

# Blood Eosinophils in Chronic Obstructive Pulmonary Disease: Is There Enough Evidence?

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There is strong evidence to support the use of blood eosinophil counts (BECs) in chronic obstructive pulmonary disease (COPD). *Post hoc*, secondary-prespecified and data-modelling analyses have shown a better response to inhaled corticosteroids in patients with increased BECs. Consequently, experts have suggested that BECs may be a useful biomarker to predict a favourable response to corticosteroid therapy. However, the literature is rich in contrasting data and there are still fundamental points that need to be clarified before sound judgement can be made. In this narrative review, we examine the evidence that supports or denies the role of BECs in COPD. Based on the available literature, we believe that the role of BEC as a valuable biomarker to guide COPD treatment in clinical practice remains unsupported.

## Keywords

Biomarker, blood count, COPD, Chronic Obstructive Pulmonary Disease, eosinophils, exacerbations, corticosteroids, inhalation therapy, pneumonia

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The lack of well-validated biomarkers for monitoring disease activity, predicting future clinical outcomes and the effect of therapeutic interventions highlights the need to find new biomarkers in chronic obstructive pulmonary disease (COPD). In recent years, extensive research has gone into identifying and attempting to validate relevant diagnostic biomarkers of disease activity and therapeutic response.<sup>1</sup>

A degree of eosinophil-associated airway inflammation can be present in both stable COPD and during acute exacerbations of COPD (AECOPDs); protection from AECOPDs with inhaled corticosteroids (ICSs) seems to be greatest in patients with higher blood eosinophil counts (BECs). Therefore, there has been increased interest in BECs as a biomarker for predicting the risk of AECOPDs and response to corticosteroid therapy.<sup>2,3</sup> The 2020 Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) report recommends using BECs to guide treatment in patients with COPD in order to select the most appropriate patients for ICS therapy.<sup>4</sup> For patients with one AECOPD per year, a peripheral blood level of  $\geq 300$  eosinophils/ $\mu$ L identifies those who are more likely to respond to long-acting  $\beta_2$ -agonists (LABA) or ICS treatment. For patients with two or more moderate exacerbations per year, or at least one severe exacerbation requiring hospitalization in the prior year, LABA/ICS treatment can be considered when BECs are  $\geq 100$  cells/ $\mu$ L, as ICS effects are more pronounced in patients with greater AECOPD frequency and/or severity. In any case, a beneficial response after the addition of ICS may be observed when BECs are  $\geq 100$  cells/ $\mu$ L, with a greater magnitude of response more likely with higher BECs.

In contrast to the GOLD report, the American Thoracic Society clinical practice guideline for the pharmacologic management of COPD is more pragmatic.<sup>5</sup> It does not make recommendations for or against ICSs as an additive therapy to long-acting bronchodilators in patients with COPD and blood eosinophilia (defined as  $\geq 2\%$  blood eosinophils or  $\geq 150$  cells/ $\mu$ L). However, the guideline does suggest ICSs as an additive therapy for patients with a history of one or more AECOPDs in the past year requiring antibiotics, oral steroids or hospitalization. This recommendation is conditional and means that treatment can be personalized for individual patients. Physicians must help each patient arrive at a management decision consistent with their values and preference.

There is strong support for the role of BECs as a biomarker in COPD; eosinophil measurement, plus clinical judgement and other patient-centred factors, have utility in developing individualized treatment plans for patients with COPD.<sup>6</sup> However, the evidence showing a better response to ICSs with increased blood eosinophils comes from *post hoc*, secondary-prespecified and data-modelling analyses, which has generated many uncertainties that must be resolved before blood eosinophil levels can be endorsed to direct broad-based clinical therapy.<sup>7,8</sup> Consequently, there is also concern regarding the real value of the BEC as a valid biomarker to predict AECOPD risk and the clinical response to ICSs.

In this critical, narrative review, we examine the evidence that supports or denies a role for blood eosinophils in COPD, taking into consideration the strengths and the weaknesses of the material under review. The general aim of a data analysis in a critical review is to analyse and examine the literature and the main ideas and relationships of an issue.<sup>9</sup> Systematic reviews are superior to critical reviews in answering specific questions. Over recent years we have conducted systematic reviews with sophisticated meta-analyses to answer specific questions, some of which will be resumed below.<sup>10</sup> However, our aim is to provide a review of the most important and critical aspects of the current knowledge of the topic. We fully share the opinion that the interpretative elements of narrative reviews – which are better suited to addressing a topic in a wider way – are necessarily subjective and that the resulting product is the starting point for further evaluation and not an endpoint.<sup>11</sup> The data analysis part of a critical review is not particularly developed according to a specific standard.<sup>12</sup> We identified references through searches of PubMed using the keywords ‘COPD’ and ‘blood eosinophils’ up to August 2020. We supplemented the bibliographic database searches with backward citation tracking of relevant publications. The most significant information has been selected.

### Eosinophilic inflammation in chronic obstructive pulmonary disease

COPD is conventionally considered a neutrophil-mediated inflammatory disease.<sup>13</sup> However, eosinophils are present in the airways, tissues and blood of some patients with COPD, whether during stable disease or AECOPD.<sup>13–16</sup> The lungs of patients with COPD contain more eosinophils than those of healthy subjects, but no more than 40% exhibit blood eosinophil levels  $\geq 200$  cells/ $\mu$ L and/or  $\geq 2\%$ .<sup>17,18</sup>

The role of eosinophils in COPD pathogenesis is still unclear, but is likely different from that in asthma and it is unknown why only some patients with COPD develop eosinophilic airway inflammation.<sup>18</sup> In patients with COPD and eosinophilia there is an increase in sputum interleukin (IL)-5 and an increase in the secretion of granulocyte-macrophage colony stimulating factor and CC chemokine ligand 5, which are central to maintaining eosinophil survival in lungs and to recruiting eosinophils, respectively, by airway epithelial cells.<sup>19</sup> Additionally, epithelial cells of COPD patients secrete thymic stromal lymphopoietin (TSLP) and IL-33, which are important for the recruitment and activation of T helper-2 and type 2 innate lymphoid cells.<sup>19</sup> There is documentation of a close association between aberrant TSLP signalling and COPD, and it has been suggested that TSLP expression by human airway smooth muscle cells may influence immune regulation by interacting with, and influencing local immune cells in, COPD airways.<sup>20</sup> Multiple findings from several studies have connected eosinophilic inflammatory responses with excess localized expression of TSLP.<sup>21</sup>

Increasing evidence suggests that the presence of an eosinophilic inflammation in COPD is associated with severe AECOPD, longer hospital stays and higher risks of readmission; however, it has been highlighted that the association of high blood eosinophil levels and increased risk of AECOPDs is not strong.<sup>18,22</sup> In a real-world COPD population study that used European data from the Adelphi Real World Respiratory Disease Specific Programme 2017 survey, more GOLD D patients had elevated BECs compared with GOLD B.<sup>23</sup> The proportions of GOLD D patients with a history of  $\geq 2$  exacerbations and BECs of  $\geq 150$ ,  $\geq 300$  and  $\geq 400$  cells/ $\mu$ L were 81.2%, 39.4% and 24.6%, respectively. In total, 10.6% of patients had  $\geq 300$  cells/ $\mu$ L and a history of  $\geq 2$  exacerbations.

We still do not know whether BEC predicts exacerbation risk independently of exacerbation phenotype, exacerbation treatment and

what factors confound interpretation of the BEC. For example, infection may be particularly problematic because viral and bacterial infections can both increase and decrease BECs in patients with COPD.<sup>24</sup> Nevertheless, an inverse relationship between bacterial counts and blood eosinophils was observed during AECOPDs but not in the stable state. Peripheral blood eosinophilia (defined as blood eosinophil levels of  $\geq 2\%$ ) must be considered a marker of non-infectious inflammatory exacerbations.<sup>25,26</sup>

### Peripheral blood eosinophils as a surrogate marker for sputum eosinophilia

Performing differential cell counts and assessing mediator concentrations in induced sputum is a valid non-invasive technique for the assessment of airway inflammation.<sup>27</sup> A significant increase in induced sputum eosinophils and eosinophil cationic protein levels was found in patients with stable COPD compared with healthy subjects.<sup>28</sup> A high sputum eosinophil count is associated with a positive response to corticosteroid treatment in stable COPD; also it is useful to titrate corticosteroid therapy to reduce AECOPDs.<sup>15,29</sup>

However, there are some problems regarding the use of induced sputum.<sup>1</sup> Sputum induction itself causes a local inflammatory response with transient, longer-lived eosinophilia, possibly due to local changes in osmolarity, activating epithelial and mast cells.<sup>30</sup> Furthermore, measuring sputum eosinophils is time consuming; some patients do not provide adequate samples because airflow obstruction that characterizes COPD cannot be totally prevented by premedication with a short-acting  $\beta_2$ -agonist (SABA) or an antimuscarinic agent, and sputum induction requires expertise and may not always be successful (the failure rate can be up to 30%).<sup>14,31</sup>

BECs have been used as a surrogate for eosinophilic airway inflammation because they are generally thought to be good predictors of eosinophil concentrations in the airways and measurements of BEC are more practical than performing eosinophil counts in induced sputum due to the ease of sample collection.<sup>32</sup> Some studies have shown correlations between BECs and eosinophil counts in sputum, and a correlation between blood eosinophil percentages and eosinophil counts in bronchial submucosal samples.<sup>14,33,34</sup>

Apparently, eosinophilic inflammation in COPD emerges at lower levels than those that characterize eosinophilia (peripheral BEC  $> 500$  cells/ $\mu$ L).<sup>6</sup> However, the threshold of BECs that defines the presence of pulmonary eosinophilic inflammation is still not clear. In fact, different thresholds, including relative values as a percentage of other cells present, of  $\geq 2\%$  or  $\geq 4\%$  or absolute values of  $\geq 100$ ,  $\geq 150$  or  $\geq 300$  cells/ $\mu$ L have been proposed, but it is difficult to recommend a single threshold because BECs vary during a 24-hour period in any subject. Also, BECs differ during stable disease, exacerbations and following treatment.<sup>32,35</sup>

### The reproducibility of blood eosinophil counts

According to a US National Science Foundation subcommittee on replicability in science, “Reproducibility is a minimum necessary condition for a finding to be believable and informative”.<sup>35</sup> Reproducibility of results (previously described as replicability) refers to obtaining the same results from the conduct of an independent study whose procedures are closely matched; however, this is difficult to obtain when we analyse studies that have explored the role of blood eosinophils in COPD.<sup>36</sup> The possibility of obtaining ‘inferential reproducibility’, which means drawing the same conclusions from either an independent replication of a study or a reanalysis of the original study, seems more realistic. However, there is

a huge discrepancy between the available data, so obtaining inferential reproducibility is difficult.<sup>36</sup>

### The stability of blood eosinophil counts

The stability and reproducibility of BEC over time is an issue that is relevant for its use as a biomarker in patients with COPD. Conceptually, the stability of BECs should reflect the eosinophilic count in the lung. However, blood eosinophils cannot be assumed to truly reflect lung tissue eosinophils, as documented by Turato et al., who were unable to find a correlation between tissue eosinophils from resected lung tissue and blood eosinophils in patients with COPD.<sup>37</sup> Nevertheless, a recent study has documented that low levels of submucosal eosinophilic airway inflammation in patients with COPD are highly stable over time, whereas high levels show increased biological variability over time.<sup>38</sup>

An analysis of the COPD-MAP observational cohort showed that approximately 70% of blood eosinophil measurements remained in the same category over 1 year using the GOLD 2019 thresholds (<100, 100–299 or ≥300 cells/μL), and 85.3% of patients with eosinophils <100 cells/μL had stable counts.<sup>39</sup> Also, the analysis of the German COPD and Systemic Consequences-Comorbidities Network (COSYCONET) cohort demonstrated that in COPD, non-eosinophilia (<150 eosinophils/μL) in blood is more robust over time than eosinophilia defined as a count of ≥300 cells/μL.<sup>40</sup> When patients were stratified into persistently-low, variable and persistently-high blood eosinophil groups, no significant differences in baseline characteristics were detected among the groups.<sup>41</sup>

However, the repeatability of the peripheral BEC has been shown to be moderate.<sup>42</sup> A population-based study that used data obtained from the Clinical Practice Research Datalink showed that the stability of peripheral BECs was significantly lower in patients with COPD compared with control subjects without COPD.<sup>43</sup> In patients with COPD the stability was approximately 85% at 6 months and 62% at 2 years' follow-up, and declined progressively thereafter. In COPD, unstable eosinophilia was reported in as many as 40.5% (threshold ≥300 cells/μL) to 49% (threshold ≥2%) of the studied population.<sup>44,45</sup> Consequently, a small proportion of patients remain with persistently elevated or lower BECs. Systemic corticosteroids, antibiotics, older age and male sex affect the stability of BECs, while the impact of ICS use or smoking status was negligible.<sup>18</sup> Since BECs present significant variability throughout the course of COPD, it has been noted that a single measurement may not be a reliable predictor of future exacerbation risk and responsiveness of patients to ICSs.<sup>46</sup>

### Eosinophil levels and relation to outcomes in patients with chronic obstructive pulmonary disease

Correlation between blood eosinophil levels and their consequences on COPD remains uncertain. *Table 1* reports conflicting data on the value of BECs in influencing outcomes in COPD.

#### Impact on lung function

The Copenhagen General Population Study documented that individuals with BECs ≥340 cells/μL had lower levels of forced expired volume in 1 second (FEV<sub>1</sub>) as a percentage of predicted value than individuals with COPD and low BECs.<sup>2</sup> Furthermore, non-eosinophilic patients presented with higher post-bronchodilator FEV<sub>1</sub> than patients with higher sputum eosinophils but not with higher blood eosinophils.<sup>47</sup> However, in another study, FEV<sub>1</sub> reversibility seemed to be weakly correlated with sputum eosinophil levels in COPD.<sup>48</sup>

Rogliani et al. examined the time course of FEV<sub>1</sub> over 4 years in patients with COPD who were allocated to two groups: either <2% or ≥2% blood eosinophils. They observed an accelerated decline in the group with eosinophil levels ≥2% at baseline, while those with eosinophil levels <2% had a significantly slower decline in FEV<sub>1</sub> and a larger increase in forced vital capacity and residual volume.<sup>49</sup> Conversely, the Hokkaido COPD Cohort Study Group investigators reported that in patients with COPD, lower BEC at baseline was associated with a rapid decline in FEV<sub>1</sub> over a 10-year period.<sup>50</sup> However, an analysis of the UK Clinical Practice Research Datalink (primary care records) and Hospital Episode Statistics (hospital records) showed that the rate of FEV<sub>1</sub> change was not significantly different when stratified by eosinophil level.<sup>51</sup> Furthermore, data on two large cohorts of well-characterized patients with COPD showed that lung function tests were similar in patients irrespective of eosinophil level or longitudinal eosinophil level behaviour.<sup>44</sup> It has been documented that peripheral eosinophil count is a poor reflection of lung function in patients with stable, steroid-naive, mild-to-moderate COPD.<sup>52</sup>

#### Impact on exacerbations

Several studies have suggested that higher blood eosinophil levels during stable disease may indicate a greater risk of exacerbation. The previously mentioned Copenhagen General Population Study documented that in a general population context among individuals with COPD, blood eosinophil levels >340 cells/μL were associated with an increased risk of moderate-to-severe exacerbations.<sup>2</sup> Additionally, in patients with blood eosinophil levels ≥2%, there was a higher rate of severe exacerbations. Furthermore, patients with moderate-to-severe COPD and BECs of ≥300 cells/μL had an increased risk of exacerbations in the COPDGene study, which was prospectively validated in the Evaluation of COPD to Longitudinally Identify Predictive Surrogate Endpoints (ECLIPSE) study (ClinicalTrials Identifier: NCT00292552), although this finding was observed only in patients with frequent exacerbations, but not in patients with 0 or 1 AECOPD in the previous year.<sup>53</sup> A study that used a primary care electronic medical record database in Catalonia, Spain, showed that the number of exacerbations was slightly higher in patients with <150 cells/μL or with ≥500 cells/μL, and in those with higher variability in BECs.<sup>22</sup>

Conversely, persistent BECs ≥300 cells/μL over 2 years were not a risk factor for COPD exacerbations.<sup>45</sup> In the Canadian Cohort Obstructive Lung Disease (CanCOLD) study (ClinicalTrials Identifier: NCT00920348), patients with blood eosinophil levels >3% frequently reported at least one exacerbation in the previous year, and BECs >300 cells/μL were associated with chronic phlegm.<sup>54</sup> In contrast, in patients with COPD and blood eosinophil levels ≥2% (regardless of the cut-off chosen) from the Initiatives BPCO French cohort, there was no difference in exacerbation rate.<sup>55</sup> Also in the SPIROMICS database, BEC alone was not a reliable biomarker for COPD exacerbations.<sup>47</sup>

A retrospective cohort study from the UK Clinical Practice Research Datalink that included patients with COPD showed that it was the history of previous exacerbations that affected the appearance of further exacerbations regardless of the level of blood eosinophils. However, it was also noted that higher blood eosinophil levels were linked with a slightly increased incidence of moderate or severe exacerbations among those with a history of exacerbations.<sup>56</sup> Intriguingly, a historical follow-up study that used longitudinal medical record data to evaluate BECs as a biomarker of exacerbation risk reported that elevated BECs might predict COPD exacerbation risk only in ex-smokers.<sup>57</sup>

Table 1: Conflicting data on the value of blood eosinophil counts in influencing outcomes in chronic obstructive pulmonary disease

	Effect	Reference
<b>Impact on lung function</b>		
Pros	<ul style="list-style-type: none"> <li>• Lower levels of FEV<sub>1</sub> % predicted value with BECs <math>\geq 340</math> cells/<math>\mu</math>L than with low BECs</li> <li>• Higher post-bronchodilator FEV<sub>1</sub> in non-eosinophilic patients than in patients with higher sputum eosinophils</li> <li>• Baseline blood eosinophil levels <math>\geq 2\%</math> associated with accelerated decline in FEV<sub>1</sub></li> <li>• Lower BECs at baseline associated with a rapid decline in FEV<sub>1</sub> over a 10-year period</li> </ul>	2 47 49 50
Cons	<ul style="list-style-type: none"> <li>• FEV<sub>1</sub> reversibility weakly correlated with sputum eosinophil levels</li> <li>• Rate of FEV<sub>1</sub> change not significantly different when stratified by eosinophil level</li> <li>• Lung function tests similar irrespective of eosinophil level or longitudinal eosinophil level behaviour</li> </ul>	47 51 44
<b>Impact on exacerbations</b>		
Pros	<ul style="list-style-type: none"> <li>• BECs <math>\geq 340</math> cells/<math>\mu</math>L associated with an increased risk of moderate-to-severe exacerbations</li> <li>• Blood eosinophil level <math>\geq 2\%</math> associated with a higher rate of severe exacerbations</li> <li>• Increased risk of exacerbations when BECs <math>\geq 300</math> cells/<math>\mu</math>L in moderate-to-severe COPD if patients are frequent exacerbators</li> <li>• At least one exacerbation in the previous year when blood eosinophil level <math>&gt; 3\%</math>, whereas <math>&gt; 300</math> cells/<math>\mu</math>L is associated with chronic phlegm</li> <li>• Elevated BECs predict COPD exacerbation risk only in ex-smokers</li> <li>• Number of exacerbations slightly higher when BEC is <math>&lt; 150</math> cells/<math>\mu</math>L or highly variable</li> <li>• Higher blood eosinophil levels linked with a slightly increased incidence of moderate or severe exacerbations among those with a history of exacerbations</li> </ul>	2 53 54 57 22 56
Cons	<ul style="list-style-type: none"> <li>• Persistent BECs <math>\geq 300</math> cells/<math>\mu</math>L over 2 years not a risk factor for COPD exacerbations</li> <li>• No difference in exacerbation rate with blood eosinophil levels <math>\geq 2\%</math> (regardless the cut-off chosen)</li> <li>• BEC alone is not a reliable biomarker for COPD severity or exacerbations</li> <li>• The history of previous exacerbations affects the appearance of further exacerbations regardless of blood eosinophil levels</li> </ul>	45 55 47 56
<b>Impact on quality of life</b>		
Pros	<ul style="list-style-type: none"> <li>• Blood eosinophil levels <math>\geq 2\%</math> are associated with significantly lower SGRQ scores, suggesting less impact of COPD</li> <li>• Annually measured SGRQ scores improved, mainly in terms of the symptoms and impact domains, in patients with persistently high BEC (<math>\geq 300</math> cells/<math>\mu</math>L) over 3 years, compared with patients with persistently low BEC (<math>&lt; 300</math> cells/<math>\mu</math>L), suggesting less impact of COPD in patients with higher eosinophil counts</li> <li>• Threshold of 67 eosinophils/mL identified for improvement in the SGRQ score in ICS/LABA versus LABA. Patients using ICS/LABA have greater improvements with higher BECs</li> </ul>	45,55 41 58
Cons	<ul style="list-style-type: none"> <li>• No treatment differences between ICS/LABA and any comparator in the change from baseline SGRQ score in either eosinophil subgroup (baseline blood eosinophil level <math>&lt; 2\%</math> and <math>\geq 2\%</math>)</li> </ul>	59
<b>Impact on survival</b>		
Pros	<ul style="list-style-type: none"> <li>• Significantly lower risk of death associated with persistently elevated BECs (<math>\geq 300</math> cells/<math>\mu</math>L) over 2 years</li> <li>• Improved overall survival in patients with a BEC <math>\geq 300</math> cells/<math>\mu</math>L compared with those with a BEC <math>&lt; 300</math> cells/<math>\mu</math>L</li> <li>• Survival period increases with increasing BEC</li> </ul>	44 61 60
Cons	<ul style="list-style-type: none"> <li>• No significant association with all-cause mortality among patients with COPD and absolute BECs <math>\geq 340</math> cells/<math>\mu</math>L versus <math>&lt; 340</math> cells/<math>\mu</math>L</li> <li>• No difference in the risk of on-treatment deaths in patients with COPD regardless of treatment using a blood eosinophil cut-off of <math>&lt; 2\%</math> versus <math>\geq 2\%</math> to categorize patients</li> <li>• Elevated BECs (<math>\geq 200</math> cells/<math>\mu</math>L) not associated with mortality when compared with patients with COPD and decreased BECs</li> </ul>	62 3 63
<b>Impact on incidence of pneumonia</b>		
Pros	<ul style="list-style-type: none"> <li>• More pneumonia events in patients with COPD with eosinophil levels <math>&lt; 2\%</math> than in those with higher counts</li> <li>• Only in severe COPD (FEV<sub>1</sub> <math>&lt; 50\%</math> predicted), BECs <math>\geq 340</math> cells/<math>\mu</math>L associated with high risk of hospitalization due to pneumonia. With FEV<sub>1</sub> <math>\geq 50\%</math> predicted, trend toward significant decrease in the risk of pneumonia</li> </ul>	64 66
Cons	<ul style="list-style-type: none"> <li>• Differences in risk of pneumonia according to BECs not observed in retrospective re-analyses of clinical trials</li> </ul>	58, 67

BEC = blood eosinophil count; COPD = chronic obstructive pulmonary disease; FEV<sub>1</sub> = forced expired volume in one second; ICS = inhaled corticosteroid; LABA = long-acting  $\beta_2$ -agonist; SGRQ = St George Respiratory Questionnaire.

### Impact on quality of life

Some studies have shown that patients with COPD and higher eosinophil levels ( $\geq 2\%$ ) have significantly lower St George Respiratory Questionnaire (SGRQ) scores.<sup>45,55</sup> Furthermore, annually measured SGRQ scores improved in patients with persistently high BECs ( $\geq 300$  cells/ $\mu$ L) over 3 years compared with patients with persistently low BECs ( $< 300$  cells/ $\mu$ L), mainly in terms of symptoms and impact domains.<sup>41</sup> These findings suggest a lower impact of COPD in patients with higher eosinophil counts.

In the retrospective re-analysis of the Foster 48-Week Trial to Reduce Exacerbations in COPD (FORWARD) (ClinicalTrials Identifier: NCT00929851) study that compared 48 weeks of treatment with ICS/LABA (extrafine beclomethasone dipropionate plus formoterol fumarate) versus LABA alone (formoterol fumarate), a threshold for improvement in the SGRQ score of 67 eosinophils/ $\mu$ L was identified and patients using ICS/LABA had greater improvements with higher BECs.<sup>58</sup> This finding contrasts with the results of re-analysed data from three randomized controlled trials of at least 1-year duration comparing ICS or ICS/LABA combination therapy



(with a long-acting muscarinic antagonist [LAMA]), LABA or placebo according to baseline eosinophil categories (baseline blood eosinophil level <2% and ≥2%). These data found no treatment differences for fluticasone propionate/salmeterol versus any comparator in change from baseline SGRQ score in either of the eosinophil subgroups.<sup>59</sup>

### Impact on survival

A significantly lower risk of death has been reported in patients with persistently elevated eosinophils over 2 years than in those whose levels were lower than the predetermined threshold of 300 cells/μL.<sup>44</sup> Utilizing data from the specialty care hospital register of the Hospital District of Southwest Finland, it was found that patients with a BEC ≥300 cells/μL had improved overall survival compared with those with a BEC <300 cells/μL.<sup>60</sup> In particular, an analysis of two different prospective COPD cohort studies in South Korea showed that survival period increased with increasing BEC.<sup>61</sup>

In contrast, there was no significant association with all-cause mortality among patients with COPD with absolute BECs ≥340 cells/μL versus <340 cells/μL in a cohort study conducted using the UK Clinical Practice Research Datalink.<sup>62</sup> Furthermore, a retrospective analysis of data from the ISOLDE study, using blood eosinophil cut-offs of <2% versus ≥2% to categorize patients, found no difference in the risk of on-treatment deaths in patients with COPD regardless of treatment in both eosinophil groups. Elevated BECs (≥200 cells/μL) were not associated with mortality when compared with patients with COPD with decreased BECs.<sup>3,63</sup>

### Impact on incidence of pneumonia

The correlation between reduced eosinophil levels and the incidence of pneumonia remains uncertain. Pavord et al. examined patient-level data from the GlaxoSmithKline clinical trial registry, and observed that patients with COPD with eosinophil levels <2% had more pneumonia events than did those with higher counts.<sup>64</sup> This finding fits well with the documentation that higher sputum eosinophil levels are associated with less bacterial colonization in the stable state and that low BECs (<100 cells/μL) are associated with increased risks of chronic bacterial infection and pneumonia.<sup>25,65</sup>

Conversely, data from the Copenhagen General Population Study showed that in patients with severe COPD (FEV<sub>1</sub> <50% predicted), BEC ≥340 cells/μL was associated with high risk of hospitalization due to pneumonia.<sup>66</sup> However, in patients with FEV<sub>1</sub> ≥50% predicted and blood eosinophilia, there was a trend toward significance in diminishing the risk of pneumonia. Differences in risk of pneumonia according to BECs were not observed in other retrospective re-analyses of clinical trials.<sup>58,67</sup>

### Blood eosinophil counts as predictors of response to inhaled corticosteroids

A number of *post hoc* analyses and meta-analyses of randomized controlled trials have demonstrated a link between higher BECs and ICS effects on AECOPD prevention.<sup>6</sup> However, it has been highlighted that in many studies where blood eosinophils were determined to predict ICS effect, a history of asthma was not systematically excluded.<sup>68</sup>

Nevertheless, Bafadehl et al. found that the effect of ICS/LABA treatment compared to LABA monotherapy on exacerbation prevention was observed at above approximately 100 eosinophils/μL at the start of the study, with increasingly larger benefits at higher BECs.<sup>69</sup> Furthermore, they reported a linear relationship between BEC and FEV<sub>1</sub> and SGRQ total score with LABA/ICS treatment; the minimum clinically important

difference in treatment effect compared with LABA alone occurred at a BEC of 270 cells/μL for FEV<sub>1</sub> and 480 cells/μL for SGRQ total score.

Another *post-hoc* analysis showed that LABA/ICS treatment offers protective effects for clinically important deteriorations, a composite endpoint consisting of three components of COPD worsening (AECOPDs, deteriorations in FEV<sub>1</sub> and increases in SGRQ total score), compared with LABA alone, with the magnitude of the effect dependent on patients' eosinophil levels.<sup>70</sup> In patients with low BECs (<100 cells/μL), the treatment benefit of LABA/ICS versus LABA, and thus the effect of ICS, was poor to minimal. It has also been documented that the protective effect of ICS/LABA/LAMA combination therapy versus LABA/LAMA combination therapy for the risk of moderate or severe AECOPD was greater in patients with higher BECs, but no significant effect modifiers were found for trough FEV<sub>1</sub>.<sup>71</sup>

A meta-analysis of five studies comprising 12,496 patients with moderate-to-very severe COPD suggested not only a modest benefit from ICS-containing treatments versus non-ICS, ICS withdrawal or placebo in reducing the annual rate of moderate/severe exacerbations, but also an increase in the incidence of pneumonia risk in patients with COPD with blood eosinophil levels ≥2% at baseline.<sup>72</sup>

More detailed information was generated by a *post hoc* analysis of the Withdrawal of Inhaled Steroids during Optimized Bronchodilator Management (WISDOM) trial (ClinicalTrials Identifier: NCT00975195), suggesting that a significant increase in the annual exacerbation rate for patients in the ICS-withdrawal group versus the ICS-continuation group can be only observed when the baseline BEC is ≥300 cells/μL or ≥4%.<sup>73</sup> ICS withdrawal in patients with COPD with BECs ≥300 cells/μL was associated with a higher exacerbation risk particularly in patients with a history of at least two exacerbations per year.<sup>74</sup> However, in the Effect of Indacaterol Glycopyrronium versus Fluticasone Salmeterol on COPD Exacerbations (FLAME) study (ClinicalTrials Identifier: NCT01782326), the annual rate of exacerbations was lower with LABA/LAMA versus LABA/ICS, independent of the baseline eosinophil level (<2% versus ≥2%).<sup>75</sup> Prespecified analyses of data from this study did not show a significant difference in the rate of AECOPDs between the LABA/LAMA and LABA/ICS groups at higher baseline eosinophil thresholds (i.e. ≥3%, ≥5% or ≥300 cells/μL).<sup>76</sup> Furthermore, a real-world primary care population, in which continuous ICS users and those who had withdrawn ICSs were stratified by absolute (340 cells/μL as a cut-off value) or relative (4% as a cut-off value) BECs, did not show an increased risk of moderate and/or severe AECOPDs or all-cause mortality among patients with blood eosinophilia who withdrew their use of ICS.<sup>62</sup>

A substantial difference was observed when comparing the data generated by randomized controlled trials with those of the observational studies. In fact, a systematic review of *post hoc* analyses of 11 randomized controlled trials and 5 observational studies that examined the association between three blood eosinophil thresholds (a relative eosinophil count of 2% and absolute counts of 150 cells/μL and 300 cells/μL) and the response of exacerbation risk to ICSs in patients with COPD found that the independent effect of ICSs on the reduction of exacerbation risk was 20% at ≥2% BEC threshold, 35% at ≥150 cells/μL BEC threshold, and 39% at ≥300 cells/μL BEC threshold.<sup>77</sup> However, no association was found in four out of five observational studies. It has been highlighted that almost all data reporting an association between BEC levels and AECOPDs or ICS response come from patients enrolled in randomized controlled trials enriched for a prior history of frequent or severe exacerbation.<sup>51</sup>

In a large British primary care cohort of patients with COPD, prevalent ICS use was associated with slower rates of FEV<sub>1</sub> decline in COPD regardless of blood eosinophil level.<sup>78</sup> Another study, that stratified ICS users and non-users with COPD into two groups based on baseline eosinophil levels ( $\geq 2\%$  and  $< 2\%$ ), found that response to bronchodilators, in terms of trough FEV<sub>1</sub>, dyspnoea and health-related quality of life, was similar in both groups.<sup>67</sup>

As already mentioned, blood eosinophil levels present significant variability throughout the course of COPD; therefore, a single measurement may not be a reliable predictor of ICS response considering that corticosteroids are able to suppress eosinophils, at least in the sputum of patients with COPD.<sup>15,46</sup> However, an analysis of the data from the InforMing the Pathway of COPD Treatment (IMPACT) trial (ClinicalTrials Identifier: NCT02164513) suggested that two blood eosinophil measurements do not appear to provide additional information to predict ICS response in COPD versus one value.<sup>79</sup>

A *post hoc* analysis of the Inhaled Steroids in Obstructive Lung Disease in Europe (ISOLDE) study has recently shown that in eosinophil suppression of  $\geq 200$  cells/ $\mu$ L, ICS use was associated with a decelerated FEV<sub>1</sub> decline rate by 32 mL/year, and a 30% reduction in the exacerbation rate. In contrast, in patients experiencing an increase in eosinophils of  $\geq 200$  cells/ $\mu$ L, ICS use was associated with an accelerated FEV<sub>1</sub> decline rate by 37 mL/year and an increased exacerbation rate by 80%.<sup>80</sup> Eosinophil change was not predictive of clinical response with regard to health status evaluated using SGRQ.

## Conclusion

Although there is increasing pressure for the use of BEC in COPD (justified by some interesting information) we agree that the evidence supporting BEC as a biomarker in patients with COPD is still too weak.<sup>35,81,82</sup> In fact, not only is the literature rich in contrasting data, but there are still fundamental points that need to be clarified before we can make sound judgement.

The pooled analysis of the Benralizumab Efficacy in Moderate to Very Severe Chronic Obstructive Pulmonary Disease with Exacerbation History (GALATHEA; ClinicalTrials Identifier: NCT02138916) and Efficacy and Safety of Benralizumab in Moderate to Very Severe Chronic Obstructive Pulmonary Disease with Exacerbation History (TERRANOVA; ClinicalTrials Identifier: NCT02155660) phase III trials was designed to evaluate the efficacy and safety of benralizumab (which targets IL-5 receptor  $\alpha$  and depletes eosinophils through antibody-dependent cellular cytotoxicity) for patients with moderate-to-very severe COPD. In patients with eosinophilic inflammation (BECs  $\geq 220$  cells/ $\mu$ L) and an increased risk of AECOPDs it was shown that elevated BEC alone was insufficient to identify treatment effect with anti-eosinophil therapy.<sup>83</sup> In fact, not only when coupled mainly with three or more AECOPDs in the previous year, but also with post-bronchodilator FEV<sub>1</sub>  $< 40\%$  of predicted normal, or post-bronchodilator response to SABAs of  $\geq 15\%$ ,

the baseline elevated blood eosinophils were strong predictors of treatment effect for AECOPD rate reduction with benralizumab.

The finding that eosinophil depletion has a minimal effect on AECOPD rate suggests that eosinophil depletion is unlikely to ameliorate exacerbation outcomes for the majority of patients with COPD. Furthermore, the effect of corticosteroids may not be related to the decrease in eosinophils in the blood, but rather to their multiple actions in the most severe forms of COPD when combined with a LABA.<sup>84</sup> According to Miravittles et al., blood eosinophils may be a reasonable predictor of risk only in patients with COPD who have frequent exacerbations; however, as already mentioned, there was a marked difference in the incidence rate of AECOPD in those with previous AECOPD compared with those with no such history, regardless of eosinophil levels.<sup>22,56</sup> Nevertheless, the evidence generated by the re-analysis of the ISOLDE results by baseline BEC, supporting the possible efficacy of ICSSs in susceptible patients in whom the eosinophil count decreased after the oral corticosteroids, complicates matters further.<sup>3</sup>

Unfortunately, we have not yet understood whether eosinophils are causally related in the pathogenesis of a patient's AECOPD risk or whether it is just an epimarker of other biological processes that predispose patients to increased exacerbation risk. However, this latter hypothesis contrasts with the documentation that in patients with significant emphysema in high-resolution computed tomography there were lower blood eosinophil levels, and these differences were present irrespective of frequent exacerbation history or the use of ICSSs.<sup>85</sup>

It is likely that the substantial contrast between randomized controlled trial data and real-world studies adds to the uncertainty regarding the value of BEC as a biomarker in COPD. A recent study examined the association between blood eosinophil levels and the subsequent rate of AECOPDs in a population-based cohort of patients with COPD managed in primary care. The study reported that a history of AECOPD and ICS use appeared to be associated with the impact of blood eosinophil levels on the rate of AECOPD.<sup>86</sup> In particular, this study suggests that ICS use may diminish the association between blood eosinophil levels and AECOPD risk, and undermines the importance of ICS-containing therapies in patients with high blood eosinophil levels. It is well known that a large percentage of patients are still inappropriately prescribed ICSSs for the management of COPD; therefore, it cannot be excluded that this incongruous use may cause the eosinophil value to differ from that found in randomized controlled trials in which there is tight control over treatments and the outcome measures are collected prospectively and carefully.<sup>87</sup>

Nonetheless, although there is a seemingly well-established conviction that studies have firmly supported BECs as a prognostic biomarker and a predictor of response to ICSSs, we believe that the use of blood eosinophil level as a valuable biomarker to guide COPD treatment in clinical practice, remains largely unsupported.<sup>81,88</sup> □

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