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Early View

Research letter

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The reproducibility of COPD blood eosinophil counts

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Post-hoc and pre-specified analyses of chronic obstructive pulmonary disease (COPD) randomised controlled trials have shown that higher blood eosinophil counts predict greater inhaled corticosteroid (ICS) effects on exacerbation prevention (1-5). COPD patients with higher blood eosinophil counts have greater eosinophil numbers in sputum, bronchoalveolar lavage and bronchial tissue, and more reticular basement membrane thickening (6). Furthermore, increased sputum eosinophil counts are associated with reduced airway presence of pathogenic bacteria in COPD (7). Eosinophilic COPD therefore has distinct biological features associated with increased ICS responsiveness.

ICS effects incrementally increase with higher eosinophil counts (1, 3-5), rather than an "all or nothing" phenomenon. Thresholds defined by ≥300, 150-<300 and <150 eosinophils/μL appear to predict high, intermediate and low ICS response respectively (8, 9). While there is currently no consensus regarding the threshold(s) for use in clinical practice, it appears that approximately 150 eosinophils/μL is a key cut-off predicting little or no ICS response.

Blood eosinophil count variability may cause movement across a threshold assigning an individual to a different ICS response category. Using historical data, we report the long term reproducibility (>2 years) of COPD blood eosinophil counts using <150 eosinophils/μL as a key ICS response prediction threshold.

Results from COPD patients aged ≥40 years, diagnosed by GOLD criteria (10), recruited for research studies at the Medicines Evaluation Unit (Manchester University NHS Hospitals Trust) were used. Patients taking oral corticosteroids or with a previous asthma diagnosis were excluded. All patients provided blood samples >4 weeks from exacerbation. This research

was approved by the local Ethics Committees (North West, Preston and Manchester South, UK; REC references: 10/H1016/2, 10/H1003/108 and 06/Q1403/156); all patients provided written informed consent.

Blood eosinophil measurements (reported to two decimal places) were performed by The Doctors Lab (London, UK) or Wythenshawe Hospital clinical laboratory (Manchester, UK); normal eosinophil ranges for both laboratories were <400 eosinophils/μL. Symptoms using the modified MRC Scale (mMRC) and the COPD assessment test (CAT), health related quality of life using the St George's Respiratory Questionnaire (SGRQ-C) and exacerbation history were recorded, and lung function measurements performed.

Comparisons of repeat measures were by Spearman's rank correlation (Prism 7.0, Graphpad, San Diego, USA), intraclass correlation coefficient (ICC) using log transformed data, Bland-Altman analysis, assessment of heterogeneous variance and repeatability coefficient analysis (11) (SPSS 22.0, IBM, Armonk, USA).

COPD patients (n=82) had a mean (SD) age 65.1 (6.3) years, FEV_1 57.7 (17.2) % predicted and FEV_1/FVC ratio 44.7% (13.3%). The median smoking history was 41.4 pack years; 62% were ex-smokers. The mean (SD) mMRC, CAT and SGRQ-C scores were 1.9 (1.1), 18.4 (9.3) and 40.3 (25.2) respectively.

Repeat blood eosinophil counts at baseline and 6 months (n=55) showed there was a significant correlation (rho=0.80, p<0.001) and an ICC=0.89. Repeat measurements at ≥2 years (n=59,

mean 2.96 years; range 2.03 - 5.19 years) also showed a significant correlation (rho=0.74, p<0.001) with ICC=0.87 (Fig1A).

Bland-Altman regression analysis (6 months: p=0.006 and >2 years: p=0.015) and analysis of heterogeneous variance indicated that mean differences and variability between visits increased with higher blood eosinophil counts. We therefore calculated repeatability coefficients for <150, 150-<300 and ≥300 eosinophils/μL; 95% of repeat measurements at 6 months fell within 106, 160 and 470 eosinophils/μL respectively, with similar data observed at ≥2 years (Fig1B). The larger eosinophil count changes were not associated with changes in ICS use.

Using the <150 eosinophils/μL threshold, at 6 months there were 48/55 (87%) results that remained stable above or below this level, while at >2 years, 51/59 (86%) results showed stability (Fig1C). The >100 eosinophils/μL threshold (proposed to predict positive ICS treatment effects (1)) also provided similar stability results (91% and 85% stability at 6 months and >2 years respectively). We evaluated reproducibility using ≥300, 150-<300 and ≤150 eosinophils/μL thresholds (Fig1C-E); at >2 years, 38/59 (64.0%) of measurements remained in the same category, with 1 (1.7%) patient moving between the lowest and highest category, 7 (12%) moving between lowest and middle categories and 13 (22%) moving between the middle and higher categories. Similar reproducibility was observed at 6 months; 39/55 (71%) of measurements remained within the same category, with 7 measurements (13%) moved between the lowest and middle category and 9 (16%) between the middle and highest category.

In summary, the majority (≥86%) of blood eosinophil measurements repeated at 6 months or >2 years remained in the same category using the 150 eosinophils/μL threshold. This indicates good long term biomarker stability in most individuals using this single threshold.

Statistical analysis showed greater variability at higher blood eosinophil counts. The decreased variability at lower eosinophil thresholds explains why the majority of results remained stable at ≤100 or <150 eosinophils/μL. The greatest variation was observed ≥300 eosinophils/μL, although only one patient moved between the lowest and highest categories (<150 and ≥300 respectively). Variation between the middle and highest categories should not cause many problems with clinical interpretation, as both categories predict a positive ICS response (3, 9).

There were 12.7% and 13.6% of results that moved between the <150 eosinophils/μL and the other categories at 6 months and >2 years respectively. These results are more difficult to interpret in clinical practice, varying between predicting a low response, suggesting ICS should not be used, to predicting a positive response favouring ICS use. Other clinical factors should also be used to make individual decisions about ICS use, including risk of side effects and any previous clinical history of ICS response. Nevertheless, our results demonstrate that >86% of repeat eosinophil counts provide a clinically similar interpretation regarding ICS response prediction using thresholds of either 100 or 150 eosinophils/μL.

Repeat measurements at 6 months and >2 years resulted in ICC values of 0.89 and 0.87, respectively. Other COPD studies have reported ICC of 0.73 at 6 months (n=145) (12) and 0.74 at 1 year (n=17,724) (13). These results all closely match our own findings. These results can be influenced by the number of decimal places to which eosinophil counts were reported.

In the ECLIPSE study, the reproducibility of 4 blood eosinophil measurements over 3 years using a 2% threshold was reported; 51% of patients did not change category (14). It is not ideal to compare results between studies using percentage and absolute counts. Multiple blood tests (as in ECLIPSE) increase the statistical probability that individuals might change category. In such instances, we suggest a practical approach to choose the category where most of the values lie.

Casanova et al (2017) reported that 15.8% of COPD patients had consistent blood eosinophil counts ≥300/µL after 12 months (CHAIN cohort), and 12% after >7.5 years (BODE cohort) (15). We observed 33% and 20% after 6 months and >2 years respectively. We had more patients with a baseline count ≥300 eosinophils/µL (39%) compared to the CHAIN (34.7%) and BODE (26.6%) cohorts. Moreover, we propose that using lower eosinophil thresholds (100 or 150 cells/µL) provides more stable categorisation of eosinophil counts over time.

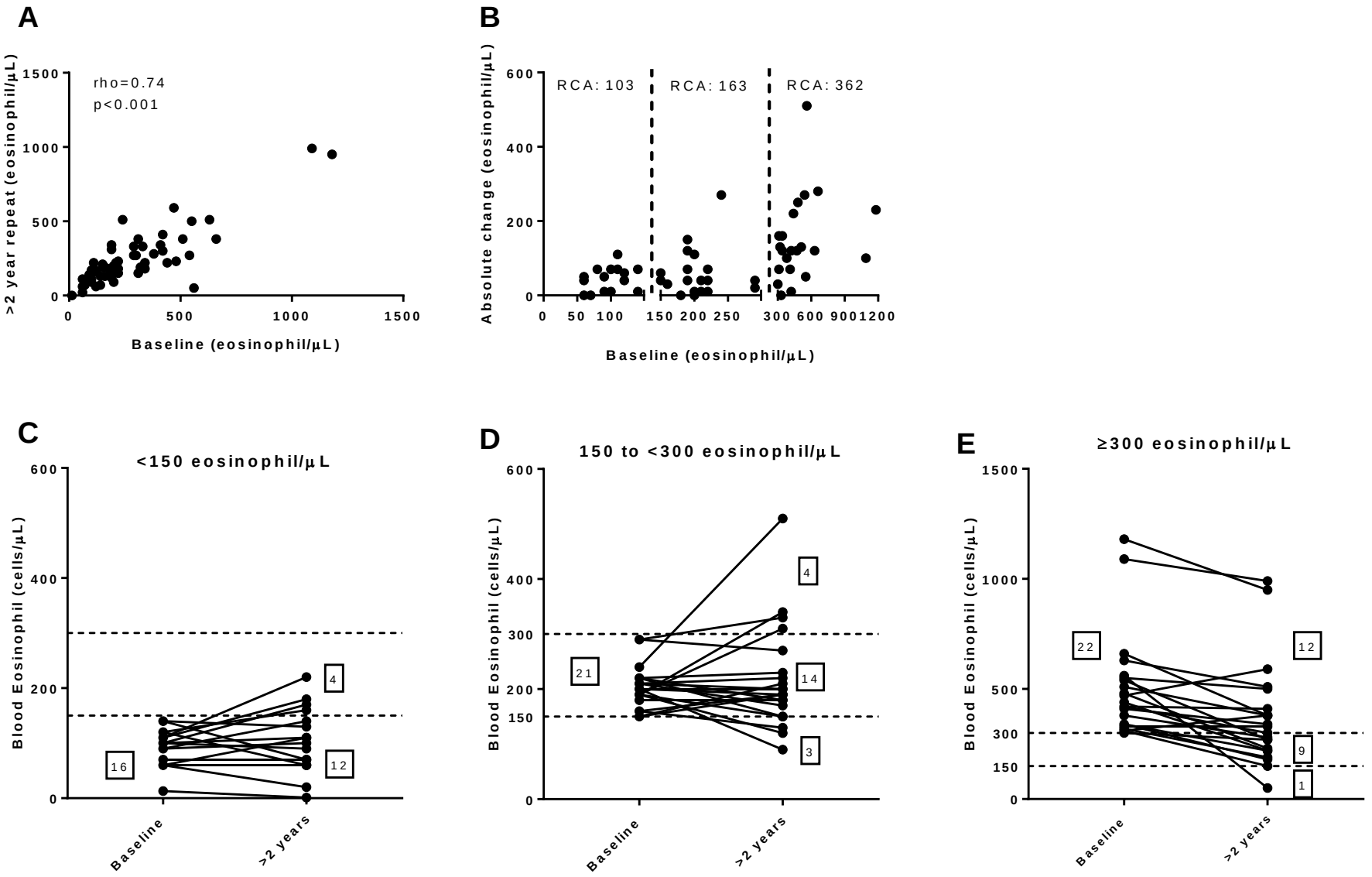
While our sample size is modest, we provide accurate information regarding movement across commonly used eosinophil threshold values. Nearly 90% of repeated measurements remain in the same category when using 150 eosinophils/µL to predict ICS response.

References:

1. Bafadhel M, Peterson S, De Blas MA, Calverley PM, Rennard SI, Richter K, et al. Predictors of exacerbation risk and response to budesonide in patients with chronic obstructive pulmonary disease: a post-hoc analysis of three randomised trials. Lancet Respir Med. 2018;6(2):117-26.
2. Vestbo J, Papi A, Corradi M, Blazhko V, Montagna I, Francisco C, et al. Single inhaler extrafine triple therapy versus long-acting muscarinic antagonist therapy for chronic obstructive pulmonary disease (TRINITY): a double-blind, parallel group, randomised controlled trial. Lancet. 2017;389(10082):1919-29.
3. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. Lancet Respir Med. 2015;3(6):435-42.
4. Calverley PMA, Tetzlaff K, Vogelmeier C, Fabbri LM, Magnussen H, Wouters EFM, et al. Eosinophilia, Frequent Exacerbations, and Steroid Response in Chronic Obstructive Pulmonary Disease. American journal of respiratory and critical care medicine. 2017;196(9):1219-21.
5. Siddiqui SH, Guasconi A, Vestbo J, Jones P, Agusti A, Paggiaro P, et al. Blood Eosinophils: A Biomarker of Response to Extrafine Beclomethasone/Formoterol in Chronic Obstructive Pulmonary Disease. American journal of respiratory and critical care medicine. 2015;192(4):523-5.
6. Kolsum U, Damera G, Pham TH, Southworth T, Mason S, Karur P, et al. Pulmonary inflammation in patients with chronic obstructive pulmonary disease with higher blood eosinophil counts. The Journal of allergy and clinical immunology. 2017;140(4):1181-4 e7.
7. Kolsum U, Donaldson GC, Singh R, Barker BL, Gupta V, George L, et al. Blood and sputum eosinophils in COPD; relationship with bacterial load. Respiratory research. 2017;18(1):88.
8. Roche N, Chapman KR, Vogelmeier CF, Herth FJF, Thach C, Fogel R, et al. Blood Eosinophils and Response to Maintenance Chronic Obstructive Pulmonary Disease Treatment. Data from the FLAME Trial. American journal of respiratory and critical care medicine. 2017;195(9):1189-97.
9. Singh D. Predicting Corticosteroid Response in Chronic Obstructive Pulmonary Disease. Blood Eosinophils Gain Momentum. American journal of respiratory and critical care medicine. 2017;196(9):1098-100.
10. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. The European respiratory journal. 2017;49(3).
11. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res. 1999;8(2):135-60.
12. Barker BL, Mistry V, Pancholi M, Brightling CE, Bafadhel M. Are Sputum and Blood Biomarkers of Inflammation Repeatable in Stable Copd? Thorax. 2012;67:A155-A6.
13. Landis SH, Suruki R, Hilton E, Compton C, Galwey NW. Stability of Blood Eosinophil Count in Patients with COPD in the UK Clinical Practice Research Datalink. COPD. 2017;14(4):382-8.
14. Singh D, Kolsum U, Brightling CE, Locantore N, Agusti A, Tal-Singer R, et al. Eosinophilic inflammation in COPD: prevalence and clinical characteristics. The European respiratory journal. 2014;44(6):1697-700.
15. Casanova C, Celli BR, de-Torres JP, Martinez-Gonzalez C, Cosio BG, Pinto-Plata V, et al. Prevalence of persistent blood eosinophilia: relation to outcomes in patients with COPD. The European respiratory journal. 2017;50(5).

Figure Legends:

Figure 1: Variation of repeated measurements of COPD blood eosinophils. (A) Blood samples were collected at baseline and >2 years later. (B) Baseline eosinophils samples were characterised as being either <150 eosinophils/μL, 150 to <300 eosinophils/μL or ≥300 eosinophils/L for repeatability coefficient analysis (RCA), which predicts where 95% of the repeat values will fall. Graphs C-E illustrate changes in these categories from baseline during repeat measurements (C: <150; D: 150 to <300; E: ≥300 eosinophils/μL). Boxed numbers describe the number of samples in each category. The dotted lines show the 150 and 300 eosinophils/μL cut-offs.


A
rho=0.74
p<0.001
>2 year repeat (eosinophil/µL)
Baseline (eosinophil/µL)
B
RCA: 103
RCA: 163
RCA: 362
Absolute change (eosinophil/µL)
Baseline (eosinophil/µL)
C
<150 eosinophil/µL
Blood Eosinophil (cells/µL)
16
4
12
Baseline
>2 years
D
150 to <300 eosinophil/µL
Blood Eosinophil (cells/µL)
21
4
14
3
Baseline
>2 years
E
≥300 eosinophil/µL
Blood Eosinophil (cells/µL)
22
12
9
1
Baseline
>2 years