgical resection for suspected lung cancer, recruited from Manchester University NHS Foundation Trust Hospital, UK. Patients provided written informed consent using protocols approved by Manchester (03/SM/396) and Northwest (20/NW/0302) ethics committees. COPD patients (mean age 68.0 years; mean smoking history 53.6 pack-years) met GOLD diagnostic criteria: 54.2% were current smokers with ⩾10-pack-year history, and had an FEV_1/forced vital capacity ratio <0.7, with mean FEV_1 64.8% predicted, comprising 10, 23 and 15 GOLD stage 1, 2 and 3 patients, respectively. Controls (mean age 66.8 years; mean smoking history 40.6 pack-years) were current (n=14, 51.9%) or ex-smokers (n=13, 48.1%) with ⩾10 pack-years and no airflow obstruction. Smoking status was self-reported and ex-smoking constituted ⩾1 year(s) of cessation. No subjects had a history of asthma. Age, pack-year history and sex were not different between groups.

Eosinophils in the small airway (< 2 mm diameter) intra- and subepithelial regions were identified by Luna staining. Intra- and subepithelial cell counts were normalised to the area of epithelium or lamina propria, respectively, and quality control checked with an interuser disagreement <10%. Differences between groups were assessed using unpaired t-tests or Mann–Whitney U-tests with significance set at $p<0.05$. Multiple linear regression analyses assessed associations between intraepithelial eosinophil counts

Shareable abstract (@ERSpublications)
Current smoking reduces small airway intraepithelial eosinophil counts in COPD patients and controls. This provides evidence of an attenuation of type-2 related inflammation in the small airways imposed by current smoking, which may affect ICS response. https://bit.ly/49YSKwG

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(dependent variable) and smoking status (independent variable), whilst controlling for potentially confounding variables: FEV_1 % predicted and ICS use in COPD.

COPD patients had higher intraepithelial eosinophil counts *versus* controls (median 93.1 *versus* 7.9 cells per mm^2 respectively, p=0.01) (figure 1a). Subepithelial eosinophil counts were not significantly different between groups (30.6 *versus* 18.5 cells per mm^2 respectively, p=0.15) (figure 1b), although numerically higher in COPD patients. There were significant associations between intra- and subepithelial eosinophil counts in COPD patients (ρ=0.52, p<0.001) and controls (ρ=0.62, p<0.001).

Intraepithelial eosinophil counts were lower in current *versus* ex-smokers, both in COPD patients (median 48.0 *versus* 189.1 cells per mm^2 respectively, p=0.04) (figure 1c) and controls (median 0.0 *versus* 52.9 cells per mm^2 respectively, p=0.02) (figure 1d). Current smoking did not influence subepithelial eosinophil counts in COPD patients (median 32.8 *versus* 28.9 cells per mm^2 for current and ex-smokers respectively, p=0.96) or controls (median 12.6 *versus* 24.7 cells per mm^2 for current and ex-smokers respectively, p=0.40). Clinical characteristics were no different between ex- *versus* current-smoking COPD patients or controls.

Univariate analysis revealed no association between eosinophil counts and FEV_1 (absolute or % predicted, or GOLD stage) or ICS use in COPD. Furthermore, the relationship between intraepithelial eosinophil

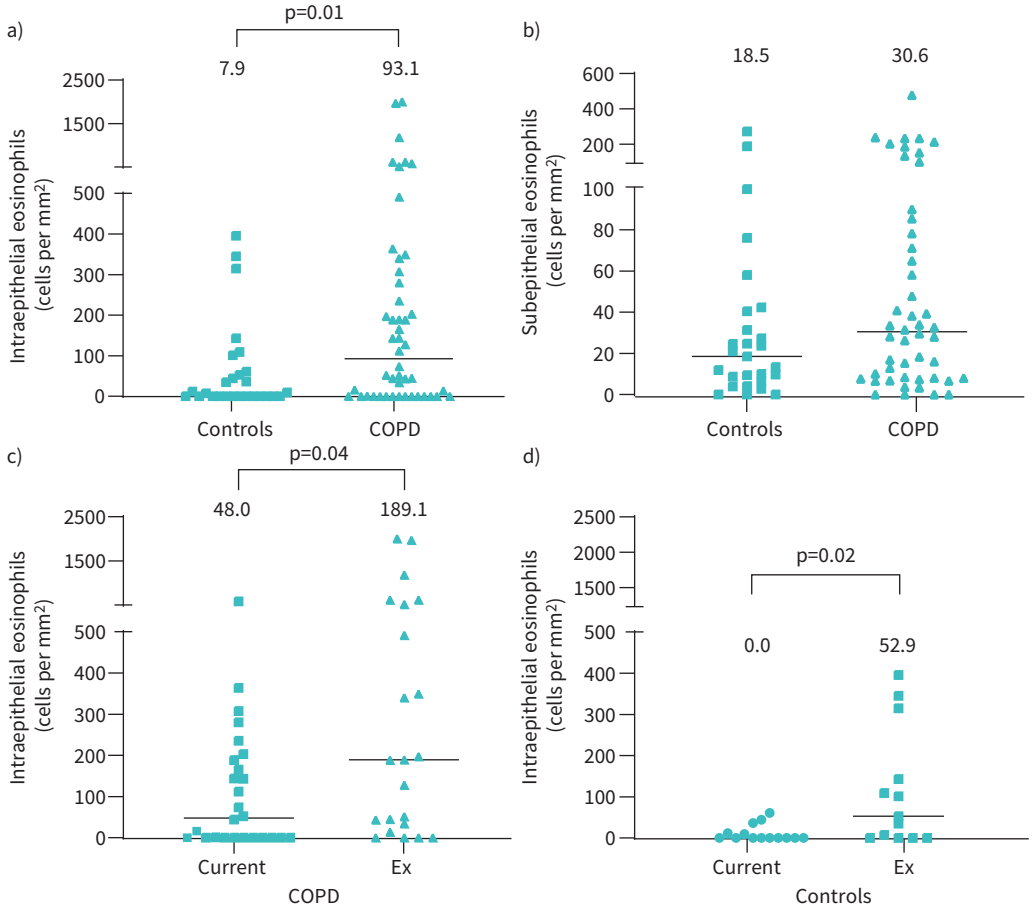

FIGURE 1 Comparison of small airway eosinophil counts between different groups defined by presence of COPD and smoking status. a) Intraepithelial eosinophil counts for COPD *versus* control, b) subepithelial eosinophil counts for COPD *versus* control (27 control subjects and 48 COPD patients), intraepithelial eosinophil counts for c) COPD current *versus* ex-smokers (n=26 and n=22, respectively), and for d) current *versus* ex-smoking controls (n=14 and n=13, respectively). Data represent individual patients or controls, black horizontal lines represent median eosinophil counts and individual datasets are labelled with median eosinophil count per mm^2.

counts and smoking status remained significant when adjusted for potential confounding factors (COPD, p=0.01; controls, p=0.02). The median within-subject difference was not statistically different between different tissue blocks, regardless of proximity to the excised tumour.

We report higher small airway intraepithelial eosinophil counts in COPD patients *versus* controls. The key novel finding was that intraepithelial eosinophil counts were lower in current smokers, observed in both COPD patients and controls.

The strengths of this study include the stratification by current smoking status, combined with the careful quantification of eosinophil numbers in both the intra- and subepithelium. Eosinophilic airway inflammation exists in a subgroup of COPD patients [1, 2]; our results suggest this is more prominent in the intraepithelial region of the small airways. We have previously demonstrated a type 2 gene expression signature, using bronchial brushings, that is associated with higher blood and bronchial tissue eosinophil counts [2]. The airway epithelium therefore appears to be an important location for the presence of type 2 inflammation in COPD.

Current smoking may reduce eosinophil numbers *per se* or alter eosinophil retention in different airway compartments. Bronchoalveolar lavage eosinophils are increased in COPD current *versus* ex-smokers, suggesting increased transit into the airway lumen, possibly due to increased activation [12, 13]. Once these signals are removed (smoking cessation), eosinophils may resume tissue homeostatic functions with longer residency in the epithelium. Limitations of this analysis include: smoking status was self-reported and we were unable to analyse confounding clinical factors due to limited information available. Matched BECs were not available for these samples. However, the relationship between BECs and small airway eosinophil counts has been recently reported, showing a positive correlation [14]. Reassuringly, eosinophil counts were repeatable when assessed from multiple tissue blocks, regardless of proximity to the excised tumour.

The present analysis shows that current smoking attenuates eosinophil counts in the small airway epithelium, which may contribute to the blunted clinical response to ICS in current smokers [5, 6].

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Conflict of interest: A. Higham has received personal fees from Chiesi. D. Singh has received personal fees from Aerogen, AstraZeneca, Boehringer Ingelheim, Chiesi, Cipla, CSL Behring, Epidendo, Genentech, GlaxoSmithKline, Glenmark, Gossamerbio, Kinaset, Menarini, Novartis, Orion, Pulmatrix, Sanofi, Synairgen, Teva, Theravance and Verona. A. Beech and S. Booth have no conflicts of interest to declare.

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