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1 **Sputum cell counts in COPD patients who use electronic cigarettes**

2

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1 *To the editor*

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

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

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

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

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

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

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

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

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

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

12

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

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

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

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

7

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13 the Department of Health.

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1

2 **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 <sup>2</sup> )	28 (5) <sup>¶</sup>	29 (5) <sup>¶¶¶</sup>	25 (4)	0.001
Exacerbation rate (1 year period)	1 [0-6]	1 [0-6]	0 [0-2]	0.2
FEV <sub>1</sub> (L)	1.6 (0.5)	1.6 (0.5)	1.6 (0.5)	1.0
FEV <sub>1</sub> % predicted	61 (15)	63 (16)	58 (15)	0.3
FEV <sub>1</sub> /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
<b>Sputum Characteristics</b>				
Induced/spontaneous	69/3	124/9	21/2	
Neutrophil (%)	72 [7-97]** <sup>¶¶¶</sup>	83 [11-99]	91 [30-99]	<0.0001
Macrophage (%)	23 [1-67]** <sup>¶¶¶</sup>	10 [1-78]	5 [1-52]	<0.0001
Eosinophil (%)	1.5 [0-18] <sup>¶</sup>	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] <sup>¶¶</sup>	1.8 [0-43.3] <sup>¶</sup>	0.8 [0-17.0]	0.004
Total cell count (x10 <sup>6</sup> /g)	5.5 [0.6-56.0]** <sup>¶¶¶</sup>	11.2 [0.6-116.0]	19.2 [0.8-57.7]	<0.0001
Neutrophil (x10 <sup>6</sup> /g)	3.9 [0.1-48.9]** <sup>¶¶¶</sup>	7.6 [0.2-112.5]	16.7 [0.2-57]	<0.0001
Macrophage (x10 <sup>6</sup> /g)	1.2 [0.04-6.8] <sup>¶</sup>	1.1 [0.1-25.8]	0.7 [0.1-3.6]	0.05
Eosinophil (x10 <sup>6</sup> /g)	0.08 [0-2.5]	0.1 [0-3.1]	0.1 [0-3.6]	0.8
Lymphocyte (x10 <sup>6</sup> /g)	0 [0-0.4]	0.02 [0-0.4]	0.01 [0-0.6]	0.06
Epithelial cells (x10 <sup>6</sup> /g)	0.13 [0-2.8]*	0.22 [0-3.9]	0.14 [0-0.6]	0.005

3

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

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



## 1 References

- 2 1. Vogelmeier CF, Criner GJ, Martinez FJ, et al. Global Strategy for the Diagnosis, Management,  
3 and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *Eur*  
4 *Respir J.* 2017;49(3).
- 5 2. Action on Smoking and Health: Use of e-cigarettes (vapes) among adults in Great Britain. 2021.  
6 [https://ash.org.uk/wp-content/uploads/2021/06/Use-of-e-cigarettes-vapes-among-adults-in-Great-](https://ash.org.uk/wp-content/uploads/2021/06/Use-of-e-cigarettes-vapes-among-adults-in-Great-Britain-2021.pdf)  
7 [Britain-2021.pdf.](https://ash.org.uk/wp-content/uploads/2021/06/Use-of-e-cigarettes-vapes-among-adults-in-Great-Britain-2021.pdf)
- 8 3. Higham A, Rattray NJ, Dewhurst JA, et al. Electronic cigarette exposure triggers neutrophil  
9 inflammatory responses. *Respir Res.* 2016;17(1):56.
- 10 4. Willemse BW, ten Hacken NH, Rutgers B, et al. Effect of 1-year smoking cessation on airway  
11 inflammation in COPD and asymptomatic smokers. *Eur Respir J.* 2005;26(5):835-45.
- 12 5. Wen Y, Reid DW, Zhang D, et al. Assessment of airway inflammation using sputum, BAL, and  
13 endobronchial biopsies in current and ex-smokers with established COPD. *Int J Chron Obstruct Pulmon*  
14 *Dis.* 2010;5:327-34.
- 15 6. Babusyte A, Stravinskaite K, Jeroch J, et al. Patterns of airway inflammation and MMP-12  
16 expression in smokers and ex-smokers with COPD. *Respir Res.* 2007;8:81.
- 17 7. Scanlon PD, Connett JE, Waller LA, et al. Smoking cessation and lung function in mild-to-  
18 moderate chronic obstructive pulmonary disease. The Lung Health Study. *Am J Respir Crit Care Med.*  
19 2000;161(2 Pt 1):381-90.
- 20 8. Higham A, Cadden P, Southworth T, et al. Leukotriene B4 levels in sputum from asthma  
21 patients. *ERJ Open Res.* 2016;2(4).
- 22 9. Beech A, Lea S, Li J, et al. Airway Bacteria Quantification Using Polymerase Chain Reaction  
23 Combined with Neutrophil and Eosinophil Counts Identifies Distinct COPD Endotypes. *Biomedicines.*  
24 2021;9(10).
- 25 10. Higham A, Beech A, Wolosianka S, et al. Type 2 inflammation in eosinophilic chronic  
26 obstructive pulmonary disease. *Allergy.* 2021;76(6):1861-4.
- 27 11. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic  
28 obstructive pulmonary disease. *N Engl J Med.* 2004;350(26):2645-53.
- 29 12. Pesci A, Majori M, Cuomo A, et al. Neutrophils infiltrating bronchial epithelium in chronic  
30 obstructive pulmonary disease. *Respir Med.* 1998;92(6):863-70.
- 31 13. Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction. Accumulation of  
32 neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis.* 1983;128(5):833-8.
- 33 14. Guzik K, Skret J, Smagur J, et al. Cigarette smoke-exposed neutrophils die unconventionally  
34 but are rapidly phagocytosed by macrophages. *Cell Death Dis.* 2011;2:e131.
- 35 15. Heijink IH, Pouwels SD, Leijendekker C, et al. Cigarette smoke-induced damage-associated  
36 molecular pattern release from necrotic neutrophils triggers proinflammatory mediator release. *Am J*  
37 *Respir Cell Mol Biol.* 2015;52(5):554-62.
- 38 16. Lee J, Luria A, Rhodes C, et al. Nicotine drives neutrophil extracellular traps formation and  
39 accelerates collagen-induced arthritis. *Rheumatology (Oxford).* 2017;56(4):644-53.
- 40 17. Xu M, Scott JE, Liu KZ, et al. The influence of nicotine on granulocytic differentiation - inhibition  
41 of the oxidative burst and bacterial killing and increased matrix metalloproteinase-9 release. *BMC Cell*  
42 *Biol.* 2008;9:19.
- 43 18. Yoshikawa H, Kurokawa M, Ozaki N, et al. Nicotine inhibits the production of proinflammatory  
44 mediators in human monocytes by suppression of I-kappaB phosphorylation and nuclear factor-  
45 kappaB transcriptional activity through nicotinic acetylcholine receptor alpha7. *Clin Exp Immunol.*  
46 2006;146(1):116-23.
- 47 19. Ghosh A, Coakley RD, Ghio AJ, et al. Chronic E-Cigarette Use Increases Neutrophil Elastase and  
48 Matrix Metalloprotease Levels in the Lung. *Am J Respir Crit Care Med.* 2019;200(11):1392-401.

1 20. Reidel B, Radicioni G, Clapp PW, et al. E-Cigarette Use Causes a Unique Innate Immune  
2 Response in the Lung, Involving Increased Neutrophilic Activation and Altered Mucin Secretion. Am J  
3 Respir Crit Care Med. 2018;197(4):492-501.

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