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To the editor

Chronic obstructive pulmonary disease (COPD) is caused by the inhalation of noxious particles, commonly from cigarette smoking [1]. Smoking cessation is an important part of COPD management. Electronic cigarettes (e-cigs) are used by COPD patients to facilitate cigarette smoking cessation [2]. E-cigs contain multiple chemicals that can cause inflammation and toxicity [3]. The potential harm caused by long term e-cig use in COPD patients is unknown.

A longitudinal study reported that inflammatory cell infiltration persisted in bronchial tissue after 1 year of cigarette smoking cessation in COPD patients, while sputum neutrophil and lymphocyte counts increased [4]. In contrast, healthy smokers showed either no change or a decrease in airway inflammation [4]. Cross-sectional analysis has also shown that COPD ex-smokers have increased sputum and bronchoalveolar lavage neutrophil counts compared to current smokers, although this has not been a consistent finding across studies [5, 6]. The mechanisms responsible for an apparent increase in airway inflammation in COPD patients who stop smoking remains unclear, particularly in light of the clinical benefits of smoking cessation including reduced rate of disease progression [7].

The effect of e-cig use on airway inflammation in COPD ex-cigarette smokers (COPDE) has not been documented. The aim of this analysis was to compare airway inflammatory cell counts in COPD current smokers (COPDS;n=72), COPDE (n=133), and COPD ex-smokers who use e-cigs (COPDE+e-cig;n=23).

This retrospective analysis used sputum cell count data collected from subjects participating in research at our centre between 2014 – 2020. All subjects provided an acceptable sputum sample (>50% leukocyte viability, <30% non-squamous cells) at stable visits, with no exacerbation samples included. The study was conducted in accordance with the Declaration of Helsinki of 1975 and was approved by the local ethics committee (NRES Committee North West; ref codes 05/Q1402/41; 10/H1016/25; 10/H1003/108; 16/NW/0836). All subjects provided written informed consent. Sputum differential cell counts (DCC) were produced from high quality sputum preparations and counts were quality control checked with an inter-user agreement of <10%. For the vast majority of patients, induced sputum was collected following saline nebulisation and processed as previously described [8]. Briefly, sputum plugs were processed using 0.2% Dithiothreitol (DTT). DTT supernatants were removed and the cell

pellet was re-suspended in phosphate buffered saline. Cytospins were prepared before being air-dried, fixed in methanol, and stained with RapiDiff (Triangle, Skelmersdale, UK) for DCC. Some patients included in this study have been used in previous publications [9, 10].

The clinical characteristics of the study population are presented in table 1. The groups were matched for gender, lung function, symptoms, exacerbation rates and prevalence of chronic bronchitis. COPDE were slightly older and COPDE+e-cig had a lower BMI. COPDS had a greater pack year history.

The percentage of sputum neutrophils were significantly higher in COPDE and COPDE+e-cig (medians 83% and 91% respectively) compared to COPDS median 72%; p=0.003 and p<0.0001 respectively), and numerically higher in COPDE+e-cig compared to COPDE, almost reaching significance (p=0.058). There were similar findings for sputum neutrophil cell count, with significantly lower cell counts in COPDS compared to COPDE and COPDE+e-cig (p=0.0002 and p=0.0004 respectively), and numerically higher in COPDE+e-cig compared to COPDE, but not significant (p=0.37). The percentage of macrophages were significantly lower in COPDE and COPDE+e-cig compared to COPDS (p<0.0001) and numerically lower in COPDE+e-cig compared to COPDE (p=0.057). The percentage of eosinophils were lower in COPDE and COPDE+e-cig compared to COPDS, reaching significance for COPDE+e-cig (p=0.1 and p=0.04 respectively). However, absolute eosinophil numbers were similar between groups, suggesting the higher neutrophil numbers in COPDE and COPDE+ecig affected the percentage calculation of sputum eosinophils, rather than an increase in eosinophil numbers in COPDS per se.

Increased sputum neutrophil counts were observed in COPDE versus COPDS, providing confirmation of previous reports of the effects of current smoking on airway inflammation in COPD patients [4, 5]. Our data also support previous findings that even following smoking cessation, small airway inflammation persists [11]. Importantly, e-cig use in COPD ex-smokers appeared to further increase neutrophil percentage counts, almost reaching significance compared to COPDE (p=0.058). These results suggest that the use of e-cigs in COPDE may alter the profile of airway inflammation.

Lung neutrophil numbers are increased by long term cigarette smoking in healthy subjects, and further increased by the development of COPD in susceptible smokers [12, 13]. At first

glance, it may seem somewhat surprising to observe lower neutrophil numbers in COPDS versus COPDE. However, in vitro studies have shown that acute cigarette smoke exposure induces neutrophil cell death and increases efferocytosis of neutrophils [14, 15]. We propose that (1) COPD patients have chronic lung neutrophilia, due to upregulated chemotaxis mechanisms in response to inhaled toxins, (2) additionally, the acute toxic effects of cigarette smoke may influence lung neutrophil numbers by causing cell death, possibly by increased formation of neutrophil extracellular traps (NETosis) in response to nicotine exposure [16]. This can explain how smoking cessation increases sputum neutrophil counts, observed here and previously [4, 5]. Alternative explanations may involve the immunosuppressant effects of nicotine which inhibit the oxidative burst of neutrophils and cytokine production from monocytes, by activating the nicotinic acetylcholine receptor alpha7 [17, 18].

The levels of neutrophil derived proteins including neutrophil elastase, matrix metalloproteinase-9 and myeloperoxidase are increased in e-cig users compared to never smokers [19, 20] and e-cig vapour extract causes neutrophil activation in vitro, without affecting neutrophil viability [3]. E-cig use is therefore associated with a neutrophilic inflammatory response in the lungs, without causing neutrophil cell death, unlike cigarette smoking [14, 15]. This is likely due to the differences in the chemical composition of the two products. In COPD ex-smokers, e-cig use may therefore further enhance neutrophil mediated inflammation due to continued exposure to the harmful chemicals in e-cig vapour extract [3]. Future studies should examine the levels of neutrophil derived proteins and inflammatory markers in these patients.

We observed some differences in the clinical characteristics in the study groups. However, these differences were not consistent between groups and are unlikely to account for the differences in the sputum differential cell counts observed. The number of participants in each study group were not equally distributed, as COPD patients who are e-cig users are a minority of the COPD population. Nevertheless, our results are consistent with previous studies comparing sputum cell counts in COPD current vs ex-smokers. Further studies are required to replicate our findings in respect to the effects of e-cig use in COPD ex-smokers. The cross-sectional design of this study has limitations, as a longitudinal design allows within individual changes due to smoking cessation or e-cig use to be monitored. Such longitudinal

studies are scarce in the literature, due to the practical difficulties of setting up cohorts for long term follow up.

This analysis has two major findings. Firstly, our results suggest that e-cig use in COPD ex-smokers causes increased sputum neutrophil percentages. Second, current cigarette smoking in COPD patients lowers sputum neutrophil counts. The former finding raises concerns over the impact of long term e-cig use on airway inflammation in COPD patients.

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Table 1. Clinical characteristics of the sputum study population

	COPDS	COPDE	COPDE+e-cig	ANOVA / p-test
n	72	133	23	n/a
Age (Years)	64 (8)***	69 (6)	65 (7)**	<0.0001
Gender: %male	60	68	65	0.5
Pack years	51 (19) ***	38 (20)	46 (13)	<0.0001
Years since stopped smoking	n/a	14 [1-65]	3 [1-9]	<0.0001
Years using e-cigs	n/a	n/a	3 [1-6]	
BMI (kg/m^2)	28 (5)¶	29 (5) ¶¶¶	25 (4)	0.001
Exacerbation rate (1 year period)	1 [0-6]	1 [0-6]	0 [0-2]	0.2
FEV_1 (L)	1.6 (0.5)	1.6 (0.5)	1.6 (0.5)	1.0
FEV_1 % predicted	61 (15)	63 (16)	58 (15)	0.3
FEV_1/FVC ratio	52 (11)	48 (11)	48 (13)	0.08
GOLD category (%)				
1	7.0	15.8	0	
2	69.4	58.6	74.0	0.5
3	22.2	24.1	21.7	
4	1.4	1.5	4.3	
CAT	19 (8)	17 (9)	18 (5)	0.3
mMRC	2 [0-4]	2 [0-12]	2 [0-4]	0.5
SGRQ (total)	49 [8-80]	39 [2-85]	40 [12-74]	0.2
Chronic bronchitis (%)	83	69	76	0.09
ICS users (%)	64	77	78	0.05
LAMA users (%)	88	78	78	0.3
LABA users (%)	69	77	91	0.09
No maintenance inhaled medication (n)	0	0	1	n/a
Sputum Characteristics				
Induced/spontaneous	69/3	124/9	21/2	
Neutrophil (%)	72 [7-97]**¶¶¶	83 [11-99]	91 [30-99]	<0.0001
Macrophage (%)	23 [1-67]***¶¶¶	10 [1-78]	5 [1-52]	<0.0001
Eosinophil (%)	1.5 [0-18]¶	0.8 [0-29]	0.5 [0-62]	0.03
Lymphocyte (%)	0.3 [0-1.5]	0.3 [0-2.8]	0.3 [0-1.5]	0.2
Epithelial cells (%)	2.3 [0-24.3]¶¶	1.8 [0-43.3]¶	0.8 [0-17.0]	0.004
Total cell count (x10^6/g)	5.5 [0.6-56.0]***¶¶¶	11.2 [0.6-116.0]	19.2 [0.8-57.7]	<0.0001
Neutrophil (x10^6/g)	3.9 [0.1-48.9]***¶¶¶	7.6 [0.2-112.5]	16.7 [0.2-57]	<0.0001
Macrophage (x10^6/g)	1.2 [0.04-6.8]¶	1.1 [0.1-25.8]	0.7 [0.1-3.6]	0.05
Eosinophil (x10^6/g)	0.08 [0-2.5]	0.1 [0-3.1]	0.1 [0-3.6]	0.8
Lymphocyte (x10^6/g)	0 [0-0.4]	0.02 [0-0.4]	0.01 [0-0.6]	0.06
Epithelial cells (x10^6/g)	0.13 [0-2.8]*	0.22 [0-3.9]	0.14 [0-0.6]	0.005

BMI, body mass index; CAT, COPD assessment test; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroids; LABA, long acting beta agonist; LAMA, long acting muscarinic antagonist; mMRC, modified medical research council questionnaire; SGRQ, St George's

respiratory questionnaire. Data presented as mean ± (standard deviation) or median ± [range]. * vs COPDE; ¶ vs COPDE+e-cig. One symbol = $p<0.05$, two symbols = $p<0.01$ and three symbols = $p<0.001$.

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