next step, this regimen will be evaluated for its *in vivo* impact on models of neutrophilic airway inflammation in humans with asthma.

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Pulmonary inflammation in patients with chronic obstructive pulmonary disease with higher blood eosinophil counts

To the Editor:

Higher eosinophil counts in induced sputum and blood predict a greater clinical response to inhaled corticosteroids (ICS) in patients with chronic obstructive pulmonary disease (COPD).[1,2] This suggests a different profile of inflammation that is corticosteroid sensitive in patients with COPD with higher blood (or sputum) eosinophil counts. However, the nature of lung inflammation in patients with COPD with higher blood eosinophil counts is not well understood.

We have characterized pulmonary inflammation in patients with COPD with higher compared with lower blood eosinophil counts. We obtained induced sputum, bronchial mucosa, and bronchoalveolar lavage (BAL) samples to investigate whether patients with COPD with higher blood eosinophil counts have increased eosinophil numbers throughout the lungs. Furthermore, we investigated differences between groups in levels of inflammatory proteins and airway remodeling markers.

Patients with COPD older than 40 years with postbronchodilator FEV_1/forced vital capacity ratio of less than 0.7 and 10 or more pack-year smoking history were recruited. Skin prick testing to the common allergens house dust mite, cat hair, and grass pollen mix was performed; patients with a positive skin prick test result to any allergen were excluded. Patients with a previous diagnosis of childhood or adult asthma were excluded. All patients provided written informed consent using protocols approved by the Greater Manchester Ethics Committees (East, 05/Q1402/41 and South, 06/Q1403/156). Patients were classified according to blood eosinophil counts as eosinophil^{low} or eosinophil^{high} (<150 cells/μL or >250 cells/μL, respectively); patients with counts between 150 cells/μL and 250 cells/μL were excluded. Clinical assessments included symptom assessment, skin prick testing, exhaled nitric oxide, spirometry with reversibility, blood and sputum sampling, followed by bronchoscopy on a separate day. A total of 102 inflammatory proteins in the serum, sputum, and BAL were measured using a multiplex panel. IL-5, CCL11, and eosinophil-derived neurotoxin were measured in sputum and BAL supernatants. Endobronchial biopsy sections were stained with hematoxylin and eosin to quantify basement membrane thickness. Eosinophils were identified by Luna staining. Immunohistochemistry was performed with primary antibodies directed against neutrophils, macrophages, CD4 helper T cells, CD8 cytotoxic T cells, mast cells, basophils, and Tenascin (TNC). Reticular basement membrane (RBM) thickness and TNC staining thickness within the RBM were calculated. Differences between groups were analyzed using unpaired *t* tests, Mann-Whitney tests, and chi-square tests as appropriate. Significant differences in multiplex protein levels between groups were defined by median fold ratio (MR) of more than ±2 and *P* value of less than .05. Full details of the methods (including manufacturer details) and statistical analysis are provided in this article's Online Repository at www.jacionline.org.

Twenty-one eosinophil^{low} and 20 eosinophil^{high} patients were recruited with median blood eosinophil counts of 0.10×10^9/L and 0.41×10^9/L, respectively ($P < .001$; see Fig E1, *A* and *B*,

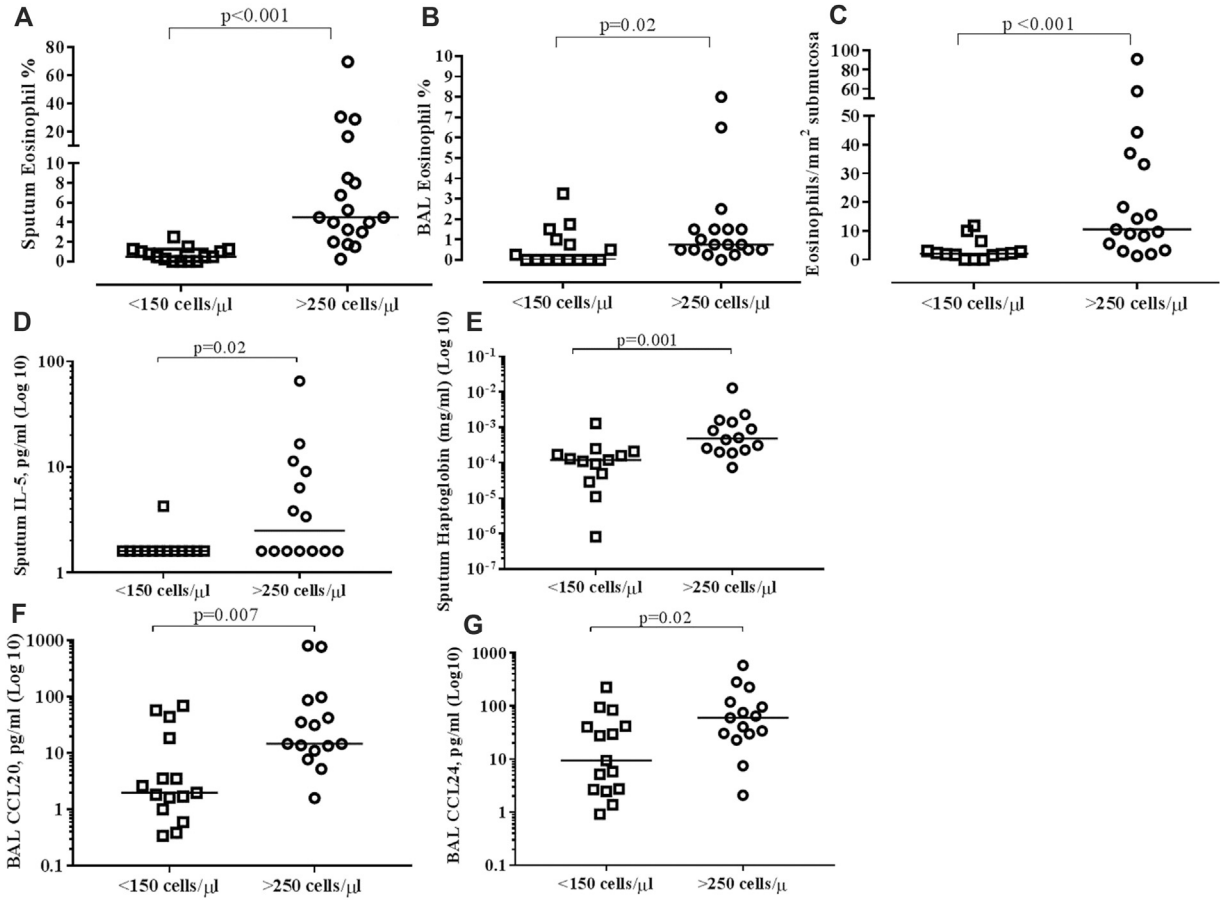

FIG 1. The levels of lung eosinophils in the sputum **(A)**, BAL **(B)**, and submucosa **(C)** and levels of inflammatory protein for sputum IL-5 **(D)**, sputum haptoglobin **(E)**, BAL CCL20 **(F)**, and BAL CCL24 **(G)** between the blood eosinophil^{low} and blood eosinophil^{high} groups. *Solid lines* in the dot plot represent median values.

in this article's Online Repository at www.jacionline.org). The groups were well matched for age, sex, smoking status, symptom scores, and exacerbation history (see Table E2 in this article's Online Repository at www.jacionline.org). The mean FEV_1 % predicted was 62.4 versus 65.5 ($P = .45$), and ICS use was 76% versus 55% ($P = .20$) for the eosinophil^{low} and eosinophil^{high} patients, respectively. Blood basophil counts were higher in eosinophil^{high} patients ($P = .01$; see Table E3 in this article's Online Repository at www.jacionline.org), with no differences between groups for other blood leukocyte subsets. The lung inflammatory cell counts are presented in Table E3. A significantly lower sputum neutrophil % (median, 62.9 vs 80.5; $P = .04$) and higher sputum eosinophil % (median, 4.5 vs 0.5; $P < .001$; Fig 1, *A*), sputum eosinophil absolute count $\times 10^6$/g (median, 0.26 vs 0.02; $P < .001$), BAL eosinophil % (median, 0.75 vs 0.00; $P = .02$; Fig 1, *B*), and submucosal eosinophil counts per mm^2 (median, 10.6 vs 2.1; $P < .001$; Fig 1, *C*, and Fig 2, *A* and *B*) were observed in the eosinophil^{high} versus eosinophil^{low} group. There were no differences between groups for other inflammatory cells.

Sputum IL-5 levels were significantly higher in the eosinophil^{high} versus eosinophil^{low} group (median, 2.5 pg/mL vs 1.6 pg/mL; $P = .02$; Fig 1, *D*), with no differences for eosinophil-derived neurotoxin or CCL11. Multiplex analysis showed that 72, 58, and 40 proteins measured in serum, BAL, and sputum, respectively, had more than 50% of samples with levels above lower limit of quantification; these proteins were selected for statistical analysis using data from all patients. Serum analyte levels were similar between groups. BAL analysis showed 40 proteins with more than 2 MR change and $P < .05$ in eosinophil^{high} patients compared with eosinophil^{low}. A different BAL aliquot was used to validate these findings; 29 proteins remained significantly increased in the eosinophil^{high} group, with strong correlations between these assays from different samples (see Table E4 in this article's Online Repository at www.jacionline.org). The highest ratio changes were observed for CCL20 (MR = 7.39; $P = .007$) and CCL24 (MR = 6.35; $P = .02$) (Fig 1, *F* and *G*). Tissue remodeling proteins including matrix metalloproteinase (MMP) 7 (MR = 6.08; $P = .004$), tissue inhibitor of metalloproteinase 1 (TIMP-1) (MR = 5.19; $P = .005$), and MMP-9 (MR = 2.57; $P = .02$) were also increased in the eosinophil^{high} group. In sputum, only haptoglobin was significantly different between groups; this was higher in blood eosinophil^{high} patients (MR = 4; $P < .001$; Fig 1, *E*) in sputum and in BAL (MR = 3.31; $P = .001$). RBM thickness (median, 8.8 μm vs 6.2 μm; $P < .001$; Fig 2, *C-E*) and TNC staining (mean, 5.0 μm vs 2.8 μm; $P = .003$; Fig 2, *F-H*) were significantly increased in eosinophil^{high} versus eosinophil^{low} patients, respectively.