Exhaled nitric oxide, eosinophils and current smoking in COPD patients

Received: 3 July 2023
Accepted: 25 Sept 2023

To the Editor:

There is increasing interest in type 2 (T2) inflammation in COPD [1]. COPD patients with higher blood eosinophil counts (BECs) have a greater T2 profile, including interleukin (IL)-13 expression, in bronchial brush and sputum samples [2–4]. IL-13 upregulates inducible nitric oxide synthase, increasing fractional exhaled nitric oxide (F_{ENO}) levels.

Current smoking suppresses F_{ENO} levels [5], reducing the potential utility of this biomarker in unselected COPD populations. Nevertheless, there is an association between sputum eosinophil counts and F_{ENO} levels in COPD patients [6, 7], although there are inconsistent results, probably due to small sample sizes and the effects of current smoking [8].

While F_{ENO} is a promising biomarker of T2 inflammation in COPD patients, at an individual level, the following questions remain in COPD: 1) whether high F_{ENO} levels can occur without high eosinophil counts; 2) whether high F_{ENO} levels are observed in a proportion of current smokers. To answer these questions, we stratified F_{ENO} levels by current smoking status and subsequently examined the relationship with BEC and sputum eosinophils.

140 COPD patients with paired F_{ENO} measurements and BEC who attended our research centre between 2012 and 2023 were analysed retrospectively. All subjects provided written informed consent using protocols approved by the local research ethics committees: South Manchester 06/Q1403/156 and North West 16/NW/0836. Patients were categorised into three groups: BEC^{low} (<100 eosinophils·μL^{-1}, median 70 eosinophils·μL^{-1}; n=19), BEC^{mid} (100–300 eosinophils·μL^{-1}; n=80; median count =195) and BEC^{high} (>300 eosinophils·μL^{-1} median 420 eosinophils·μL^{-1}; n=41; p<0.001). F_{ENO} measurements were performed in accordance with European Respiratory Society guidelines [9]. $F_{ENO}{}^{high}$ patients were classed as >20 ppb, which identifies individuals at higher exacerbation risk [10]. The groups were well matched for clinical characteristics (mean age 64 years, mean forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio 0.5 and mean FEV_1 65% predicted, with 78% inhaled corticosteroid (ICS) users), apart from fewer females in the BEC^{high} (20%) *versus* BEC^{mid} and BEC^{low} groups (both 42%) (p=0.047). The number of atopic individuals (positive skin prick to cat dander, grass pollen or dust mite) trended higher in BEC^{high} compared to BEC^{mid} and BEC^{low} groups (30% *versus* 17% and 0%, respectively; p=0.07). Approximately half were current smokers (mean 40 pack-years). Ex-smokers were defined as >12 months smoking cessation. No subjects had a history of asthma. Patients receiving oral corticosteroids or antibiotics within 6 weeks prior to sampling were excluded.

71 patients had induced sputum cell count data available, obtained by saline nebulisation and processing [11]. Sputum eosinophil percentages were significantly higher in the BEC^{high} group (median counts: BEC^{low} 0.5%, BEC^{mid} 1% and BEC^{high} 5%; p<0.001).

In the entire COPD cohort (n=140), F_{ENO} levels were significantly higher in ex-smokers (n=75) compared to current smokers (n=65) (p<0.0001; figure 1). F_{ENO} levels were significantly higher in ex-smokers *versus* current smokers in the BEC^{mid} and BEC^{high} groups (p<0.0001 and p=0.004, respectively; figure 1), with no difference in the BEC^{low} group (p=0.3). The proportion of $F_{ENO}{}^{high}$ in ex-smokers was 30%, 45% and 48% in the BEC^{low}, BEC^{mid} and BEC^{high} groups, respectively, with no significant difference in F_{ENO}

Shareable abstract (@ERSpublications)
High F_{ENO} can occur despite low blood eosinophil counts in ex-smokers, while a minority of current smokers have elevated F_{ENO} that is not related to eosinophil counts. F_{ENO} levels may be related to noneosinophilic mechanisms in a subgroup of COPD. https://bit.ly/3PSWvM2

Cite this article as: Higham A, Beech A, Dean J, *et al*. Exhaled nitric oxide, eosinophils and current smoking in COPD patients. *ERJ Open Res* 2023; 9: 00686-2023 [DOI: 10.1183/23120541.00686-2023].

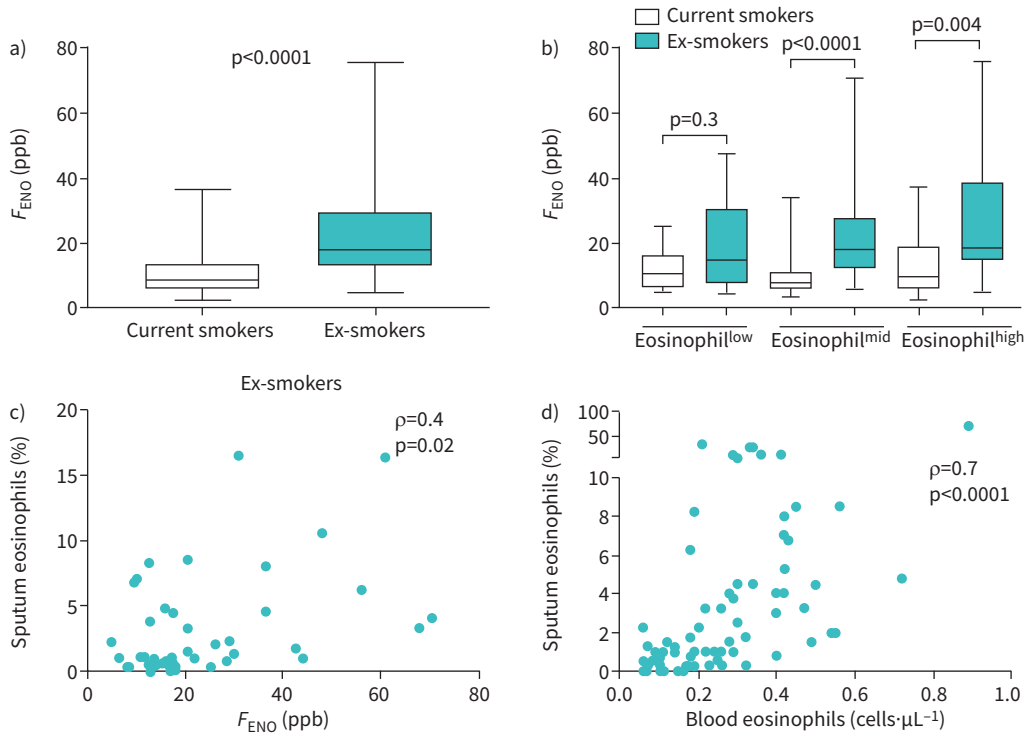

FIGURE 1 Exhaled nitric oxide, eosinophils and current smoking in COPD patients. The effect of smoking status on fractional exhaled nitric oxide (F_{ENO}) levels was compared in a) COPD patients unselected for blood eosinophil counts and b) COPD patients separated according to blood eosinophil counts. Data are presented as median (interquartile range). c) The relationship between F_{ENO} and sputum eosinophil percentage was investigated in ex-smokers. d) The relationship between sputum eosinophil percentage and blood eosinophil counts was investigated in the whole cohort.

levels between these ex-smoker subgroups (Kruskal–Wallis p=0.4; figure 1). The proportion of F_{ENO}^{high} in the BEC^{low}, BEC^{mid} and BEC^{high} current smokers was 11%, 6% and 25%, respectively (overall 12%) with no difference between groups (Kruskal–Wallis p=0.2; figure 1).

In both current and ex-smokers, there were no significant correlations (p>0.05) between BEC and F_{ENO} levels (data not shown). There was a significant correlation between sputum eosinophil percentage and F_{ENO} levels in ex-smokers (ρ=0.4, p=0.02; figure 1), but not current smokers (ρ=0.3, p=0.2; data not shown). Using the Youden index ((sensitivity+specificity) – 1) generated from received operating characteristic curves from the ex-smoker group (area under the curve 0.7, p=0.04), the optimal F_{ENO} cut-off value that predicts >3% sputum eosinophils was >30.5 ppb. Applying this value, the proportion of ex-smokers with F_{ENO} levels >30.5 ppb was 20%, 16% and 33% in the BEC^{low}, BEC^{mid} and BEC^{high} groups, respectively.

There was a significant correlation between blood and sputum eosinophils in the whole cohort (ρ=0.7, p<0.0001; figure 1) and when separated into current (ρ=0.6, p=0.0002) and ex-smokers (ρ=0.5, p=0.0002). Blood and sputum eosinophil counts were not different between current and ex-smokers in any of the groups.

In the entire cohort, F_{ENO} levels were not different in ICS users *versus* non-ICS users (p=0.5) and when patients were separated into BEC^{low}, BEC^{mid} and BEC^{high} groups (p>0.05 for all analyses). We restricted this analysis to ex-smokers and found no differences in F_{ENO} levels between ICS users and non-ICS users (p=0.2). F_{ENO} levels were similar in non-exacerbators *versus* exacerbators (at least one exacerbation in previous year) in the whole cohort and when separated into current and ex-smokers (p>0.05).

In the BEC^{high} group, there was no difference in BEC (p=0.2) and a nonsignificant increase in F_{ENO} levels in atopic *versus* nonatopic patients (median 17.6 *versus* 13.5 ppb, p=0.07).

On a group level, our integrated analysis confirms that increased F_{ENO} levels are associated with elevated pulmonary eosinophilic inflammation in COPD ex-smokers. However, F_{ENO}^{high} occurred in some patients with low BEC. While we observed a strong correlation between BEC and sputum eosinophils, the relationship was imperfect, with discordant blood and sputum eosinophil results observed [1]. This discordance may be due to BEC variability, and can explain why 30% of BEC^{low} ex-smokers were F_{ENO}^{high}, as some of these individuals may have discordant high sputum eosinophil counts. Alternatively, high F_{ENO} levels may occur without high levels of eosinophils, such as through IL-13 driven pathophysiology, which can also cause goblet cell remodelling [12].

Despite suppression of F_{ENO} by smoking, some COPD current smokers were F_{ENO}^{high}, and this was greatest in the BEC^{high} group (25%). We speculate that these individuals have a higher level of T2 inflammation, enriched by high BEC, and F_{ENO} levels remain elevated despite smoking. However, the lack of a difference in F_{ENO} levels between current smokers in different BEC groups, and the lack of a relationship between sputum eosinophils and F_{ENO} in current smokers, argues in favour of a non-eosinophil-related mechanism being responsible for increased F_{ENO} in these current smokers, such as IL-13-driven pathways.

We did not observe a difference between ICS users and nonusers, including a subgroup analysis in ex-smokers. The magnitude of suppression in previous studies is often small [13], and our current study was not able to demonstrate this small effect. Alternatively, we cannot discount poor adherence or poor inhaler technique as an explanation. The retrospective study design prevents this analysis.

Our findings suggest that atopy may influence F_{ENO} levels to some degree in our patients, but this requires confirmation in larger datasets.

The recent BOREAS study in COPD patients with a history of exacerbations plus BEC >300 cells·μL^{-1} reported that dupilumab reduced exacerbation rates by 30%, with improvements observed for lung function and quality of life [14]. In the F_{ENO}^{high} subgroup, there appeared to be a greater benefit for lung function improvement. These results suggest potential for F_{ENO} as a predictive biomarker of treatment response with anti-IL-13 biologicals in COPD. While the BOREAS study focused on BEC^{high} COPD patients, we now show that BEC^{low} patients may be F_{ENO}^{high}. The use of F_{ENO} may have applications in identifying individuals with T2 inflammation who are more likely to respond to targeted therapies.

Andrew Higham [1], Augusta Beech [1,2], James Dean [1,2] and Dave Singh[1,2]

[1]Division of Immunology, Immunity to Infection and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester and Manchester University NHS Foundation Trust, Manchester, UK. [2]Medicines Evaluation Unit, The Langley Building, Manchester, UK.

Corresponding author: Andrew Higham (andrew.higham@manchester.ac.uk)

Provenance: Submitted article, peer reviewed.

Acknowledgements: A. Beech and D. Singh are supported by the by the National Institute for Health Research (NIHR) Manchester Biomedical Research Centre (BRC). The authors acknowledge the North West Lung Centre Charity for supporting this project. In addition, we thank the study participants for their contribution. This report is independent research and the views expressed in this publication are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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 J. Dean have no conflicts of interest to declare.

Ethics statement: This study was conducted in accordance with the amended Declaration of Helsinki. Sample collection was approved by the local research ethics committees (REC): South Manchester REC 06/Q1403/156 and NRES Committee North West Preston 16/NW/0836. All subjects provided written informed consent.

References

1 Singh D, Agusti A, Martinez FJ, *et al.* Blood eosinophils and chronic obstructive pulmonary disease: a Global Initiative for Chronic Obstructive Lung Disease science committee 2022 review. *Am J Respir Crit Care Med* 2022; 206: 17–24.

2 Higham A, Beech A, Wolosianka S, *et al.* Type 2 inflammation in eosinophilic chronic obstructive pulmonary disease. *Allergy* 2021; 76: 1861–1864.

3 Higham A, Dungwa J, Pham TH, *et al.* Increased mast cell activation in eosinophilic chronic obstructive pulmonary disease. *Clin Transl Immunology* 2022; 11: e1417.

4 George L, Taylor AR, Esteve-Codina A, *et al.* Blood eosinophil count and airway epithelial transcriptome relationships in COPD *versus* asthma. *Allergy* 2020; 75: 370–380.

5 Silkoff PE, Singh D, FitzGerald JM, *et al.* Inhaled steroids and active smoking drive chronic obstructive pulmonary disease symptoms and biomarkers to a greater degree than airflow limitation. *Biomark Insights* 2017; 12: 1177271917730306.

6 Chou KT, Su KC, Huang SF, *et al.* Exhaled nitric oxide predicts eosinophilic airway inflammation in COPD. *Lung* 2014; 192: 499–504.

7 Vincken S, Sylvia V, Daniel S, *et al.* The role of F_{ENO} in stable COPD patients with eosinophilic airway inflammation. *Respir Med* 2021; 181: 106377.

8 Antus B, Paska C, Barta I. Predictive value of exhaled nitric oxide and blood eosinophil count in the assessment of airway eosinophilia in COPD. *Int J Chron Obstruct Pulmon Dis* 2020; 15: 2025–2035.

9 Horváth I, Barnes PJ, Loukides S, *et al.* A European Respiratory Society technical standard: exhaled biomarkers in lung disease. *Eur Respir J* 2017; 49: 1600965.

10 Alcázar-Navarrete B, Ruiz Rodriguez O, Conde Baena P, *et al.* Persistently elevated exhaled nitric oxide fraction is associated with increased risk of exacerbation in COPD. *Eur Respir J* 2018; 51: 701457.

11 Higham A, Cadden P, Southworth T, *et al.* Leukotriene B4 levels in sputum from asthma patients. *ERJ Open Res* 2016; 2: 00088-2015.

12 Atherton HC, Jones G, Danahay H. IL-13-induced changes in the goblet cell density of human bronchial epithelial cell cultures: MAP kinase and phosphatidylinositol 3-kinase regulation. *Am J Physiol Lung Cell Mol Physiol* 2003; 285: L730–L739.

13 Lim CS, Rani FA, Tan LE. Response of exhaled nitric oxide to inhaled corticosteroids in patients with stable COPD: a systematic review and meta-analysis. *Clin Respir J* 2018; 12: 218–226.

14 Bhatt SP, Rabe KF, Hanania NA, *et al.* Dupilumab for COPD with type 2 inflammation indicated by eosinophil counts. *N Engl J Med* 2023; 389: 205–214.