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Early View

Research letter

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Blood eosinophil counts in COPD patients compared to controls

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In patients with chronic obstructive pulmonary disease (COPD), blood eosinophil counts can predict the effects of inhaled corticosteroids (ICS) on exacerbation rates [1-4]. Blood eosinophil counts are now recommended by the Global initiative for the management of Obstructive Lung Disease (GOLD) as a biomarker to help guide ICS use in clinical practice [5].

It is estimated up to 40% of COPD patients have increased numbers of eosinophils in the sputum [6, 7] . There is also an association between lung and blood eosinophil counts [8-11], and blood eosinophil counts have been used as a surrogate marker for eosinophil numbers in the lungs [12]. However, it is not clear whether COPD peripheral blood eosinophil counts are increased above levels observed in healthy controls. If true, this would implicate systemic eosinophilia in COPD as a cause of increased lung eosinophils in COPD patients. We tested the hypothesis that peripheral blood eosinophil counts are raised in COPD patients by retrospectively analysing blood eosinophil counts collected from COPD patients and older healthy controls (age >40 years) at our research centre.

Results from COPD patients (n=209), healthy smokers (HS; n=46) and healthy non-smokers (HNS; n=81) aged ≥40 years who participated in research studies at the Medicines Evaluation Unit (Manchester University NHS Hospitals Trust, UK) were used. COPD was diagnosed according to GOLD recommendations[13] and subjects with a previous clinical diagnosis of any asthma were excluded. All healthy subjects had normal spirometry. COPD patients and HS had smoking history ≥10 pack-years. Subjects taking oral corticosteroids were excluded. All subjects provided blood samples >4 weeks from exacerbations or colds. This research was approved by the local Ethics Committees; all subjects provided written informed consent.

Blood eosinophil measurements (reported to an accuracy of 10 cells/μl) were performed by The Doctors Lab (London, UK) or Manchester University NHS Foundation Trust Hospital laboratory (Manchester, UK); normal eosinophil ranges for both laboratories were <400 eosinophils/μL. Atopy was determined using skin prick testing and clinical history of childhood asthma, eczema or hayfever.

Statistical analyses were performed using ANOVA or Kruskall-Wallis tests with post-tests as appropriate and Spearman's correlation using GraphPad Prism version 7.00 (San Diego, California; USA). $P<0.05$ was considered statistically significant.

The mean (SD) ages were 65.2 (6.8), 58.4 (9.6) and 55.8 (10.2) years in the COPD, HS and HNS groups respectively ($p<0.0001$), with 60%, 41% and 57% respectively being male. FEV_1 % predicted was 58.7%, 106.1% and 104.4% in the COPD, HS and HNS groups respectively. The median smoking history was 42.5 pack years for COPD patients and 28.5 pack years for HS. The majority (155/209) of COPD patients were taking ICS treatment. The mean (SD) mMRC, CAT and SGRQ-C scores in COPD patients were 2.4 (1.2), 20.2 (7.3) and 49.4 (20.8) respectively, while 35, 24 and 41% had 0, 1 or ≥ 2 exacerbations respectively in the last 12 months.

Blood eosinophil counts were significantly higher in COPD patients compared to HS ($p<0.01$) and HNS ($p<0.001$) (see Figure 1a); the median blood eosinophil counts were 210, 140 and 120 cells/μl in COPD, HS and HNS respectively. Total leucocyte count, neutrophil count and monocyte counts were increased in COPD ($7.5x10^9$/L, $4.43x10^9$/L and $0.61x10^9$/L respectively) versus HNS ($6.05x10^9$/L, $3.47x10^9$/L and $0.50x10^9$/L respectively, all

p<0.0001) but not HS ($7.75x10^9$/L, $4.24x10^9$/L and $0.61x10^9$/L respectively). In COPD patients there was no correlation between blood neutrophil and eosinophil counts (p=0.26).

In the COPD group, the proportion of patients with blood eosinophil counts <100 cells/µl, 100-300 cells/µl and ≥300 cells/µl was 13%, 56% and 31% respectively. For the control groups, a lower proportion of individuals had blood eosinophil counts ≥300 cells/µl; 30%, 48% and 22% of HS blood eosinophil counts <100 cells/µl, 100-300 cells/µl and ≥300 cells/µl respectively, while, for HNS the proportions were 40%, 55% and 5% respectively. 18.7% of COPD patients compared to 8.7% and 2.5% of HS and HNS subjects respectively had a blood eosinophil count above the HNS upper reference interval of 400cells/µl. There were no significant correlations between age and blood eosinophil counts in each group (all p >0.05). There were 155/209, 30/46 and 64/81 COPD patients, HS and HNS without atopy. Blood eosinophil counts within non-atopic patients showed similar results to the whole group (Figure 1b), with significant differences between COPD patients and HNS (p<0.0001), and a trend towards significance between COPD and HS (p=0.06). Blood eosinophil counts were higher in COPD patients using ICS (medians: 220 versus 165 cells/µl, p=0.04). Blood eosinophil counts were similar in COPD current and ex-smokers (225 cells/µl vs. 200 cells/µl respectively, p=0.2)

We have shown that blood eosinophil counts are increased in COPD patients compared to healthy controls. Even when atopic patients were removed from the analysis, similar results were obtained despite a reduction in statistical power that likely contributed to the lack of significance for COPD patients versus HS. The increased eosinophil numbers in the systemic circulation may be a reason for increased lung eosinophil numbers observed in a subgroup of COPD patients [6, 11].

The strengths of this study are that the subjects were well characterised (e.g. atopic status, exacerbation history and ICS use) and that all blood counts were obtained in the stable state. Importantly, we also tried to match the ages of the groups. We included all results in subjects aged >40 years, but found approximately 7 and 10 year age differences between COPD patients compared to HS and HNS respectively. We do not believe that this influenced the results, as there was no correlation between age and eosinophil counts. A limitation of this analysis is that the sample sizes for control groups were smaller than the COPD group.

Oral corticosteroids are known to reduce blood eosinophil counts[14]. Here, we observed higher blood eosinophil counts in patients taking ICS. This does not rule out an effect of ICS on blood eosinophil counts, as the cross-sectional nature of our study does not include paired measurements before and after starting ICS treatment. We speculate that perhaps the higher eosinophil counts in ICS users are due to higher eosinophil counts being associated with higher exacerbation rates (observed in other cohort studies[15]), coupled with ICS being used to treat patients with more exacerbations.

We have previously shown that COPD patients with higher blood eosinophil counts also have increased lung eosinophil numbers, and other differences in airway biology including greater reticular basement thickening [11]. The current results support the concept of a subgroup of "eosinophilic COPD" patients, with increased blood and lung eosinophil counts. Eosinophilic COPD appears to be a subgroup (endotype) with unique pulmonary and systemic manifestations, and a differential response to drugs [1-4].

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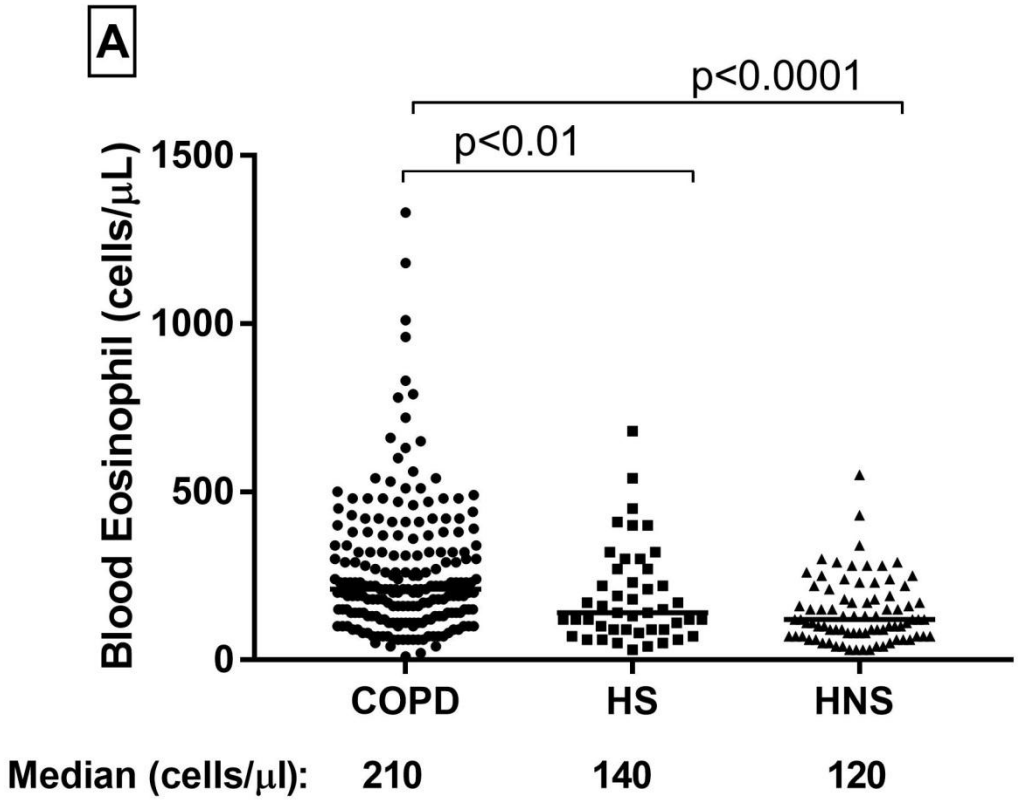
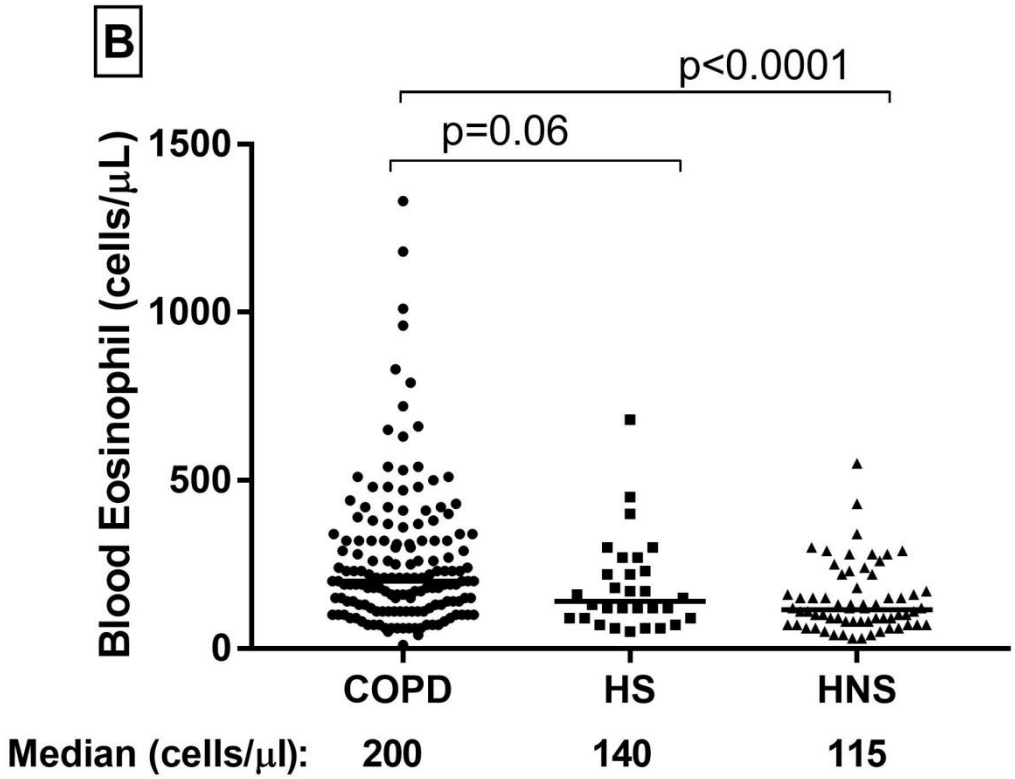
Figure Legend

Figure 1: The comparison of blood eosinophil counts between COPD, Healthy Smoker and Healthy non-smoker groups. (A) Dot plot includes all subjects including those with a history of atopy (B) Dot plot represents subjects without any history of atopy. Statistical analysis was performed using Kruskal-Wallis and Dunn's multiple comparisons post testing.

HS = Healthy smokers; HNS = Healthy non-smokers


A
p<0.0001
p<0.01
Blood Eosinophil (cells/μL)
1500
1000
500
0
COPD
HS
HNS
Median (cells/μl):
210
140
120


B
p<0.0001
p=0.06
Blood Eosinophil (cells/μL)
1500
1000
500
0
COPD
HS
HNS
Median (cells/μl):
200
140
115