

Clinical Management Review

Eosinophils in COPD—Current Concepts and Clinical Implications

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In recent years, heterogeneity in chronic obstructive pulmonary disease (COPD) inflammatory patterns has been recognized as a basis for more precise treatment interventions because current therapies have limited effectiveness. Eosinophilic airway inflammation in COPD has become a subject of research interest as a potential treatment target for inhaled corticosteroid therapy. However, the role of eosinophils in COPD is still unclear, and it is unknown why only some patients with COPD develop eosinophilic airway inflammation. Induced sputum analysis is the most common method of assessing the type of airway inflammation. Accessibility to sputum induction, however, is limited in clinical practice, and blood eosinophils have been proposed to serve as a surrogate marker and treatment guide. Blood eosinophil count has been shown to poorly predict sputum eosinophilia, and, moreover, it seems to be fairly unstable and affected by various factors. Nevertheless, in several trials, blood eosinophil count appeared to predict good response to inhaled corticosteroids. However, biologics targeting eosinophils do not appear to be effective in COPD. In this review, we briefly summarize the current knowledge on eosinophils in COPD pathogenesis. Then, we discuss the use of blood eosinophil count in COPD in relation to the recent Global Initiative for Chronic Obstructive Pulmonary Disease recommendations, their ability to predict sputum eosinophilia, and their potential role in guiding treatment. © 2020 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)

Key words: COPD; inflammation; eosinophil; biomarker

INTRODUCTION

Heterogeneity in inflammatory patterns in chronic obstructive pulmonary disease (COPD) has recently gained considerable attention.¹ The growing interest in identifying the inflammatory patterns is closely related to the concept of treatable traits, that is, the features of the disease that could be a target of a tailored therapeutic intervention.² These targets can be similar in different diseases and their identification would allow specific treatment to those patients for whom it would be most beneficial and to avoid it in cases where the benefits would be insignificant and/or the risk of side effects high.

Eosinophilic airway inflammation, a typical feature of asthma, has also been proposed as a treatable trait in some patients with COPD. This inflammatory pattern can be found in patients with COPD either as a result of a coexisting asthma (asthma-COPD overlap) or poorly understood mechanisms that may lead to airway eosinophilia in patients with COPD without any features of asthma. Depending on the diagnostic criteria, asthma-COPD overlap is present in 3.8% (Spanish criteria) to 18.4% (clinician's diagnosis) of patients with obstructive lung diseases (both COPD and asthma),³ whereas eosinophilic COPD constitutes 32% to 40% of the COPD population.⁴⁻⁷ Over the past decade, a number of studies have investigated eosinophilic airway inflammation in COPD as a promising target for inhaled corticosteroid (ICS) therapy. It has been documented that in patients with COPD, ICS titration to normalize sputum eosinophil count resulted in a reduction of a severe exacerbation rate.⁸ However, sputum analysis is not widely available and requires specific technical expertise. Therefore, blood eosinophil count has been extensively evaluated as a surrogate marker of eosinophilic airway inflammation because it is easily accessible. This review presents current concepts on eosinophilic COPD and the potential of blood eosinophils to serve as a biomarker and predictor of a favorable response to therapeutic intervention.

EOSINOPHILIC AIRWAY INFLAMMATION IN COPD Eosinophil correlations between different respiratory samples

Airway inflammation can be defined on the basis of evaluation of various airway samples, for example, induced sputum (IS), bronchial mucosa biopsy, or bronchoalveolar lavage fluid (BALF).^{9,10} The eosinophil count and percentage vary among different respiratory compartments,¹¹ and therefore, different thresholds for elevated sputum levels have been established for each type of sample. Sputum eosinophilia is usually defined as 3% or more eosinophils of all nonepithelial sputum cells,¹² whereas the increased eosinophil percentage in the BALF is defined as more than 1%.¹³

The relationship between eosinophil counts in different compartments is poor. A study by Hartjes et al demonstrated a weak correlation between the eosinophil counts in the IS and bronchial biopsy.¹⁴ This correlation could be related to the fact that IS and

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Abbreviations used

BALF- Bronchoalveolar lavage fluid
 COPD- Chronic obstructive pulmonary disease
 GOLD- Global Initiative for Chronic Obstructive Pulmonary Disease
 ICS- Inhaled corticosteroid
 IS- Induced sputum
 LABA- Long-acting β_2 -agonist
 LAMA- long-acting muscarinic antagonist
 MMP-12- Matrix-metalloproteinase-12
 SGRQ- St George's Respiratory Questionnaire

bronchial biopsy represent adjacent compartments. In turn, the eosinophil counts in BALF and bronchial biopsies do not seem to correlate.^{10,11,14} Because the acquisition of IS, BALF, and bronchial biopsy requires expertise and is contraindicated in patients with a severe or unstable disease, peripheral blood sampling and assessment of blood eosinophil counts became a subject of considerable interest.

Phenotypes of pulmonary eosinophils

Heterogeneity of eosinophils in humans has been described as early as in the 1980s.^{15,16} Two types of eosinophils were reported: normodense and hypodense. Hypodense eosinophils were found in increased numbers in blood in hypereosinophilic diseases. It was hypothesized that they derived from immature cells released from the bone marrow, presented a greater cytotoxic activity, and were metabolically more active compared with normodense eosinophils. However, the role of these 2 eosinophil types in eosinophilic COPD has not yet been established.^{15,16} More recently, Mesnil et al¹⁷ in a murine model of allergic inflammation characterized 2 distinct types of eosinophils in the lungs. Eosinophils located in the lung parenchyma, referred to as residual eosinophils, had a ring-shaped nucleus, were independent from IL-5, and manifested L-selectin expression. It has been suggested that residual eosinophils could be involved in maintaining immune homeostasis in the lungs by hindering T_H2 -related responses. In contrast, eosinophils found in the peribronchial area and in BALF (inflammatory eosinophils) had a segmented nucleus, were IL-5-dependent and L-selectin negative, and had a high surface expression of CD101. It is possible that inflammatory eosinophils observed by Mesnil et al and activated hypodense eosinophils could represent the same eosinophil phenotype that is involved in inflammatory response. Mesnil et al also described differences in the eosinophil characteristics between residual eosinophils in lung parenchyma from those without asthma and inflammatory eosinophils found in the sputum of patients with asthma. The differences were similar to those found in the murine model.¹⁷ However, it must be specified that the human material used to assess the eosinophils in the lung parenchyma was taken from the healthy margins of lung carcinoma of those without asthma¹⁷ and, therefore, does not fully represent the molecular environment in healthy lungs.

Biological mechanisms involved in eosinophilic airway inflammation in COPD

Eosinophilic airway inflammation is less common and less pronounced in patients with COPD than in patients with

asthma,^{4,5,18} and its mechanism is still not well understood. It was reported that impaired function of macrophages to clear apoptotic eosinophils in COPD led to the release of intracellular proinflammatory cytokines and was associated with an increased severity and frequency of exacerbations.¹⁹ Thymic stromal lymphopoietin and IL-33, secreted by epithelial cells, are involved in recruitment and activation of T_H2 and type 2 innate lymphoid cells (Figure 1).²² It was demonstrated that both patients with COPD and patients with asthma have an increased expression of IL-33 in bronchial mucosa compared with control subjects.¹⁸ Moreover, higher IL-33 concentrations in sputum or blood were found in patients with COPD with sputum eosinophilia.²³ T_H2 cells, together with type 2 innate lymphoid cells, produce numerous T_H2 cytokines, particularly IL-4, IL-5, and IL-13. IL-5 stimulates recruitment and activation of eosinophils and leads to tissue eosinophilia. In a murine model, IL-33 was shown to induce IL-13 expression in airway eosinophils.²⁴ In turn, eosinophil-derived IL-13 was found to stimulate alveolar macrophages to produce matrix-metalloproteinase-12 (MMP-12).^{25,26} Matrix-metalloproteinases are proteases that cause destruction of the alveolar septa and in consequence lead to emphysema.²⁵ A higher level of sputum MMP-12 was found in patients with eosinophilic COPD with coexisting emphysema compared with patients with noneosinophilic COPD without emphysema.²⁵ Taking into account the fact that both patients with COPD and patients with asthma have increased sputum MMP-12 levels and that the relationship between sputum MMP-12 level and the extent of emphysema was found only in patients with COPD,²⁷ it is likely that MMP-12 could act differently in both diseases.

Clinical characteristics of patients with COPD with eosinophilic airway inflammation

There are limited data on the potential differences in clinical characteristics of patients with COPD with eosinophilic and neutrophilic airway inflammation. Studies that characterized patients with high and low sputum eosinophil counts yielded conflicting results. Some authors reported higher postbronchodilator FEV₁ in noneosinophilic patients compared with patients with higher sputum eosinophils.^{5,28} In contrast, in other studies there were no differences in the severity of airway obstruction between eosinophilic and non-eosinophilic patients.^{7,29} However, Chou et al²⁹ found significantly higher sputum eosinophil percentages in patients with very positive bronchodilator reversibility (defined as FEV₁ increase >15% and >400 mL) (median, 6.11%) than in patients without very positive bronchodilator reversibility (median, 1.02%).²⁹ In contrast, differences in sputum eosinophil levels in relation to the presence of positive bronchodilator reversibility (FEV₁ increase >12% and >200 mL) were not found.^{29,30} In a different study, in both patients with COPD and patients with asthma, the postbronchodilator change was lower with the increasing sputum eosinophil count.³¹ In contrast to asthma,³² the relationship between bronchial hyperresponsiveness and eosinophils in COPD is still equivocal.^{31,33} However, a comprehensive discussion of this topic is beyond the scope of this review.

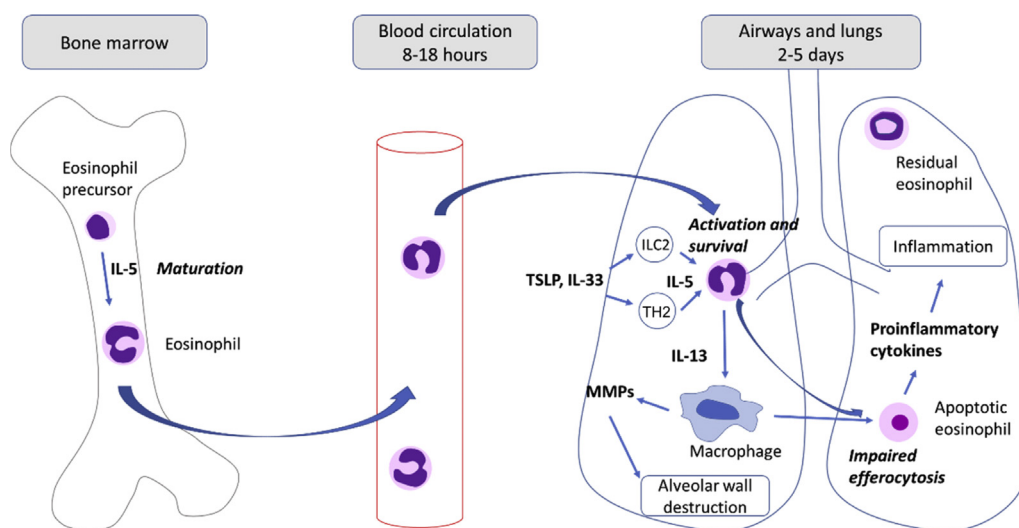


FIGURE 1. Eosinophil life cycle in the bone marrow, blood, airways, and lungs. Eosinophil maturation in the bone marrow is mediated by IL-3, IL-5, and GM-CSF. Then, mature eosinophils enter the blood circulation on the way to tissues. There is a significant difference between the duration of eosinophil stay in the circulation, which lasts between 8 and 18 hours, and their survival time in tissues, which may be up to several days.²⁰ Survival of eosinophils in the tissues can be enhanced by various cytokines (eg, IL-3 and IL-5), and this effect is mediated by NF- κ B–induced upregulation of Bcl-xL.²¹ It is worth noting that the number of eosinophils in the tissues is about 100-fold greater than in the blood, and blood circulation serves merely as a track from the bone marrow to the tissues.²⁰ Therefore, it is rather questionable whether blood eosinophil counts have the power to predict airway eosinophil levels. *ILC2*, Type 2 innate lymphoid cell; *MMPs*, matrix-metalloproteinases; *TSLP*, thymic stromal lymphopoietin.

PERIPHERAL BLOOD EOSINOPHILS IN COPD

Current Global Initiative for Chronic Obstructive Pulmonary Disease recommendations on the role of blood eosinophils in the treatment of COPD

The latest Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) 2020 report recommends using blood eosinophil counts to guide treatment in patients with COPD, for both new diagnoses classified as GOLD D and for those who exacerbate despite bronchodilatation (Figure 2).³⁴ It has been proposed that in patients with COPD GOLD D starting therapy, a cutoff of greater than or equal to 300 cells/ μ L should identify those who may benefit from ICS/long-acting β_2 -agonist (LABA) combination. In patients in whom treatment with bronchodilators is not adequate to prevent exacerbations, an addition of ICSs should be considered depending on the number of exacerbations and the eosinophil count. Among patients with only 1 exacerbation per year, those with eosinophils greater than or equal to 300 cells/ μ L have the greatest likelihood to benefit from a treatment regimen that includes ICSs. In turn, when there are at least 2 moderate or 1 severe exacerbations per year observed, a cutoff of greater than or equal to 100 cells/ μ L may indicate a benefit from ICS treatment. A cutoff of less than 100 cells/ μ L however could select patients who are less likely to benefit from ICSs and therefore should have their treatment modified, for example, by considering roflumilast or azithromycin as an add-on therapy. Importantly, the cutoff of 100 cells/ μ L lies within the normal range for both general COPD population^{7,35,36} and frequent exacerbators.³⁷ That means that according to GOLD 2020 recommendations, ICS treatment should be considered for most patients with exacerbations. In turn, this could lead to prescription of ICSs for patients who

might not profit from this treatment. The prevalence of frequent exacerbations in patients with blood eosinophil count greater than or equal to 100 cells/ μ L is unknown, but it is estimated that approximately 10% of patients with COPD with eosinophilia greater than or equal to 300 cells/ μ L have at least 2 exacerbations.³⁷

Prevalence and stability of elevated blood eosinophil count in COPD

The prevalence of high peripheral eosinophil levels in patients with COPD varies across the studies because of, for example, different thresholds (Table 1) or different inclusion criteria. Importantly, peripheral eosinophilia could be present in other nonpulmonary disorders, for instance, in helminth infections and in various allergic diseases.⁴⁵ It seems however that peripheral eosinophilia in COPD has a similar prevalence to that in the general population. For comparison, 24% and 65.5% of subjects older than 40 years with normal lung function had eosinophil levels greater than or equal to 300 cells/ μ L and greater than 2%, respectively.⁴⁶ These data may suggest that there is no increased eosinophil recruitment to the blood circulation in COPD.

If blood eosinophil counts are to be used as a guide for ICS therapy in COPD, the stability of blood eosinophil counts seems to be an important issue. It has been shown that blood eosinophil counts in asthma are variable over time.⁴⁷ In COPD, unstable eosinophilia was reported in as many as 40.5% (threshold ≥ 300 cells/ μ L)⁴⁸ to 49% (threshold $\geq 2\%$)⁴⁹ of patients. Consequently, a smaller proportion of patients remains with persistently elevated or lower eosinophil counts. Over a 4-year observation, 19% of patients with COPD with eosinophils less than or equal to 2% and only 4% of patients with eosinophils

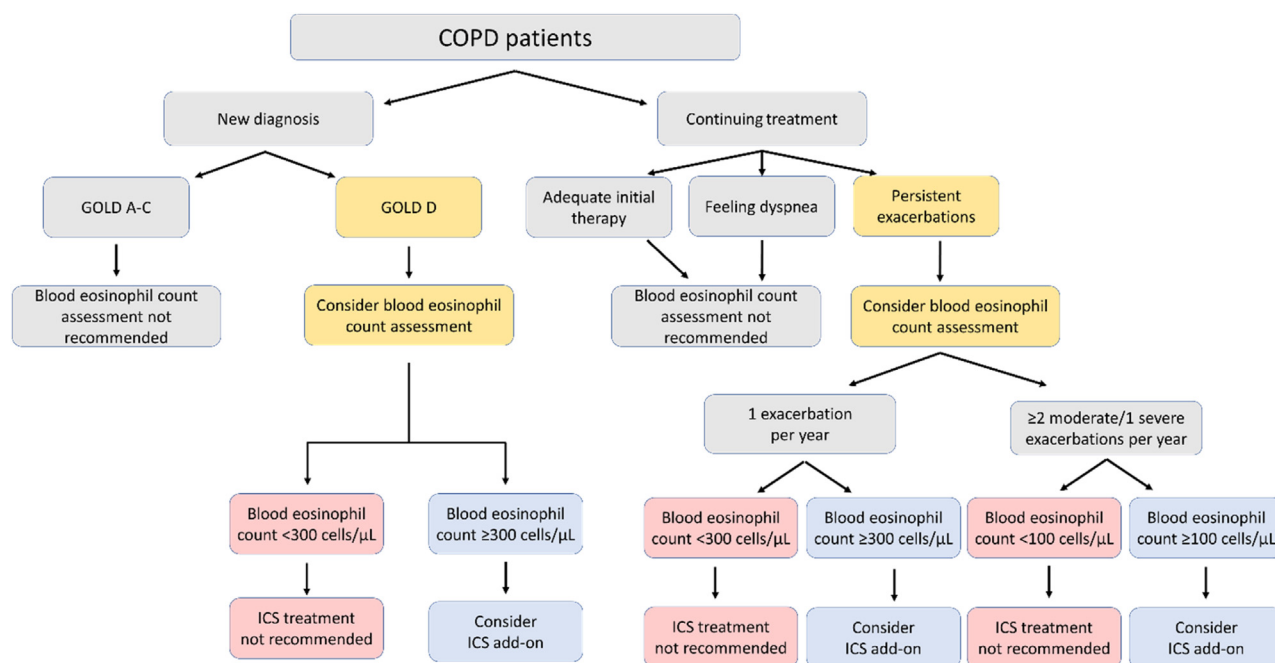


FIGURE 2. Current COPD GOLD recommendations on the blood eosinophil count assessment to guide treatment with ICSs.

TABLE I. Prevalence of high blood eosinophil counts for different thresholds in patients with COPD

	Threshold for high blood eosinophil count			
	150 cells/μL	300 cells/μL	2%	3%
Prevalence	52% ³⁸	22% ⁴⁰	32% ⁴³	37% ⁴⁰
	57% ³⁹	24% ⁴²	57% ⁴⁴	45% ⁴²
	61% ⁴⁰		61% ^{40,44}	
	67% ⁴¹		66% ⁴¹	
			68% ⁴	
			74% ⁴²	
			75% ⁴⁴	

*Persistent eosinophilia after prednisone.

greater than 2% stayed in their respective groups.⁵⁰ The stability of blood eosinophil levels in subsequent measurements appears to be better in patients with COPD with lower than with higher eosinophil counts^{48,51-53} and it was shown to be affected by systemic corticosteroids and antibiotics, older age, and male sex but the impact of ICS use or smoking status was negligible.^{40,41,52-54}

Can blood eosinophils predict sputum eosinophilia?

Blood eosinophil counts have been extensively investigated as a surrogate marker for eosinophilic airway inflammation. A positive correlation between sputum and peripheral eosinophils in asthma has been documented in several studies.^{43,44,55-59} In COPD, however, the existing data on that correlation are equivocal. This might be partly explained by technical issues, for example, differences in sputum acquisition and analysis in multicenter studies or different inclusion criteria. Positive findings have been shown in several studies.^{6,28} However, it must be noted that they included patients with coexisting asthma^{6,28} or patients classified as GOLD 0, that is, patients having respiratory

symptoms but normal spirometry.²⁸ In studies that included patients with pure COPD, the presence and strength of correlation seem additionally dependent on the severity of the disease. In stable mild to moderate COPD, no such correlation was observed.^{7,55} Moreover, although in moderate to severe disease a weak correlation was found, peripheral eosinophils could not predict sputum eosinophilia.^{14,35} In contrast, during COPD exacerbations stronger correlations between sputum and blood eosinophil counts were reported and blood eosinophils were able to reliably predict sputum eosinophilia.^{35,58} Despite these rather poor correlations in stable COPD, blood eosinophil counts have been proposed as a marker to guide ICS treatment.

WHAT IS THE EVIDENCE THAT BLOOD EOSINOPHILIA IN COPD CAN PREDICT GOOD RESPONSE TO ICS TREATMENT?

The first data on the relationship between blood eosinophil counts and the efficacy of ICS therapy in COPD came from post hoc and retrospective analyses.^{41,44,43,59} Those studies compared the effectiveness of ICS therapy combined with LABA to ICS, LABA, or long-acting muscarinic antagonists (LAMAs) alone or placebo. The end points were exacerbation rate and improvement in FEV₁ and quality of life measured by the St George's Respiratory Questionnaire (SGRQ) or Clinical COPD Questionnaire scores. Recently, the so-called triple therapy (ICS/LAMA/LABA combination) came into the spotlight as a potential treatment in patients with severe symptomatic COPD who are at increased risk of exacerbation despite the LAMA/LABA or LABA/ICS therapies. However, in most of the studies on the relationship between the effectiveness of triple therapy and blood eosinophil count published to date, the observation period does not exceed 1 year.^{38,60-62} Differences in study designs, thresholds defining blood eosinophilia, and drugs used in the interventions, and the fact that most analyses were supported

TABLE II. Efficacy of ICSs in reducing exacerbations in patients with COPD with higher and lower eosinophil levels

Threshold for high blood eosinophil count	Efficacy of ICSs in reducing exacerbation rates	
	Elevated blood eosinophil count*	Lower blood eosinophil count†
150 cells/ μ L	• Reduction of moderate to severe exacerbation rate with ICS/LABA vs LABA⁴¹ • No difference in exacerbation rate with LAMA/LABA vs ICS/LABA⁴⁰ • LAMA/LABA more effective in prolonging the time to the first moderate to severe exacerbation compared with ICS/LABA (in the 150-300 cells/μL group)⁴⁰ • Reduction of total moderate to severe exacerbation rates with ICS/LAMA/LABA vs LAMA/LABA or ICS/LABA⁶²	• No reduction in moderate to severe exacerbation rate with ICS/LABA vs LABA⁴¹ • Greater reduction and effectiveness in prolonging the time to the first moderate to severe exacerbation with LAMA/LABA vs ICS/LABA⁴⁰ • Reduction of total moderate to severe exacerbation rates with ICS/LAMA/LABA vs LAMA/LABA or ICS/LABA⁶²
300 cells/ μ L	• No difference in exacerbation rate with LAMA/LABA vs ICS/LABA groups⁴⁰ • No difference in exacerbation rate with LAMA/LABA vs ICS/LABA⁴⁰ • Increased moderate to severe exacerbation rate after ICS withdrawal^{61,66}	• ICS withdrawal has no impact on moderate to severe exacerbation rate^{57,67}
2%	• Reduction in moderate to severe exacerbation rate with ICS/LABA vs LAMA⁴⁴ • LAMA/LABA therapy was more effective in reducing and prolonging the time to the first moderate to severe exacerbation compared with ICS/LABA⁴⁰ • Reduction of moderate to severe exacerbation rate in ICS/LAMA/LABA vs LAMA/LABA groups⁶⁰	• No reduction in moderate to severe exacerbation rate with ICS/LABA vs LAMA⁴⁴ • LAMA/LABA therapy was more effective in reducing and prolonging the time to the first moderate to severe exacerbation compared with ICS/LABA⁴⁰ • No reduction in moderate to severe exacerbation rate in ICS/LAMA/LABA vs LAMA/LABA groups⁶⁰
3%	• No difference in exacerbation rate and time to first exacerbation with LAMA/LABA vs ICS/LABA⁴⁰	• Greater reduction in moderate to severe exacerbation rate in groups with LAMA/LABA vs ICS/LABA⁴⁰ • LAMA/LABA more effective in prolonging the time to the first moderate to severe exacerbation compared with ICS/LABA⁴⁰

*At the level or above the threshold indicated in the first column.

†VBelow the threshold indicated in the first column.

by pharmaceutical companies make the interpretation of data challenging.

The relationship between blood eosinophil count and effect of ICSs on moderate to severe COPD exacerbations

Three prospective studies have shown that patients with COPD with increased baseline blood eosinophil levels (≥ 300 cells/ μ L) were at a greater risk of disease exacerbations.^{42,50,63} However, the baseline eosinophil count does not seem to impact exacerbation severity in hospitalized patients.⁶⁴ Surprisingly, patients with persistent peripheral eosinophilia seem to have a better survival rate in comparison to persistently non-eosinophilic patients.^{48,65} The plausible explanation for a better prognosis in eosinophilic patients is that those patients benefit from ICSs that reduce the exacerbation rate.^{38,39,41,59} Table II presents thresholds for elevated peripheral eosinophil counts that were used in several clinical trials and their ability to identify patients who could benefit from ICSs.

Recognition of eosinophilic inflammatory pattern in patients with COPD has led to the hypothesis that ICSs could be effective in suppressing airway inflammation in patients with COPD with peripheral eosinophilia. Consequently, several post hoc analyses of earlier studies on ICS efficacy in patients with COPD were performed. Pascoe et al⁴¹ showed that only patients with baseline eosinophil count greater than or equal to 2% had a significant reduction in their annual moderate to severe exacerbation rate when treated with ICS/LABA versus LABA monotherapy. The effect was even more pronounced in the groups with blood eosinophils greater than or equal to 4% and greater than or equal to 6%. In a post hoc analysis of the FORWARD study by Siddiqui et al,⁵⁹ an increasingly greater reduction in the exacerbation rate was found in patients with higher blood eosinophil count classified into quartiles. The reduction was significant ($P < .05$) in patients in the third and fourth quartiles (eosinophil count, 181.6–279.8 and $>279.8/\mu$ L, respectively).⁵⁹ The more recent analyses have confirmed that the beneficial effect of ICS/LABA compared with LABA increases with higher eosinophil counts.^{39,67}

Interestingly, the beneficial effect of ICSs on the exacerbation rate does not seem to be present in patients who remained eosinophilic after the course of oral prednisone.⁴³ During a 3-year observation, ICSs were shown to be ineffective in reducing moderate to severe exacerbations in patients with severe COPD and a relatively high eosinophil count ($\geq 2\%$) after a 2-week oral prednisone treatment. In contrast, a significant reduction compared with placebo was observed when ICSs were applied to patients with lower eosinophil percentage ($<2\%$). That could suggest that ICSs might be mainly effective in susceptible patients, in whom the eosinophil count decreased after the oral corticosteroids.

Only a few trials assessed the effect of ICSs added to LAMA/LABA on exacerbation rate in patients with COPD: Informing the Pathway of COPD Treatment (IMPACT, which did not exclude patients with a history of asthma),⁶² TRIBUTE,⁶⁰ and KRONOS³⁸ (the latter 2 excluded patients with asthma). In the IMPACT study, the reduction in the exacerbation rate compared with that found in patients treated with LAMA/LABA was greater in the triple therapy group regardless of the eosinophil level.⁶² The TRIBUTE trial, however, which enrolled patients with more severe COPD compared with the IMPACT study,

showed the reduction in moderate to severe exacerbation rate only in patients with blood eosinophils greater than or equal to 2%, whereas in patients with eosinophils greater than or equal to 200 cells/ μ L or those with lower eosinophil count ($<2\%$ or <200 cells/ μ L) the difference was insignificant.⁶⁰ The moderate to severe exacerbation rate was a secondary outcome of another triple therapy trial—KRONOS.³⁸ Although the study included symptomatic patients with a low exacerbation rate in the preceding year (88% had ≤ 1 exacerbation), it showed that patients with blood eosinophils greater than 75 cells/ μ L treated with ICS/LAMA/LABA had lower exacerbation rates compared with those treated with LAMA/LABA.³⁸ Two different meta-analyses assessed the efficacy of triple therapy versus ICS/LABA⁶⁸ and single (LAMA or LABA) or dual (LAMA/LABA) bronchodilation.⁶⁹ Calzetta et al⁶⁸ found that the efficacy of ICS/LAMA/LABA therapy versus ICS/LABA was not affected by baseline eosinophil counts. In contrast, the meta-analysis by Cazzola et al⁶⁹ showed that the addition of ICSs to bronchodilators provided benefits in eosinophilic patients. ICSs as anti-inflammatory agents have a potential to suppress airway inflammation⁵ and, therefore, might have an additional effect in patients with airway eosinophilia, contrary to LAMAs.

Importantly, it has been demonstrated that ICS withdrawal in patients with COPD with blood eosinophils greater than or equal to 300 cells/ μ L was associated with a higher exacerbation risk.^{61,66} This was particularly pronounced in patients with a history of at least 2 exacerbations per year.⁷⁰ However, more data are needed to fully ascertain the safety of ICS cessation in selected patients with COPD.

Impact on FEV₁

There were several studies that compared lung function in patients with COPD with higher and lower number of blood eosinophils. In general, none of these studies showed significant differences between these 2 groups in terms of FEV₁,^{7,48,71} bronchial reversibility,⁴⁰ or hyperinflation.¹⁴ Similar findings with regard to airway eosinophilia further support the hypothesis that there might be some dissociation between the lung function and eosinophils in COPD.

The impact of ICS therapy on FEV₁ is unclear. Post hoc analysis by Pavord et al⁴⁴ showed that ICSs did not improve FEV₁ in patients with blood eosinophil percentage greater than or equal to 2%. However, Barnes et al⁴³ reported that ICSs reduced the rate of FEV₁ decline in persistently eosinophilic patients ($\geq 2\%$). A recent study by Bafadhel et al⁶⁷ showed that the treatment with higher doses of ICS/LABA was associated with a significant FEV₁ improvement in patients with COPD with blood eosinophil count greater than or equal to 270 cells/ μ L and no history of asthma and that the effect was more pronounced with increasing eosinophil levels. Similar results were found by other authors, who demonstrated that in patients with higher eosinophil blood levels ICS/LABA therapy was associated with a more significant FEV₁ improvement than that found in patients treated with LABA alone.^{39,59} Of note, triple therapy significantly improved prebronchodilator FEV₁ in patients with COPD with peripheral eosinophils greater than or equal to 150 cells/ μ L and without a history of asthma.³⁸

In the recent SUNSET trial, the effect of the treatment deescalation from the triple therapy to dual bronchodilation was assessed in terms of the lung function decline.⁶¹ An important finding in this study was that nonfrequent exacerbators without a

history of asthma but with eosinophil count greater than or equal to 300 cells/ μ L or greater than or equal to 2% had a greater reduction in postbronchodilator FEV₁ after ICS cessation in comparison to patients who continued ICS therapy. In contrast, the study showed that patients with lower eosinophil counts had no decline in FEV₁ after ICS withdrawal.

Quality of life

Although the quality of life has been measured in several trials evaluating the efficacy of ICSs in patients with COPD, only a few of them aimed to relate the results to the blood eosinophil level.^{14,39,44,67} Blood eosinophil percentages were not found to be associated with symptom level in the Clinical COPD Questionnaire score and neither higher nor lower eosinophil percentages (<2% and $\geq$ 2%) were related to the change in the Clinical COPD Questionnaire or SGRQ scores during ICS treatment.^{14,44} However, when absolute eosinophil counts were considered, the clinically important differences in the health status between patients treated with ICS/LABA versus LABA were found at higher eosinophil levels in favor of ICS/LABA.^{39,67} In the analysis by Siddiqui et al³⁹ of the FORWARD study, the SGRQ score was modeled over a continuous range of eosinophils (0-1000 cells/ μ L). The authors demonstrated that a threshold for improvement in the SGRQ score in ICS/LABA versus LABA arms was 67 eosinophils/ μ L and patients using ICS/LABA had greater improvements with higher eosinophil counts.

ICS and the risk of pneumonia

Despite the apparent efficacy of ICSs in reduction of the exacerbation rate in patients with eosinophilic COPD, it has been shown that ICSs have a major drawback—an increased risk of pneumonia^{62,72,73}—which seems to be present irrespective of the blood eosinophil level.⁷⁴ Thus, even in patients with COPD with blood eosinophilia, the positive effects of ICS therapy should be carefully weighed against the risk of pneumonia. In this context, the exacerbation rate and their severity play a major role. In an editorial by Suissa and Ernst,⁷⁴ the authors suggest that the exacerbation risk associated with not applying ICSs outweighs the ICS-related risk of pneumonia only in patients with blood eosinophils greater than 4%. Bearing that in mind, it is reasonable to consider other therapies not containing ICSs in the treatment of eosinophilic COPD. For example, LAMAs were shown to be equally effective as ICS/LABA, the latter being superior only in patients with high blood eosinophil count (>4% or >300 cells/ μ L).⁷⁵ Moreover, the FLAME study demonstrated that dual bronchodilation (LAMA/LABA) was superior to ICS/LABA in terms of reducing the annual exacerbation rate and prolongation of the interval to the first moderate to severe exacerbation in both patients with higher ($\geq$ 2%) and lower (<2%) blood eosinophil count.^{40,76}

However, it is unclear whether elevated blood eosinophil levels in patients with COPD are associated with pneumonia susceptibility independently of ICSs. In a study by Vedel-Krogh et al,⁷⁷ blood eosinophilia ($\geq$ 340 cells/ μ L) in patients with COPD with predicted FEV₁ less than 50% was associated with a higher risk of pneumonia. The risk ceased to be significant after exclusion of patients using ICSs. Moreover, in patients with predicted FEV₁ greater than or equal to 50% and blood eosinophilia, there was a trend toward significance in diminishing the risk of pneumonia, suggesting that eosinophils might have, in fact, a protective

role.⁷⁸ Of note, ICS-related pneumonia in patients with COPD is not associated with increased mortality.⁷⁹

COULD ASTHMA BIOLOGICS BE EFFECTIVE IN EOSINOPHILIC COPD?

Because eosinophils might play a role in COPD pathogenesis, it has been investigated whether asthma biologics targeting eosinophils could be effective in COPD. Clinical trials to date have studied the efficacy of several mAbs in COPD: anti-IL-5 (mepolizumab), anti-IL-5 receptor (benralizumab) and anti-IL-13 (lebrikizumab).

The analysis by Pavord et al⁸⁰ of 2 trials, METREX and METREO, aimed to evaluate the efficacy of mepolizumab as an add-on to triple therapy in patients with eosinophilic COPD with exacerbation history. Eosinophilic COPD was defined as having greater than or equal to 150 blood eosinophils/ μ L at baseline or greater than or equal to 300 blood eosinophils/ μ L in the previous year. Study participants were randomised to receive 100 mg mepolizumab or placebo in the METREX study and 100 mg mepolizumab, 300 mg mepolizumab, or placebo in the METREO study. The primary end point, that is, the reduction in the exacerbation rate, was met only in the METREX study (annual exacerbation rate was 1.40 in the mepolizumab group and 1.71 in the placebo group; rate ratio, 0.82; 95% CI, 0.68-0.98; adjusted $P = .04$). In the METREO study, the reduction in exacerbation rate was not found to be significant (annual exacerbation rate was 1.19 in the 100 mg mepolizumab group, 1.27 in the 300 mg mepolizumab group, and 1.49 per year in the placebo group).

In a prospective study by Brightling et al,⁸¹ benralizumab was shown to reduce eosinophil numbers in blood and sputum in patients with moderate to severe COPD with no history of asthma. The study population included patients who had sputum eosinophilia ($\geq$ 3%) and had at least 1 moderate to severe exacerbation in the year preceding the study onset, regardless of the underlying inhaled therapy. Although the reduction in eosinophil levels did not lead to a reduction in the exacerbation rate, an improvement in both prebronchodilator and post-bronchodilator FEV₁ was observed in the group treated with 100 mg benralizumab versus placebo. In more recent GALATHEA and TERRANOVA studies, benralizumab or placebo was administered to patients with COPD with moderate to severe airflow limitation and exacerbation history who were on dual or triple inhaled therapy.⁸² In a pooled post hoc analysis of those studies, 30 mg and 100 mg benralizumab was shown to be ineffective in reducing the annual exacerbation rate in patients with elevated eosinophil count ($\geq$ 220/ μ L). However, further exploration of possible cocharacteristics has identified subgroups that could benefit from treatment with benralizumab. It was observed that in patients with elevated eosinophil count who had 1 of the following: (1) at least 3 exacerbations in the previous year, (2) post-FEV₁ less than 40% predicted values, or (3) postbronchodilator response greater than or equal to 15%, 100 mg benralizumab significantly reduced the exacerbation rate versus placebo. In contrast, a lower dose of benralizumab (30 mg) showed no such treatment effect. However, the findings from GALATHEA and TERRANOVA studies need to be tested prospectively.

Lebrikizumab has been assessed in patients with COPD with exacerbations despite ICS treatment (NCT02546700).

However, preliminary data suggest that it does not have an effect on exacerbation rate versus placebo.⁸³ Studies that investigated other mAbs, that is, anti-IL-4 and anti-IL-13 (dupilumab) and anti-IL-33, are still at recruitment stage.

The results of the trials with biological therapies give us further insight into the potential role of eosinophils and show the complexity of the association between eosinophilic airway inflammation and a clinical course of COPD. The benefit of anti-IL-5(R) therapy in exacerbation reduction was not consistent between the studies and generally, blood eosinophil counts *per se* had poor predictability of good outcomes with biological therapies. It seems that some additional factors might impact the efficacy of those therapies in patients with eosinophilic COPD, for example, frequent (≥ 3 per year) exacerbations or poor lung function. Moreover, it is possible that response to biologics could depend on eosinophil activation status.

PERSPECTIVES AND SUMMARY

In recent years, eosinophils have gained a considerable interest as one of the important factors involved in COPD pathogenesis and as a potential biomarker. It is still largely unknown why only a certain proportion of patients with COPD have eosinophilic airway inflammation and how eosinophils impact the course of the disease. The subpopulations identified as potentially benefiting need to be prospectively studied.

It seems that the role of eosinophils in COPD pathogenesis is different from that in asthma. Further studies should not only consider eosinophil levels in different compartments when assessing airway inflammation but also address the activation status or phenotypes of pulmonary eosinophils, which might be the key to better understanding of the role of these cells in COPD. It seems important to compare the levels of different eosinophil phenotypes in the airways, as well as in the peripheral blood, in eosinophilic COPD and asthma. Moreover, it is unknown how ICSs impact eosinophils in patients with COPD. It was shown that ICSs have a small impact or none on the peripheral eosinophil counts. Blood eosinophils are variable over time and are affected by various factors, yet it appears that elevated eosinophils could reliably predict the profile of frequent COPD exacerbators who benefit from ICSs.

REFERENCES

- Barnes PJ. Inflammatory endotypes in COPD. *Allergy* 2019;74:1249-56.
- McDonald VM, Fingleton J, Agusti A, Hiles SA, Clark VL, Holland AE, et al. Treatable traits: a new paradigm for 21st century management of chronic airway diseases: Treatable Traits Down Under International Workshop report. *Eur Respir J* 2019;53:1802058.
- Barczyk A, Maskey-Warzechowska M, Gorska K, Barczyk M, Kuziemski K, Sliwinski P, et al. Asthma-COPD overlap—a discordance between patient populations defined by different diagnostic criteria. *J Allergy Clin Immunol Pract* 2019;7:2326-2336.e5.
- Kim VL, Coombs NA, Staples KJ, Ostridge KK, Williams NP, Wootton SA, et al. Impact and associations of eosinophilic inflammation in COPD: analysis of the AERIS cohort. *Eur Respir J* 2017;50:1700853.
- Leigh R, Pizzichini MM, Morris MM, Maltais F, Hargreave FE, Pizzichini E. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. *Eur Respir J* 2006;27:964-71.
- Negewo NA, McDonald VM, Baines KJ, Wark PA, Simpson JL, Jones PW, et al. Peripheral blood eosinophils: a surrogate marker for airway eosinophilia in stable COPD. *Int J Chron Obstruct Pulmon Dis* 2016;11:1495-504.
- Proboszcz M, Mycroft K, Paplinska-Goryca M, Gorska K, Nejman-Gryz P, Jankowski P, et al. Relationship between blood and induced sputum eosinophils, bronchial hyperresponsiveness and reversibility of airway obstruction in mild-to-moderate chronic obstructive pulmonary disease. *COPD* 2019;16:354-61.
- Siva R, Green RH, Brightling CE, Shelley M, Hargadon B, McKenna S, et al. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. *Eur Respir J* 2007;29:906-13.
- George L, Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. *Ther Adv Chronic Dis* 2016;7:34-51.
- Gorska K, Maskey-Warzechowska M, Nejman-Gryz P, Korczynski P, Prochorec-Sobieszek M, Krenke R. Comparative study of periostin expression in different respiratory samples in patients with asthma and chronic obstructive pulmonary disease. *Pol Arch Med Wewn* 2016;126:124-37.
- Rutgers SR, Timens W, Kaufmann HF, van der Mark TW, Koeter GH, Postma DS. Comparison of induced sputum with bronchial wash, bronchoalveolar lavage and bronchial biopsies in COPD. *Eur Respir J* 2000;15:109-15.
- Weiszhar Z, Horvath I. Induced sputum analysis: step by step. *Breathe* 2013;9:300-6.
- Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. *Am J Respir Crit Care Med* 2012;185:1004-14.
- Hartjes F, Vonk JM, Faiz A, Hiemstra PS, Lapperre TS, Kerstjens HAM, et al. Predictive value of eosinophils and neutrophils on clinical effects of ICS in COPD. *Respirology* 2018;23:1023-31.
- Fukuda T, Gleich GJ. Heterogeneity of human eosinophils. *J Allergy Clin Immunol* 1989;83:369-73.
- Shult PA, Lega M, Jadidi S, Vrtis R, Warner T, Graziano FM, et al. The presence of hypodense eosinophils and diminished chemiluminescence response in asthma. *J Allergy Clin Immunol* 1988;81:429-37.
- Mesnil C, Raulier S, Paulissen G, Xiao X, Birrell MA, Pirotin D, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest* 2016;126:3279-95.
- Gorska K, Nejman-Gryz P, Paplinska-Goryca M, Korczynski P, Prochorec-Sobieszek M, Krenke R. Comparative study of IL-33 and IL-6 levels in different respiratory samples in mild-to-moderate asthma and COPD. *COPD* 2018;15:36-45.
- Eltboli O, Bafadhel M, Hollins F, Wright A, Hargadon B, Kulkarni N, et al. COPD exacerbation severity and frequency is associated with impaired macrophage efferocytosis of eosinophils. *BMC Pulm Med* 2014;14:112.
- Park YM, Bochner BS. Eosinophil survival and apoptosis in health and disease. *Allergy Asthma Immunol Res* 2010;2:87-101.
- Schwartz C, Willebrand R, Huber S, Rupec RA, Wu D, Locksley R, et al. Eosinophil-specific deletion of I κ B α in mice reveals a critical role of NF- κ B-induced Bcl-xL for inhibition of apoptosis. *Blood* 2015;125:3896-904.
- Ying S, O'Connor B, Ratoff J, Meng Q, Fang C, Cousins D, et al. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. *J Immunol* 2008;181:2790-8.
- Tworek D, Majewski S, Szewczyk K, Kiszalkiewicz J, Kurmanowska Z, Gorski P, et al. The association between airway eosinophilic inflammation and IL-33 in stable non-atopic COPD. *Respir Res* 2018;19:108.
- Jacobsen EA, Doyle AD, Colbert DC, Zellner KR, Protheroe CA, LeSuer WE, et al. Differential activation of airway eosinophils induces IL-13-mediated allergic Th2 pulmonary responses in mice. *Allergy* 2015;70:1148-59.
- Doyle AD, Mukherjee M, LeSuer WE, Bittner TB, Pasha SM, Frere JJ, et al. Eosinophil-derived IL-13 promotes emphysema. *Eur Respir J* 2019;53:1801291.
- Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ Jr, et al. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 2000;106:1081-93.
- Chaudhuri R, McSharry C, Brady J, Donnelly I, Grierson C, McGuinness S, et al. Sputum matrix metalloproteinase-12 in patients with chronic obstructive pulmonary disease and asthma: relationship to disease severity. *J Allergy Clin Immunol* 2012;129:655-63. e8.
- Hastie AT, Martinez FJ, Curtis JL, Doerschuk CM, Hansel NN, Christenson S, et al. Association of sputum and blood eosinophil concentrations with clinical measures of COPD severity: an analysis of the SPIROMICS cohort. *Lancet Respir Med* 2017;5:956-67.
- Chou K-T, Su K-C, Hsiao Y-H, Huang S-F, Ko H-K, Tseng C-M, et al. Post-bronchodilator reversibility of FEV1 and eosinophilic airway inflammation in COPD. *Arch Bronconeumol* 2017;53:547-53.
- Reid DW, Wen Y, Johns DP, Williams TJ, Ward C, Walters EH. Bronchodilator reversibility, airway eosinophilia and anti-inflammatory effects of inhaled fluticasone in COPD are not related. *Respirology* 2008;13:799-809.
- Gorska K, Krenke R, Korczyński P, Kościuch J, Domagała-Kulawik J, Chazan R. Eosinophilic airway inflammation in chronic obstructive pulmonary disease and asthma. *J Physiol Pharmacol* 2008;59:261-70.

32. Gao J, Zhou W, Chen B, Lin W, Wu S, Wu F. Sputum cell count: biomarkers in the differentiation of asthma, COPD and asthma-COPD overlap. *Int J Chron Obstruct Pulmon Dis* 2017;12:2703-10.
33. Lapperre TS, Snoeck-Stroband JB, Gosman MM, Stolk J, Sont JK, Jansen DF, et al. Dissociation of lung function and airway inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004;170:499-504.
34. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: updated 2020. Available from: <http://www.goldcopd.org>. Accessed January 31, 2020.
35. Kolsum U, Donaldson GC, Singh R, Barker BL, Gupta V, George L, et al. Blood and sputum eosinophils in COPD: relationship with bacterial load. *Respir Res* 2017;18:88.
36. Turato G, Semenzato U, Bazzan E, Biondini D, Tine M, Torrecilla N, et al. Blood eosinophilia neither reflects tissue eosinophils nor worsens clinical outcomes in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2018;197:1216-9.
37. Vogelmeier CF, Kostikas K, Fang J, Tian H, Jones B, Morgan CL, et al. Evaluation of exacerbations and blood eosinophils in UK and US COPD populations. *Respir Res* 2019;20:178.
38. Ferguson GT, Rabe KF, Martinez FJ, Fabbri LM, Wang C, Ichinose M, et al. Triple therapy with budesonide/glycopyrrolate/formoterol fumarate with co-suspension delivery technology versus dual therapies in chronic obstructive pulmonary disease (KRONOS): a double-blind, parallel-group, multicentre, phase 3 randomised controlled trial. *Lancet Respir Med* 2018;6:747-58.
39. Siddiqui SH, Pavord ID, Barnes NC, Guasconi A, Lettis S, Pascoe S, et al. Blood eosinophils: a biomarker of COPD exacerbation reduction with inhaled corticosteroids. *Int J Chron Obstruct Pulmon Dis* 2018;13:3669-76.
40. 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. *Am J Respir Crit Care Med* 2017;195:1189-97.
41. 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;3:435-42.
42. Yun JH, Lamb A, Chase R, Singh D, Parker MM, Saferali A, et al. Blood eosinophil count thresholds and exacerbations in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2018;141:2037-2047.e10.
43. Barnes NC, Sharma R, Lettis S, Calverley PM. Blood eosinophils as a marker of response to inhaled corticosteroids in COPD. *Eur Respir J* 2016;47:1374-82.
44. Pavord ID, Lettis S, Locantore N, Pascoe S, Jones PW, Wedzicha JA, et al. Blood eosinophils and inhaled corticosteroid/long-acting β -2 agonist efficacy in COPD. *Thorax* 2016;71:118-25.
45. Weller PF, Spencer LA. Functions of tissue-resident eosinophils. *Nat Rev Immunol* 2017;17:746-60.
46. DiSantostefano RL, Hinds D, Le HV, Barnes NC. Relationship between blood eosinophils and clinical characteristics in a cross-sectional study of a US population-based COPD cohort. *Respir Med* 2016;112:88-96.
47. Mathur SK, Fichtinger PS, Evans MD, Schwantes EA, Jarjour NN. Variability of blood eosinophil count as an asthma biomarker. *Ann Allergy Asthma Immunol* 2016;117:551-3.
48. 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. *Eur Respir J* 2017;50:1701162.
49. Singh D, Kolsum U, Brightling CE, Locantore N, Agusti A, Tal-Singer R. Eosinophilic inflammation in COPD: prevalence and clinical characteristics. *Eur Respir J* 2014;44:1697-700.
50. Schumann DM, Tamm M, Kostikas K, Stolz D. Stability of the blood eosinophilic phenotype in stable and exacerbated chronic obstructive pulmonary disease. *Chest* 2019;156:456-65.
51. Southworth T, Beech G, Foden P, Kolsum U, Singh D. The reproducibility of COPD blood eosinophil counts. *Eur Respir J* 2018;52:1800427.
52. Oshagbemi OA, Burden AM, Braeken DCW, Henskens Y, Wouters EFM, Driessen JHM, et al. Stability of blood eosinophils in patients with chronic obstructive pulmonary disease and in control subjects, and the impact of sex, age, smoking, and baseline counts. *Am J Respir Crit Care Med* 2017;195:1402-4.
53. 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;14:382-8.
54. Kreindler JL, Watkins ML, Lettis S, Tal-Singer R, Locantore N. Effect of inhaled corticosteroids on blood eosinophil count in steroid-naïve patients with COPD. *BMJ Open Respir Res* 2016;3:e000151.
55. Gorska K, Paplinska-Goryca M, Nejman-Gryz P, Goryca K, Krenke R. Eosinophilic and neutrophilic airway inflammation in the phenotyping of mild-to-moderate asthma and chronic obstructive pulmonary disease. *COPD* 2017;14:181-9.
56. Westerhof GA, Korevaar DA, Amelink M, de Nijs SB, de Groot JC, Wang J, et al. Biomarkers to identify sputum eosinophilia in different adult asthma phenotypes. *Eur Respir J* 2015;46:688-96.
57. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. *J Allergy Clin Immunol* 2015;135:822-824.e2.
58. Bafadhel M, McKenna S, Terry S, Mistry V, Pancholi M, Venge P, et al. Blood eosinophils to direct corticosteroid treatment of exacerbations of chronic obstructive pulmonary disease: a randomized placebo-controlled trial. *Am J Respir Crit Care Med* 2012;186:48-55.
59. 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. *Am J Respir Crit Care Med* 2015;192:523-5.
60. Papi A, Vestbo J, Fabbri L, Corradi M, Prunier H, Cohuet G, et al. Extrafine inhaled triple therapy versus dual bronchodilator therapy in chronic obstructive pulmonary disease (TRIBUTE): a double-blind, parallel group, randomised controlled trial. *Lancet* 2018;391:1076-84.
61. Chapman KR, Hurst JR, Frent SM, Larbig M, Fogel R, Guerin T, et al. Long-term triple therapy de-escalation to indacaterol/glycopyrronium in patients with chronic obstructive pulmonary disease (SUNSET): a randomized, double-blind, triple-dummy clinical trial. *Am J Respir Crit Care Med* 2018;198:329-39.
62. Lipson DA, Barnhart F, Brealey N, Brooks J, Criner GJ, Day NC, et al. Once-daily single-inhaler triple versus dual therapy in patients with COPD. *N Engl J Med* 2018;378:1671-80.
63. Vedel-Krogh S, Nielsen SF, Lange P, Vestbo J, Nordestgaard BG. Blood eosinophils and exacerbations in chronic obstructive pulmonary disease. The Copenhagen General Population Study. *Am J Respir Crit Care Med* 2016;193:965-74.
64. MacDonald MI, Osadnik CR, Bulfin L, Hamza K, Leong P, Wong A, et al. Low and high blood eosinophil counts as biomarkers in hospitalised acute exacerbations of chronic obstructive pulmonary disease. *Chest* 2019;156:92-100.
65. Shin SH, Park HY, Kang D, Cho J, Kwon SO, Park JH, et al. Serial blood eosinophils and clinical outcome in patients with chronic obstructive pulmonary disease. *Respir Res* 2018;19:134.
66. Watz H, Tetzlaff K, Wouters EFM, Kirsten A, Magnussen H, Rodriguez-Roisin R, et al. Blood eosinophil count and exacerbations in severe chronic obstructive pulmonary disease after withdrawal of inhaled corticosteroids: a post-hoc analysis of the WISDOM trial. *Lancet Respir Med* 2016;4:390-8.
67. 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;6:117-26.
68. Calzetta L, Cazzola M, Matera MG, Rogliani P. Adding a LAMA to ICS/LABA therapy: a meta-analysis of triple combination therapy in COPD. *Chest* 2019;155:758-70.
69. Cazzola M, Rogliani P, Calzetta L, Matera MG. Triple therapy versus single and dual long-acting bronchodilator therapy in COPD: a systematic review and meta-analysis. *Eur Respir J* 2018;52:1801586.
70. 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. *Am J Respir Crit Care Med* 2017;196:1219-21.
71. Zysman M, Deslee G, Caillaud D, Chanez P, Escamilla R, Court-Fortune I, et al. Relationship between blood eosinophils, clinical characteristics, and mortality in patients with COPD. *Int J Chron Obstruct Pulmon Dis* 2017;12:1819-24.
72. Dransfield MT, Bourbeau J, Jones PW, Hanania NA, Mahler DA, Vestbo J, et al. Once-daily inhaled fluticasone furoate and vilanterol versus vilanterol only

- for prevention of exacerbations of COPD: two replicate double-blind, parallel-group, randomised controlled trials. *Lancet Respir Med* 2013;1:210-23.
73. Suissa S, Patenaude V, Lapi F, Ernst P. Inhaled corticosteroids in COPD and the risk of serious pneumonia. *Thorax* 2013;68:1029-36.
 74. Suissa S, Ernst P. Precision medicine urgency: the case of inhaled corticosteroids in COPD. *Chest* 2017;152:227-31.
 75. Suissa S, Dell'Aniello S, Ernst P. Comparative effectiveness of LABA-ICS versus LAMA as initial treatment in COPD targeted by blood eosinophils: a population-based cohort study. *Lancet Respir Med* 2018;6:855-62.
 76. Wedzicha JA, Banerji D, Chapman KR, Vestbo J, Roche N, Ayers RT, et al. Indacaterol-glycopyrronium versus salmeterol-fluticasone for COPD. *N Engl J Med* 2016;374:2222-34.
 77. Vedel-Krogh S, Nordestgaard BG, Lange P, Vestbo J, Nielsen SF. Blood eosinophil count and risk of pneumonia hospitalisations in individuals with COPD. *Eur Respir J* 2018;51:1800120.
 78. Ernst P. Blood eosinophils in COPD and the future risk of pneumonia. *Eur Respir J* 2018;52:1800981.
 79. Festic E, Scanlon PD. Incident pneumonia and mortality in patients with chronic obstructive pulmonary disease: a double effect of inhaled corticosteroids? *Am J Respir Crit Care Med* 2015;191:141-8.
 80. Pavord ID, Chanez P, Criner GJ, Kerstjens HAM, Korn S, Lugogo N, et al. Mepolizumab for eosinophilic chronic obstructive pulmonary disease. *N Engl J Med* 2017;377:1613-29.
 81. Brightling CE, Bleecker ER, Panettieri RA, Bafadhel M, She D, Ward CK, et al. Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a randomised, double-blind, placebo-controlled, phase 2a study. *Lancet Respir Med* 2014;2:891-901.
 82. Criner GJ, Celli BR, Singh D, Agusti A, Papi A, Jison M, et al. Predicting response to benralizumab in chronic obstructive pulmonary disease: analyses of GALATHEA and TERRANOVA studies. *Lancet Respir Med* 2020; 8:150-70.
 83. Yousuf A, Ibrahim W, Greening NJ, Brightling CE. T2 Biologics for chronic obstructive pulmonary disease. *J. Allergy Clin Immunol Pract* 2019;7: 1405-16.