The reproducibility of COPD blood eosinophil counts

To the Editor:

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 of ⩾300, 150–<300 and <150 eosinophils per 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 ∼150 eosinophils per 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 per μL as a key ICS response prediction threshold.

Results from COPD patients aged ⩾40 years, diagnosed by Global Initiative for Chronic Obstructive Lunge Disease criteria [10], recruited for research studies at the Medicines Evaluation Unit (Manchester University NHS Foundation Trust, Manchester, UK) 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 per μL. Symptoms using the modified Medical Research Council scale (mMRC) and the COPD Assessment Test (CAT), health-related quality of life using the St George's Respiratory Questionnaire for COPD Patients (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, CA, 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, NY, USA).

COPD patients (n=82) had a mean±SD age of 65.1±6.3 years, a forced expiratory volume in 1 s (FEV_1) of 57.7±17.2% predicted and an FEV_1/forced vital capacity ratio of 44.7±13.3%. The median smoking history was 41.4 pack-years; 62% were ex-smokers. The 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 of 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 an ICC of 0.87 (figure 1a).

@ERSpublications
Blood eosinophil counts in COPD are stable over the long term
http://ow.ly/aDlN30jVBCg

Cite this article as: Southworth T, Beech G, Foden P, *et al.* The reproducibility of COPD blood eosinophil counts. *Eur Respir J* 2018; 52: 1800427 [https://doi.org/10.1183/13993003.00427-2018].

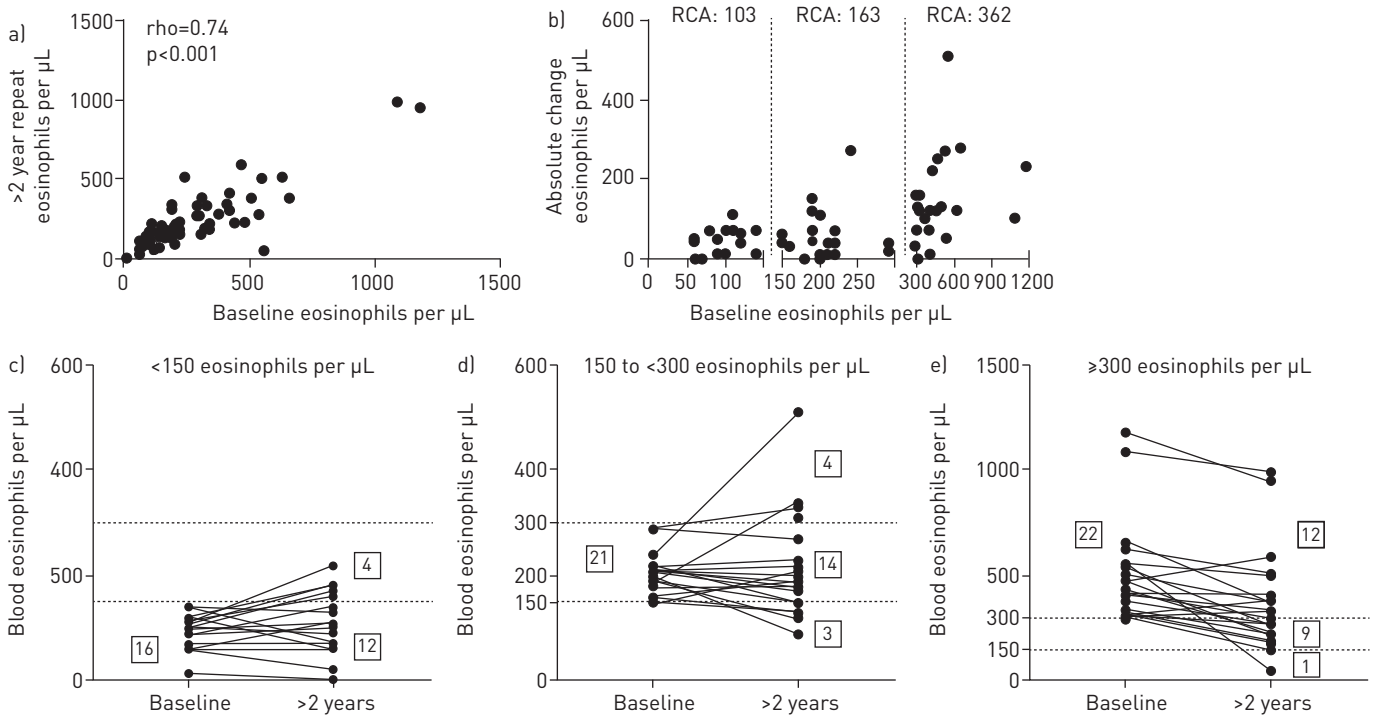

FIGURE 1 Variation of repeated measurements of chronic obstructive pulmonary disease blood eosinophils. a) Blood samples were collected at baseline and >2 years later. b) Baseline eosinophil samples were characterised as being either <150, 150–<300 or ⩾300 eosinophils per µL for repeatability coefficient analysis (RCA), which predicts where 95% of the repeat values will fall. c–e) Changes in these categories from baseline during repeat measurements (c) <150, d) 150 to <300 and e) ⩾300 eosinophils per µL). Boxed numbers describe the number of samples in each category. The dotted lines show the 150 and 300 eosinophils per µL cut-offs.

Bland–Altman regression analysis (6 months: p=0.006; >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 per µL; 95% of repeat measurements at 6 months fell within 106, 160 and 470 eosinophils per µL respectively, with similar data observed at ⩾2 years (figure 1b). The larger eosinophil count changes were not associated with changes in ICS use.

Using the <150 eosinophils per µL threshold, at 6 months there were 48 (87%) out of 55 results that remained stable above or below this level, while at >2 years, 51 (86%) out of 59 results showed stability (figure 1c). The >100 eosinophils per 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 the ⩾300, 150–<300 and ⩽150 eosinophils per µL thresholds (figure 1c–e); at >2 years, 38 (64.0%) out of 59 measurements remained in the same category, with one (1.7%) patient moving between the lowest and highest category, seven (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 (71%) out of 55 measurements remained within the same category, with seven (13%) measurements moving between the lowest and middle categories, and nine (16%) between the middle and highest categories.

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 per 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 per µL. The greatest variation was observed ⩾300 eosinophils per µL, although only one patient moved between the lowest and highest categories (<150 and ⩾300 eosinophils per µL 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].

12.7% and 13.6% of results that moved between the <150 eosinophils per µL category 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 per 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 ICCs 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 four 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.* [15] reported that 15.8% of COPD patients had consistent blood eosinophil counts ⩾300 per μL after 12 months (CHAIN cohort) and 12% after >7.5 years (BODE cohort). We observed 33% and 20% after 6 months and >2 years respectively. We had more patients with a baseline count ⩾300 eosinophils per 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 per μL) provides more stable categorisation of eosinophil counts over time.

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

Thomas Southworth[1], Gussie Beech[1], Philip Foden[2], Umme Kolsum[1] and Dave Singh[1]
[1]The University of Manchester, Medicines Evaluation Unit, Manchester University NHS Foundation Trust, Manchester, UK. [2]Medical Statistics Dept, Manchester University NHS Foundation Trust, Manchester, UK.

Correspondence: Dave Singh, The University of Manchester, Medicines Evaluation Unit, The Langley Building, Manchester University NHS Foundation Trust, Southmoor Road, Manchester, M23 9QZ, UK. E-mail: dsingh@meu.org.uk

Received: March 01 2018 | Accepted after revision: April 24 2018

Conflict of interest: D. Singh reports receiving personal fees from Apellis, Genentech, Cipla, Peptinnovate and Skyepharma, and grants and personal fees from Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Glenmark, Menarini, Merck, Mundipharma, Novartis, Pfizer, Pulmatrix, Teva, Therevance, Verona and AstraZeneca, outside the submitted work.

References

1 Bafadhel M, Peterson S, De Blas MA, *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: 117–126.
2 Vestbo J, Papi A, Corradi M, *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: 1919–1929.
3 Pascoe S, Locantore N, Dransfield MT, *et al.* 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: 435–442.
4 Calverley PMA, Tetzlaff K, Vogelmeier C, *et al.* Eosinophilia, frequent exacerbations, and steroid response in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2017; 196: 1219–1221.
5 Siddiqui SH, Guasconi A, Vestbo J, *et al.* Blood eosinophils: a biomarker of response to extrafine beclomethasone/formoterol in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2015; 192: 523–525.
6 Kolsum U, Damera G, Pham TH, *et al.* Pulmonary inflammation in patients with chronic obstructive pulmonary disease with higher blood eosinophil counts. *J Allergy Clin Immunol* 2017; 140: 1181–1184.
7 Kolsum U, Donaldson GC, Singh R, *et al.* Blood and sputum eosinophils in COPD; relationship with bacterial load. *Respir Res* 2017; 18: 88.
8 Roche N, Chapman KR, Vogelmeier CF, *et al.* Blood eosinophils and response to maintenance chronic obstructive pulmonary disease treatment. Data from the FLAME Trial. *Am J Respir Crit Care Med* 2017; 195: 1189–1197.
9 Singh D. Predicting corticosteroid response in chronic obstructive pulmonary disease. Blood eosinophils gain momentum. *Am J Respir Crit Care Med* 2017; 196: 1098–1100.
10 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.
11 Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res* 1999; 8: 135–160.

12 Barker BL, Mistry V, Pancholi M, *et al.* Are sputum and blood biomarkers of inflammation repeatable in stable COPD? *Thorax* 2012; 67: A155–A1A6.
13 Landis SH, Suruki R, Hilton E, *et al.* Stability of blood eosinophil count in patients with COPD in the UK Clinical Practice Research Datalink. *COPD* 2017; 14: 382–388.
14 Singh D, Kolsum U, Brightling CE, *et al.* Eosinophilic inflammation in COPD: prevalence and clinical characteristics. *Eur Respir J* 2014; 44: 1697–1700.
15 Casanova C, Celli BR, de-Torres JP, *et al.* Prevalence of persistent blood eosinophilia: relation to outcomes in patients with COPD. *Eur Respir J* 2017; 50: 1701162.

Copyright ©ERS 2018