Sputum cell counts in COPD patients who use electronic cigarettes

Copyright ©The authors 2022. For reproduction rights and permissions contact permissions@ersnet.org

Received: 25 Nov 2021
Accepted: 7 Feb 2022

To the Editor:

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-cigarettes) are used by COPD patients to facilitate cigarette smoking cessation [2]. E-cigarettes contain multiple chemicals that can cause inflammation and toxicity [3]. The potential harm caused by long term e-cigarette 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-cigarette 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-cigarettes (COPDE+e-cig; n=23).

This retrospective analysis used sputum cell count data collected from subjects participating in research at our centre between 2014 and 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; reference codes 05/Q1402/41, 10/H1016/25, 10/H1003/108 and 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 PBS. 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 body mass index. COPDS had a greater pack-year smoking history.

The percentage of sputum neutrophils was 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 significantly (p=0.37). The percentage of macrophages

Shareable abstract (@ERSpublications)
Electronic cigarette use in COPD ex-smokers causes increased sputum neutrophils
https://bit.ly/3HPIisR

Cite this article as: Higham A, Beech A, Jackson N, *et al*. Sputum cell counts in COPD patients who use electronic cigarettes. *Eur Respir J* 2022; 59: 2103016 [DOI: 10.1183/13993003.03016-2021].

TABLE 1 Clinical characteristics of the sputum study population

	COPDS	COPDE	COPDE+e-cig	ANOVA p-value
Subjects (n)	72	133	23	NA
Age (years)	64±8***	69±6	65±7**	<0.0001
Males (%)	60	68	65	0.5
Smoking history (pack-years)	51±19***	38±20	46±13	<0.0001
Years since stopped smoking	NA	14 (1–65)	3 (1–9)	<0.0001
Years using e-cigarettes	NA	NA	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 (%)				0.5
1	7.0	15.8	0	
2	69.4	58.6	74.0	
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	NA
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 (×10^6 per g)	5.5 (0.6–56.0)***,¶¶¶	11.2 (0.6–116.0)	19.2 (0.8–57.7)	<0.0001
Neutrophil (×10^6 per g)	3.9 (0.1–48.9)***,¶¶¶	7.6 (0.2–112.5)	16.7 (0.2–57)	<0.0001
Macrophage (×10^6 per g)	1.2 (0.04–6.8)¶	1.1 (0.1–25.8)	0.7 (0.1–3.6)	0.05
Eosinophil (×10^6 per g)	0.08 (0–2.5)	0.1 (0–3.1)	0.1 (0–3.6)	0.8
Lymphocyte (×10^6 per g)	0 (0–0.4)	0.02 (0–0.4)	0.01 (0–0.6)	0.06
Epithelial cells (×10^6 per g)	0.13 (0–2.8)*	0.22 (0–3.9)	0.14 (0–0.6)	0.005

Data are presented as mean±SD or median (range), unless otherwise stated. COPDS: COPD current smokers; COPDE: COPD ex-cigarette smokers; COPDE+e-cig: COPD ex-smokers who use e-cigarettes; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAT: COPD Assessment Test; mMRC: modified Medical Research Council questionnaire; SGRQ: St George's Respiratory Questionnaire; ICS: inhaled corticosteroids; LAMA: long-acting muscarinic antagonist; LABA: long-acting β-agonist; NA: not applicable. *: p<0.05, **: p<0.01, ***: p<0.001 *versus* COPDE; ¶: p<0.05, ¶¶: p<0.01, ¶¶¶: p<0.001 *versus* COPDE+e-cig.

was 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 was 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-cigarette 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-cigarettes 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, and 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 alpha 7 [17, 18].

The levels of neutrophil-derived proteins, including neutrophil elastase, matrix metalloproteinase-9 and myeloperoxidase, are increased in e-cigarette users compared to never smokers [19, 20] and e-cigarette vapour extract causes neutrophil activation *in vitro*, without affecting neutrophil viability [3]. E-cigarette 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-cigarette use may therefore further enhance neutrophil-mediated inflammation due to continued exposure to the harmful chemicals in e-cigarette 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 numbers of participants in each study group were not equally distributed, as COPD patients who are e-cigarette users are a minority of the COPD population. Nevertheless, our results are consistent with previous studies comparing sputum cell counts in COPD current *versus* ex-smokers. Further studies are required to replicate our findings in respect to the effects of e-cigarette 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-cigarette 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-cigarette 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-cigarette use on airway inflammation in COPD patients.

Andrew Higham [1], Augusta Beech [1], Natalie Jackson[2], Simon Lea [1] 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, Manchester University NHS Foundation Trust, Manchester, UK.

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

Acknowledgements: A. Beech and D. Singh are supported by the National Institute for Health Research (NIHR) Manchester Biomedical Research Centre (BRC). This research was supported by the North West Lung Centre Charity, Manchester. 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, A. Beech, N. Jackson and S. Lea declare no competing interests. D. Singh declares consulting fees from Aerogen, AstraZeneca, Boehringer Ingelheim, Chiesi, Cipla, CSL Behring, Epiendo, Genentech, GlaxoSmithKline, Glenmark, Gossamerbio, Kinaset, Menarini, Novartis, Pulmatrix, Sanofi, Teva, Theravance and Verona, in the 36 months prior to manuscript submission.

References

1 Vogelmeier CF, Criner GJ, Martinez FJ, *et al.* Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *Eur Respir J* 2017; 49: 1700214.

2 Action on Smoking and Health. Use of e-cigarettes (vapes) among adults in Great Britain. 2021. https://ash.org.uk/wp-content/uploads/2021/06/Use-of-e-cigarettes-vapes-among-adults-in-Great-Britain-2021.pdf

3 Higham A, Rattray NJ, Dewhurst JA, *et al.* Electronic cigarette exposure triggers neutrophil inflammatory responses. *Respir Res* 2016; 17: 56.

4 Willemse BW, ten Hacken NH, Rutgers B, *et al.* Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. *Eur Respir J* 2005; 26: 835–845.

5 Wen Y, Reid DW, Zhang D, *et al.* Assessment of airway inflammation using sputum, BAL, and endobronchial biopsies in current and ex-smokers with established COPD. *Int J Chron Obstruct Pulmon Dis* 2010; 5: 327–334.

6 Babusyte A, Stravinskaite K, Jeroch J, *et al.* Patterns of airway inflammation and MMP-12 expression in smokers and ex-smokers with COPD. *Respir Res* 2007; 8: 81.

7 Scanlon PD, Connett JE, Waller LA, *et al.* Smoking cessation and lung function in mild-to-moderate chronic obstructive pulmonary disease. The Lung Health Study. *Am J Respir Crit Care Med* 2000; 161: 381–390.

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

9 Beech A, Lea S, Li J, *et al.* Airway bacteria quantification using polymerase chain reaction combined with neutrophil and eosinophil counts identifies distinct COPD endotypes. *Biomedicines* 2021; 9: 1337.

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

11 Hogg JC, Chu F, Utokaparch S, *et al.* The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004; 350: 2645–2653.

12 Pesci A, Majori M, Cuomo A, *et al.* Neutrophils infiltrating bronchial epithelium in chronic obstructive pulmonary disease. *Respir Med* 1998; 92: 863–870.

13 Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis* 1983; 128: 833–838.

14 Guzik K, Skret J, Smagur J, *et al.* Cigarette smoke-exposed neutrophils die unconventionally but are rapidly phagocytosed by macrophages. *Cell Death Dis* 2011; 2: e131.

15 Heijink IH, Pouwels SD, Leijendekker C, *et al.* Cigarette smoke-induced damage-associated molecular pattern release from necrotic neutrophils triggers proinflammatory mediator release. *Am J Respir Cell Mol Biol* 2015; 52: 554–562.

16 Lee J, Luria A, Rhodes C, *et al.* Nicotine drives neutrophil extracellular traps formation and accelerates collagen-induced arthritis. *Rheumatology (Oxford)* 2017; 56: 644–653.

17 Xu M, Scott JE, Liu KZ, *et al.* The influence of nicotine on granulocytic differentiation - inhibition of the oxidative burst and bacterial killing and increased matrix metalloproteinase-9 release. *BMC Cell Biol* 2008; 9: 19.

18 Yoshikawa H, Kurokawa M, Ozaki N, *et al.* Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-kappaB phosphorylation and nuclear factor-kappaB transcriptional activity through nicotinic acetylcholine receptor alpha7. *Clin Exp Immunol* 2006; 146: 116–123.

19 Ghosh A, Coakley RD, Ghio AJ, *et al.* Chronic E-cigarette use increases neutrophil elastase and matrix metalloprotease levels in the lung. *Am J Respir Crit Care Med* 2019; 200: 1392–1401.

20 Reidel B, Radicioni G, Clapp PW, *et al.* E-cigarette use causes a unique innate immune response in the lung, involving increased neutrophilic activation and altered mucin secretion. *Am J Respir Crit Care Med* 2018; 197: 492–501.