Blood eosinophil levels as a biomarker in COPD

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Abstract

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disorder and patients respond differently to treatment. Blood eosinophils are a potential biomarker to stratify patient subsets for COPD therapy. We reviewed the value of blood eosinophils in predicting exacerbation risk and response to corticosteroid treatment in the available literature (PubMed articles in English; keywords: "COPD" and "eosinophil"; published prior to May 2017). Overall, clinical data suggest that in patients with a history of COPD exacerbations, a higher blood eosinophil count predicts an increased risk of future exacerbations and is associated with improved response to treatment with inhaled corticosteroids (in combination with long-acting bronchodilator[s]). Blood eosinophils are therefore a promising biomarker for phenotyping patients with COPD, although prospective studies are needed to assess blood eosinophils as a biomarker of corticosteroid response for this.

Keywords

Eosinophils, COPD, corticosteroids, exacerbations

INTRODUCTION

According to World Health Organization estimates, chronic obstructive pulmonary disease (COPD) is currently the fourth leading cause of death globally (3.2 million deaths in 2015), and is associated with significant morbidity (WHO fact sheet 2017). Mainstays of COPD treatment include bronchodilators (particularly long-acting beta-agonists [LABAs] and long-acting muscarinic antagonists [LAMAs]) and inhaled corticosteroids (ICS), aimed at alleviating symptoms, reducing exacerbations, and maintaining or improving health status (GOLD guidelines 2017). Long-term treatment with ICS plus long-acting bronchodilators is recommended for patients at high risk of exacerbations (GOLD guidelines 2017). However, the evidence for the use of inhaled or systemic corticosteroids in COPD suggests variable benefit, which needs to be offset against the risk of adverse events, including those that occur with ICS treatment, e.g., oropharyngeal candidiasis, hoarseness and pneumonia (Matera 2015). The increasing recognition of these adverse effects, together with the wider recognition that COPD is a heterogeneous disease and the availability of alternative treatment options, has led to increasing interest in a more targeted approach to ICS treatment. As the number of treatment options for COPD has grown in recent years, so has the need to identify patients who will benefit most from each option (Brightling 2013).

Biomarkers are molecules, genes or characteristics that can be objectively measured as indicators of the distinct pathophysiological processes underlying an individual patient's disease. Improved understanding of COPD pathologies has identified blood eosinophils as a potential candidate biomarker. Until recently, COPD was considered to be a mainly neutrophil-mediated inflammatory disease (in contrast to asthma, a mainly Th2-mediated, eosinophilic disease). However, a degree of eosinophil-associated airway inflammation can be present in both stable COPD (Balbi 1994, Keatings 1997, Papi 2000) and during COPD exacerbations (Saetta 1994, Bathoorn 2008, Gao 2013). Airway biopsies collected during COPD exacerbations have been shown to contain 30-fold more eosinophils compared with samples taken during stable COPD (Saetta 1994). An example of the change in eosinophil level between stable COPD and COPD exacerbation is shown in Figure 1 (Fujimoto 2005). Other studies have also shown that a raised level of blood eosinophils is associated with an increased risk of COPD exacerbations (Vedel-Krogh 2016). These findings have led to the hypothesis that blood eosinophil levels could be used as a prognostic biomarker for establishing the risk of exacerbations.

Further to this, blood eosinophil levels may be of value as a biomarker to predict response to corticosteroid treatment. The variable efficacy of corticosteroids in the overall population with COPD can be contrasted with the more consistent efficacy of corticosteroids in asthma. The benefit of corticosteroids in the treatment of asthma has been attributed to reductions in eosinophil-associated airway inflammation, but corticosteroids are largely ineffective at reducing neutrophil-associated inflammation (Trevor 2014). Thus, in 'neutrophil-mediated' COPD, corticosteroids may be ineffective, but in patients with an eosinophilic component to their COPD, corticosteroids may provide efficacy.

We reviewed the available literature on blood eosinophils as a potential biomarker for (a) predicting exacerbation risk, and (b) predicting response to corticosteroid treatment.

SEARCH STRATEGY AND SELECTION CRITERIA

References for this review were identified through searches of PubMed for articles published with the keywords "COPD" and "eosinophil" up to May 2017. English-language articles resulting from these searches and relevant references cited in those articles were reviewed. The focus of this review article is blood eosinophils (rather than sputum eosinophils). The results are summarised in the Table.

EOSINOPHILS AND AIRWAY INFLAMMATION

In healthy individuals, eosinophils are present in small numbers in the peripheral blood and tissue; they are closely associated with parasitic infections (Scott 2006) and have also been associated with allergic and autoimmune diseases (Scott 2006, Diny 2017). Eosinophils can be found in lung tissue, adhering to the endothelium, and in sputum. When recruited or activated, eosinophils provide potent pro-inflammatory effects, including activation of mediators that contribute to airway damage, such as: major basic protein, which may disrupt the epithelial barrier allowing the penetration of inhaled antigens; eosinophilic cationic protein, which causes apoptosis of airway epithelial cells; eosinophil peroxidase, which causes oxidative tissue injury; and cytokines, which contribute to the ongoing inflammatory response (Eltboli 2013). In COPD, up-regulation of the chemokine RANTES (CCL5) in the epithelium may contribute to increased eosinophil levels (Zhu 2001), as may inflammation caused by smoking (Taylor 1985).

EOSINOPHIL-ASSOCIATED AIRWAY INFLAMMATION IN COPD

Eosinophil-associated airway inflammation can be identified by measuring sputum eosinophil levels or blood eosinophil levels. In the general population, sputum eosinophil levels are typically <1.1% (of total white blood cells) (Belda 2000) and blood eosinophil levels are generally below an absolute count of 300 cells/µL of blood. Several studies have shown a correlation between sputum and blood eosinophil levels (Bafadhel 2011, Hastie 2016, Singh 2014). Blood eosinophils are a more accessible marker of eosinophil-associated airway inflammation due to the ease of sample collection (relative to induced sputum samples or bronchoscopic biopsies).

A 2% cut-off point for blood eosinophils (approximately equivalent to 150 cells/µL absolute count) (Landis 2016), which is within the published normal range, has been used as the specified threshold in the majority of studies to date, and is sensitive for predicting eosinophil-associated airway inflammation and eosinophil-associated exacerbations (Bafadhel 2011).

In the observational, large-cohort, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) 3-year study, 37.4% (n=554/1483) of patients with COPD had blood eosinophil counts ≥2% at all visits, and 13.6% (n=201/1483) had <2% at all visits (Singh 2014). An analysis of cross-sectional data derived from the National Health and Nutrition Examination Survey (NHANES 2007–2010) cohort (n=948) demonstrated that 70% of US adults with COPD had blood eosinophil levels >2% (DiSantostefano 2016). In a retrospective cohort study of patients with COPD using the UK Clinical Practice Research Datalink (CPRD), a primary care electronic medical records database, the majority of patients with COPD (75%) had at least one blood eosinophil count ≥150 cells/µL over a 12-month period (Landis 2016).

Patients with COPD with eosinophil-associated airway inflammation cannot easily be distinguished based on patient and clinical characteristics alone (Hardin 2015). In a prospective cohort study

(SPIROMICS) of approximately 3,200 participants (never smokers, smokers without COPD, patients with mild/moderate COPD, and those with severe COPD), 1,091 participants with available data were stratified by blood eosinophil count (<1%, 1–3%, >3%), with a trend towards the highest eosinophil group patients being older and male (Weir 2014). The ECLIPSE study also reported that patients with blood eosinophil levels persistently ≥2% tended to be older, with a greater proportion of males (Singh 2014). In the group with ≥2% blood eosinophils, there were also fewer current smokers, higher forced expiratory volume in 1 second (FEV_1) (but not clinically relevantly so), higher fat-free-mass index, lower Modified Medical Research Council (mMRC) Dyspnoea Scale score, and lower BODE index (body mass index, airflow obstruction, dyspnoea, exercise capacity; a higher score indicates more severe disease and worse prognosis) (Singh 2014). Similar to ECLIPSE, analysis of the 7,225 patients with COPD in the Copenhagen General Population study reported that more men and fewer smokers had ≥2% eosinophils (Vedel-Krogh 2016), but there were no differences in FEV_1 or mMRC score. The UK CPRD analysis suggested that a higher eosinophil count was associated with male gender, obesity, a history of COPD exacerbations, and a history of asthma (Landis 2016). In two parallel, randomised, controlled trials (NCT01009463 and NCT01017952), Pascoe et al (2015) noted the absence of a correlation between bronchodilator response and blood eosinophils. Additionally, in TRISTAN, which had the highest proportion of patients with elevated eosinophils, the majority of patients (88%) were not atopic, indicating that atopy was not a predictor of blood eosinophils in COPD (Pavord 2016). In summary, there is no easily identifiable group of patient or clinical characteristics, other than gender and body mass index and possibly older age, that identifies patients with a high blood eosinophil count; it should also be noted that asthma or Asthma COPD Overlap (ACO) are not satisfactory surrogates for identifying this characteristic.

EOSINOPHIL COUNTS IN STABLE COPD PREDICT EXACERBATION RISK

A degree of eosinophil-associated airway inflammation can be present in both stable COPD (Balbi 1994, Keatings 1997, Papi 2000) and during COPD exacerbations (Saetta 1994, Bathoorn 2008, Gao 2013). Higher blood eosinophil levels during stable disease may also indicate a greater risk of exacerbation (Vedel-Krogh 2016). Data from prospective, randomised, controlled trials indicate that patients with moderate-to-severe COPD who were not taking ICS and had blood eosinophil levels ≥2% or ≥150 cells/µL experienced higher exacerbation frequency than patients with lower blood eosinophil levels (Pascoe 2015).

However, these data are from clinical trials of patients with a history of exacerbations. When the broader general population is considered, there may be confounding factors, including varying history of exacerbations and ICS treatment, that may influence the best cut-off point for predicting exacerbations. For example, in a population cohort study that used receiver operating characteristic curves to identify patients at higher risk of exacerbations, a cut-off point of 340 cells/µL (3.3%) was identified as optimal to predict exacerbation risk (Vedel-Krogh 2016). In the same study and over the same follow-up period, in 203 patients with clinically confirmed COPD and a history of at least one exacerbation, blood eosinophil levels >340 cells/µL were associated with a 3.21-fold increased risk of severe exacerbations, and levels >2% were associated with a 1.85-fold increased risk of severe exacerbations (Vedel-Krogh 2016). These data highlight that a history of exacerbations and an increased blood eosinophil count may increase the risk of future exacerbations in patients with COPD. Finally, an analysis of primary care data from a follow-up study suggested that a high blood eosinophil level (≥500 cells/µL) was an independent predictor of exacerbation risk (Kerkhof 2015). Further work is needed to identify the optimal blood eosinophil cut-off point for phenotyping

patients with COPD, and different cut-off points may be required depending on the context of use, i.e., to predict exacerbation risk and/or to predict response to treatment. Importantly, the predictive value of blood eosinophil counts in patients with COPD may interact with the frequency of exacerbations in the previous year.

However, some observational cohort studies have not shown a strong relationship between blood eosinophil levels and exacerbation risk, possibly because the majority of patients in these cohorts were non-exacerbators, or were receiving ICS-containing treatments, which may suppress baseline exacerbation rates, particularly in those with higher blood eosinophils. For example, in ECLIPSE, in which approximately 90% of patients were receiving ICS at baseline, no difference in exacerbation rates was observed (either prior to study entry or over the 3-year observational period) for patients with ≥2% versus <2% blood eosinophils (Singh 2014). Baseline data from SPIROMICS showed that patients with lower blood eosinophil counts (0–0.99%) had a higher exacerbation frequency compared with patients with eosinophil counts above this threshold, although data may have been influenced by ICS use (Weir 2014).

The mechanism by which eosinophilic inflammation may contribute to exacerbations is unclear, but it may be a combination of oedema, airway remodelling, mucus production and changes in airway geometry (Eltboli 2013). Reduced clearance of eosinophils may also be a contributing factor: the relationship between eosinophil-associated airway inflammation and eosinophil clearance is poorly understood, although it has been shown that macrophage phagocytosis of eosinophils is impaired in COPD, and is related to severity and frequency of COPD exacerbations (Eltboli 2014). In addition, the chemokine eotaxin-1 is involved in the activation of eosinophils and has also been associated with disease progression in COPD (D'Armiento 2009).

Currently, the best predictor of COPD exacerbation is a previous history of exacerbation, which has led to the concept of frequent versus infrequent exacerbators (Hurst 2010). Given the role of eosinophils in COPD exacerbations, these classifications may actually reflect differences in underlying pathology. It is clear that a subset of COPD patients has raised eosinophil levels (Bafadhel 2011, Singh 2014, DiSantostefano 2016), and that having higher eosinophil levels may be associated with an increased risk of exacerbations (Vedel-Krogh 2016). Thus, blood eosinophil levels may be considered a prognostic biomarker, i.e., used to predict those patients at increased risk of exacerbations, but further research is needed regarding the exact threshold levels and implications for clinical care. Whether blood eosinophils are a strong predictor of frequent exacerbations independent of prior exacerbation history needs to be further explored, and it would be important to determine this prior to their widespread use in this context in order to understand what additional value the test brings to the practicing clinician.

EOSINOPHILS AND RESPONSE TO CORTICOSTEROID TREATMENT IN COPD

COPD eosinophilic inflammation could, in theory, be modified by corticosteroid treatment (as happens in asthma). Early, short-term studies suggested that sputum 'eosinophilia' predicted response to corticosteroid therapy in COPD (Bacci 2006, Pizzichini 1998), with a sputum eosinophil level ≥3% being associated with a 'good' response (Pizzichini 1998). Data from a randomised study has shown proof of concept for treating eosinophilic inflammation in COPD. Eighty-two patients with COPD were randomised to receive either (a) treatment according to traditional British Thoracic Society (BTS) guidelines, or (b) therapy that aimed to minimise eosinophil-associated airway inflammation (assessed by induced sputum eosinophil count) (Siva 2007). In the group who had

eosinophil-associated airway inflammation minimised by ICS, there was a mean reduction of 62% in the number of severe exacerbations resulting in hospitalisation compared with the BTS treatment group (Siva 2007). These initial data have led to analysis of larger datasets, which have demonstrated relationships between blood eosinophil levels and several efficacy measures of inhaled/oral corticosteroid treatment.

Oral Corticosteroids

Oral corticosteroids (OCS) are used in the acute management of an exacerbation of COPD. A retrospective analysis of data from the London COPD Cohort noted that a raised stable blood eosinophil count (≥2%) was associated with a shorter exacerbation time (in 184 patients with an exacerbation), in response to OCS therapy (Donaldson 2015). In addition, a case-control study reported that patients with blood eosinophil counts ≥2% responded better to OCS and had shorter hospital stays than those with <2% eosinophils (Serafino-Agrusa 2016). Similar findings regarding length of hospital stays have been reported: one study reported that patients with blood eosinophil counts >2% receiving OCS in emergency departments had significantly shorter hospital stays than those patients with ≤2% eosinophils (Russel, 2016); a further study showed that prednisolone-treated patients with eosinophil levels ≥200 cells/µL had shorter hospital stays than patients with non-eosinophilic exacerbations (Bafadhel 2016).

A recent randomised, biomarker-directed, double-blind trial further explored the relationship between blood eosinophils and OCS (Bafadhel 2012a). Patients in the 'standard' arm received oral prednisolone to treat COPD exacerbations, while patients in the biomarker-directed arm received prednisolone or placebo according to eosinophil count (>2 versus ≤2% blood eosinophils, respectively). In the biomarker-directed group, 49% of patients did not receive prednisolone. The Chronic Respiratory Questionnaire scores were similarly improved in the standard and biomarker-directed groups, and these data suggested that a reduction in the number of inappropriate OCS prescriptions was possible (Bafadhel 2012a). The same authors also retrospectively applied groupings of ≥2% versus <2% blood eosinophils and presence/absence of prednisolone treatment to three randomised, controlled trials with available data (243 patients) (Bafadhel 2014). Patients presenting with an acute COPD exacerbation and a blood eosinophil count ≥2% had significantly reduced treatment failure rates with prednisolone, compared with placebo. In contrast, there was little evidence that prednisolone modified outcomes or provided benefit in patients with a blood eosinophil count <2%. The authors concluded that a biomarker-directed corticosteroid treatment strategy using the blood eosinophil count measured at the onset of an exacerbation may be a promising approach to maximise benefit and minimise unnecessary systemic corticosteroid use (Bafadhel 2014).

Inhaled Corticosteroids

Inhaled corticosteroid-containing treatments play a key role in reducing exacerbations in COPD. Several post-hoc analyses of large studies have demonstrated a relationship between baseline blood eosinophil levels and the ability of ICS to reduce COPD exacerbations.

In a post-hoc analysis of data from two randomised, 12-month, double-blind trials comparing once-daily vilanterol with vilanterol plus fluticasone furoate in patients with moderate-to-severe COPD and a history of one or more exacerbations in the previous year, 2083/3177 patients (66%) had an eosinophil count ≥2% at study entry (Pascoe 2015). Across all doses of ICS evaluated, fluticasone

furoate and vilanterol significantly reduced the rate of moderate/severe exacerbations compared with vilanterol alone in patients with eosinophil counts ≥2%. Exacerbations were reduced by: 10% in patients with baseline eosinophil counts <2%; 24% with baseline counts 2% to <4%; 32% with baseline counts of 4% to <6%; and by 42% for those with baseline counts ≥6% (Figure 2).

The Foster 48-week Trial to Reduce Exacerbations in COPD (FORWARD) study was similar in terms of design, patient population and duration (Siddiqui 2015). In this large, randomised, double-blind, parallel-group study (n=1184), 48 weeks of treatment with beclomethasone dipropionate plus formoterol fumarate was compared with formoterol fumarate alone (Siddiqui 2015). In a post-hoc analysis, in which patients were stratified by baseline blood eosinophil count, the adjusted treatment difference in terms of COPD exacerbation rate was greatest in the group with the highest eosinophil quartile (≥279.8 cells/µL), indicating better treatment response to the formoterol fumarate/beclomethasone combination (Siddiqui 2015).

In a review of studies of ≥1-year duration of fluticasone propionate/salmeterol in patients with COPD showed that, in those with ≥2% blood eosinophils, fluticasone propionate/salmeterol was associated with significant reductions in exacerbation rates versus tiotropium (INSPIRE study; n=719) and versus placebo (TRISTAN study; n=1049). No significant difference was seen in the <2% blood eosinophil subgroup in either study (Pavord 2016).

The WISDOM study was a 12-month randomised, parallel-group trial in which patients received triple therapy (fluticasone propionate, salmeterol and tiotropium) for 6 weeks and were then randomly assigned to continue to receive ICS, or to have ICS therapy gradually withdrawn over 12 weeks (Watz 2016). A post-hoc analysis showed that patients with blood eosinophil levels >4% had more frequent exacerbations on cessation of ICS treatment. The moderate or severe exacerbation rate was higher in the ICS-withdrawal group compared with the ICS-continuation group in patients with eosinophil counts ≥2% (Watz 2016). Continuation of ICS in the WISDOM trial was shown to be superior to withdrawal of ICS in severe COPD patients with blood eosinophil counts ≥300 cells/µL and a history of frequent exacerbations (≥2 exacerbations in the previous year) (Watz 2016).

A post-hoc analysis of a small trial of patients (N=248) with COPD also showed that exacerbation rate was significantly decreased in patients with blood eosinophil levels ≥3% who were treated with either high- (1000 µg/day) or low-dose (500 µg/day) fluticasone, than in patients with blood eosinophils <3% (Cheng, 2016).

In a pooled analysis of two 26-week, double-blind studies, patients with blood eosinophil levels >300 cells/µL achieved a greater reduction in exacerbations following ICS-containing treatment (fluticasone propionate/salmeterol) compared with bronchodilators (the combination indacaterol/glycopyrronium bromide); notably, these studies were not primarily designed to measure exacerbations and did not include a high exacerbation risk population (Wedzicha 2015).

Findings from the FLAME study, a 52-week, randomised, double-blind, double-dummy, non-inferiority study, which compared a dual bronchodilator (indacaterol plus glycopyrronium) with an ICS/LABA combination (fluticasone/salmeterol), differed to those from other studies discussed here. The effect of indacaterol plus glycopyrronium versus fluticasone/salmeterol on the rate of COPD exacerbations was said to be independent of the baseline blood eosinophil count although close analysis of the data shows a diminishing benefit of indacaterol plus glycopyrronium versus

fluticasone/salmeterol in patients with a higher baseline blood eosinophil count (Wedzicha 2016). However, in contrast to the other studies, the degree of bronchodilation differed between the treatment groups (the ability of the indacaterol plus glycopyrronium combination to provide bronchodilation being more pronounced than that of fluticasone/salmeterol). Both indacaterol and glycopyrronium can individually reduce exacerbations, which may have masked any predictive values of the blood eosinophil level. In the FLAME study, patients with a blood eosinophil count >600 cells/µL at screening were excluded, as well as patients with a history of asthma or current allergic rhinitis. Moreover, the vast majority of patients (80%) enrolled into the FLAME study had only one exacerbation in the previous year (Roche 2017).

Models of future COPD exacerbation rates based on baseline blood eosinophils in clinical trials with fluticasone furoate/vilanterol and formoterol fumarate/beclomethasone have been developed, and suggest a continuous relationship between increased baseline eosinophil level and risk of exacerbation in patients with a history of at least one exacerbation in the previous year (Figure 3) (Siddiqui 2015).

These findings have been prospectively tested in the TRINITY study, which compared treatment with extrafine beclometasone dipropionate, formoterol fumarate and glycopyrronium bromide (BDP/FF/GB; fixed triple) with tiotropium, and BDP/FF plus tiotropium (open triple) over a 1 year period (Vestbo 2017). In patients receiving BDP/FF/GB vs tiotropium, in the lower eosinophil group (<2%) exacerbations were reduced by 7–8% (not significant) whereas in the >2% group exacerbations were reduced by 30% (95% CI 15–42, statistical significance achieved) (Figure 4) (Vestbo 2017).

CORTICOSTEROIDS AND EOSINOPHIL-ASSOCIATED INFLAMMATION

The exact mechanisms by which ICS provide improved treatment efficacy in patients with high blood eosinophil levels is still unclear. Corticosteroid treatment may reduce eosinophilic inflammation by reducing gross numbers of eosinophils in COPD, and OCS have been shown to reduce the blood eosinophil count by a mean of 71 cells/µL (Kreindler 2015). The evidence for ICS is mixed (Kreindler 2015, Barnes 2006, Bourbeau 2007).

EOSINOPHILS AND ASTHMA COPD OVERLAP

ACO is an umbrella term that lacks a consistent definition in the literature; ACO includes (but is not limited to) patients with eosinophil-associated airway inflammation in COPD. This population has an increased mortality risk versus patients with asthma or COPD alone (Gibson 2015).

In asthma, blood eosinophil levels are known to drop following ICS treatment (Evans 1993). However, this has not been shown in COPD (Pavord 2016), and no correlation has been observed between baseline blood eosinophil levels and the extent of reversibility of FEV_1 (Pascoe 2015). This suggests that reversible airflow limitation and blood eosinophil levels could indicate distinct underlying mechanisms, which could also potentially be responsive to different treatments. Guidelines recommend that patients with COPD and features of asthma should receive at least ICS-containing treatment, regardless of their blood eosinophil levels (GOLD Guidelines 2017). However, evidence to support this recommendation is limited, since most COPD trials exclude patients with a current history of asthma. In a study of 63 patients with COPD, patients with asthmatic symptoms

responded well to ICS, which correlated with sputum eosinophil count; FEV_1 increases in response to ICS were significantly higher in patients with COPD and asthma, compared with COPD alone, and patients with COPD plus asthma also had higher peripheral and sputum eosinophil levels (Kitaguchi 2012). In the WISDOM study, the presence of atopic markers and raised serum IgE did not help to identify patients who had more frequent exacerbations on cessation of ICS treatment. Thus, the inclusion of patients with allergic asthma was unlikely to introduce bias (Watz 2016). This adds to the growing evidence that eosinophil-associated airway inflammation is not limited to patients with a diagnosis of asthma.

EOSINOPHIL LEVELS AND LUNG FUNCTION

In a post-hoc analysis of data from two randomised, 12-month, double-blind trials that reported differences in exacerbations according to blood eosinophil level, improvement in trough FEV_1 with fluticasone furoate and vilanterol compared with vilanterol alone was not associated with blood eosinophil level (Pascoe 2015). Similarly, in the analysis of the INSPIRE, TRISTAN and SCO30002 (fluticasone propionate/salmeterol versus tiotropium or placebo) trials, no relationship was apparent between blood eosinophil levels and treatment effect on FEV_1 (Pavord 2016). However, a post-hoc analysis of data from FORWARD did suggest an association between blood eosinophil levels and treatment effect on FEV_1; there was a consistent pattern of greatest treatment effect achieved in patients with the highest blood eosinophil levels (Siddiqui 2015). A post-hoc analysis was also conducted on data from the ISOLDE study, in which 738 patients were randomised to receive fluticasone propionate 500 µg twice daily or placebo. Patients were assessed over 3 years to determine treatment effect on rate of decline in post-bronchodilator FEV_1 (Figure 5). Patients with blood eosinophil levels ≥2% receiving fluticasone propionate had a slower rate of decline of lung function (p=0.003) versus placebo; however, this was not observed in the <2% group (Barnes 2016). To our knowledge, these findings have not been replicated in any other post-hoc analyses measuring rate of decline in lung function for ICS-containing treatments compared with non-ICS-containing treatments, because there have been no other long-term studies evaluating blood eosinophil levels at baseline. In a prospective, observational cohort study of 279 patients, 'higher' blood eosinophil levels (cut-off level not stated, nor whether patients received ICS-containing treatment or not) were again associated with slower annual decline in FEV_1 (p=0.002) (Suzuki 2015).

EOSINOPHIL LEVELS AND QUALITY OF LIFE

In the FORWARD study, patients receiving beclomethasone dipropionate plus formoterol fumarate with the highest blood eosinophil levels (upper quartile; ≥279.8/µL) had a mean change in St. George's Respiratory Questionnaire (SGRQ) score of −5.6 units, compared with +0.3 units in the formoterol fumarate only group, with smaller differences shown in the other quartiles (Siddiqui 2015). Data from ECLIPSE showed that over the 3-year follow-up period, the total SGRQ score was significantly lower in the group with blood eosinophil levels ≥2% versus <2% (p=0.002) (Singh 2014). Data from the SPIROMICS cohort (Weir 2014) and the post-hoc analysis of INSPIRE and TRISTAN (Pavord 2016) showed no relationship between blood eosinophil levels and treatment effect on SGRQ.

EOSINOPHILS AND RESPONSE TO OTHER THERAPIES IN COPD

Beyond corticosteroids, the efficacy of other therapeutic agents evaluated according to blood eosinophil levels has shown mixed results.

Bronchodilators

Response to LABA and/or LAMA in COPD has been investigated according to high and low blood eosinophil levels (Iqbal 2014). In a post-hoc analysis of umeclidinium/vilanterol (in combination or alone), response in terms of trough FEV_1, dyspnoea and health-related quality of life was similar for patients with baseline blood eosinophil levels ≥2 or <2% (Iqbal 2014). Similarly, in an analysis of data from three long-term, placebo-controlled tiotropium studies, no relationship was observed between baseline blood eosinophil levels and the number of patients with exacerbations in the tiotropium or placebo groups (Calverley 2015).

Anti-IL5

IL5 is pivotal in eosinophil development and anti-IL5 treatment is known to decrease blood eosinophil levels in patients with asthma (Leckie 2000), with anti-IL5 therapy shown to significantly reduce exacerbations (Ortega 2014, Pavord 2012). However, in COPD, the relationship between IL5 and exacerbations is unclear. In one study, 18 bronchial biopsies were examined to ascertain whether the airway eosinophilia present in asthma and chronic bronchitis during exacerbations was associated with IL5 expression in bronchial mucosa. Compared with controls, eosinophil levels increased similarly in both asthma and in exacerbations of chronic bronchitis; however, the number of IL5 immunopositive cells increased significantly only in asthma (Saetta 1996). A study profiling sputum inflammatory mediators showed no difference in sputum IL5 levels between asthma and COPD patients (n=54 and n=49, respectively) (Bafadhel 2012b). Interestingly, an analysis stratified by eosinophilic and non-eosinophilic airway inflammation demonstrated a significantly higher level of sputum IL5 in patients with eosinophilic airway inflammation, independent of the clinical diagnosis of asthma or COPD. Despite this, when the anti-IL5 monoclonal antibody benralizumab was investigated for COPD, it did not reduce the rate of acute exacerbations relative to placebo in the overall population (Brightling 2014). In patients with high sputum eosinophil levels (≥3%), numerical differences (benefits) were observed with the active treatment for several lung function measures; a progressive increase in treatment benefit was demonstrated with increasing eosinophil levels (Brightling 2014).

The anti-IL5 agent mepolizumab has been approved for the treatment of severe eosinophilic asthma, reducing exacerbation rates in this subset of asthma patients (Pavord 2012, Haldar 2009). Studies investigating mepolizumab in COPD (in patients with sputum eosinophils >3% [NCT01463644] or blood eosinophils ≥150 cells/µL [NCT02105961]) are underway.

PRACTICAL APPLICATION OF EOSINOPHIL LEVELS AS A BIOMARKER

Overall, evidence reported here suggests that blood eosinophil levels could potentially be used as a biomarker to predict (a) which patients with COPD will be more prone to exacerbations, and (b) which patients will respond best to corticosteroid therapy. Clinical management and treatment could then be directed accordingly. However, a number of key evidence gaps remain that need to be addressed ahead of widespread adoption by clinicians, including the need for prospective confirmation of these data. Furthermore, there are several practical aspects to measuring blood eosinophil levels that need to be considered to ensure its utility as a robust and reliable biomarker.

Variability and stability

Eosinophil counts can fluctuate because their half-life in blood is short and they show a diurnal variation, with a peak in the evening; therefore, they should ideally be measured at the same time

each day (Winkel 1981, Szefler 2012). External factors can also affect blood eosinophil levels; exercise and smoking may increase the blood eosinophil count, but their specific impact in COPD is unknown (Szefler 2012, Mensinga 1992). Ethnicity appears to have minimal influence; one study reported no differences between four ethnic groups (black, Asian [Indian], Asian [non-Indian], and white) (Bain 1984).

The stability of eosinophil levels over longer periods of time will influence whether one or more blood tests are necessary to guide clinical decisions. An analysis of 27,557 patients with stable COPD treated in UK primary care evaluated the stability of blood eosinophil levels over 1 year (mean of 2.2 eosinophil tests per patient). This analysis reported an intra-class correlation coefficient of 0.61 for all eosinophil measurements, and this increased to 0.69 when patients who had received an antibiotic or systemic corticosteroid during follow-up were removed (Landis 2017). These intra-class correlation coefficients suggest good repeatability of the eosinophil measurement (Landis 2017) and are similar to coefficients observed in previous studies involving blood eosinophil measurements (Negewo 2016), as well as to previous studies on other widely used biomarkers, such as serum cholesterol (Shekelle 1981). A post-hoc analysis of blood eosinophil levels in TRISTAN (Pavord 2016), where repeated measurements were available, showed some variability between measurements for individuals (Figure 6).

Measuring blood eosinophils

Our review of the literature identified studies in COPD that examined blood eosinophil level thresholds defined by absolute counts and/or percentage cell counts. It has not yet been defined which measurement is most appropriate for use as a biomarker in COPD. A National Institutes of Health (NIH) and federal agencies expert group has suggested that the percentage of eosinophils should not be reported unless specific reasons exist for knowing the proportions of eosinophils compared with other cells (Szefler 2012). However, strong correlation between these measures has been reported (rho=0.92; $p<0.001$), with 88% concordance between samples classified using 2% and 150 cells/µL cut-off values (Singh 2014). Most studies in COPD have used the 2% cut-off, but it has not been confirmed whether this is optimal or if the blood eosinophil count is a continuous variable with no sharp cut-off point. The optimal cut-off may also depend on the context of use, i.e., whether to predict exacerbation risk or to predict response to treatment, and different cut-offs may be needed for different endpoints. So far, the majority of data suggest that blood eosinophil levels may predict ICS response on a continuous rather than a dichotomous scale.

Measurement of eosinophil levels can be carried out by standard laboratory cell counters, but ensuring repeatability and accuracy will be important to implement the test effectively in clinical practice. The NIH and federal agencies expert group proposed guidelines recommending standard methodology and noted that although automated counting systems were accurate, they could produce errors in samples with high blood eosinophil levels (Szefler 2012). Manual counting is not recommended due to the potential for inaccuracy.

FUTURE PERSPECTIVES

Preliminary evidence suggests that while blood eosinophil levels may predict response to ICS, the target of ICS treatment is not to decrease the eosinophil count in blood (Kreindler 2015). This means that blood eosinophil levels appear to be a biomarker for predicting treatment response to ICS, without being modified by ICS treatment. There are other examples of similar biomarkers that

identify responder patients, without being a biological target for the treatment, e.g., FeNO in asthma. As discussed previously, the clinical levels used to define eosinophilia and the eosinophil levels for use as a biomarker are distinct; thus, 'treatment' of eosinophilia by ICS is not a clinical aim.

The use of blood eosinophils as a biomarker to direct COPD treatment shows great promise, but further work is needed to understand the full clinical implications. Large, randomised, biomarker-directed trials should be undertaken to confirm the findings reported in this review, and also examine other outcomes (e.g., economic). Meta-analysis of data from several sources could permit greater power to explore a variety of cut-off points, mainly dependent on the medical history (frequency of previous exacerbations) and the point of care (population-based, primary care, secondary or tertiary care). As meta-analysis of large datasets is also a component of regulatory qualification for drug development tools (Amur 2015), a working group dedicated to blood eosinophils has been formed by the COPD Biomarker Qualification Consortium (CBQC) (Miller 2016). There are also other unanswered questions: Can blood eosinophil levels guide the timing of preventative ICS/OCS interventions in a high-risk population? Is there a relationship between blood eosinophil levels and risk of ICS-associated pneumonia or other co-morbidities? What treatment combinations are best for eosinophil-associated exacerbations? Does blood eosinophil count predict exacerbation risk independently of exacerbation phenotype and exacerbation treatment (antibiotics and/or steroids)? And what factors confound interpretation of eosinophil count (e.g., infection)? Infection may be particularly troublesome, as viral and bacterial infections can both increase and decrease eosinophil counts in patients with COPD (Kolsum 2017, Papi 2006), suggesting increased awareness of the potential caveats on the use of eosinophil levels as a biomarker in COPD is warranted.

CONCLUSIONS

Blood eosinophil levels show strong potential as a prognostic and theragnostic biomarker in the clinical management of COPD; however, further research is needed to determine the precise implications for the clinical care of patients with COPD. Overall, the growing body of evidence from clinical trials and routine clinical care suggests that in COPD patients with a history of exacerbations (i.e. Global Initiative for Chronic Obstructive Lung Disease [GOLD] groups C and D), higher blood eosinophil levels are associated with an increased exacerbation risk in the absence of concomitant ICS use. Available evidence also suggests that high blood eosinophil levels predict response to ICS in COPD patients with a history of exacerbations, allowing the potential for a more targeted approach to treatment with ICS. However, future prospective studies will be required to confirm the utility of blood eosinophils as a biomarker in COPD, and practical aspects such as reproducibility and optimal number of measurements need to be determined.

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Figures

Figure 1. Changes in (a) absolute eosinophil counts and (b) relative eosinophil counts in 1 g sputum obtained in a stable phase and during an acute exacerbated phase from individual patients with unstable COPD (n=30). During exacerbation, the absolute eosinophil counts and relative eosinophil counts showed significant increases from those in the stable phase ($p<0.05$) (Fujimoto 2005).

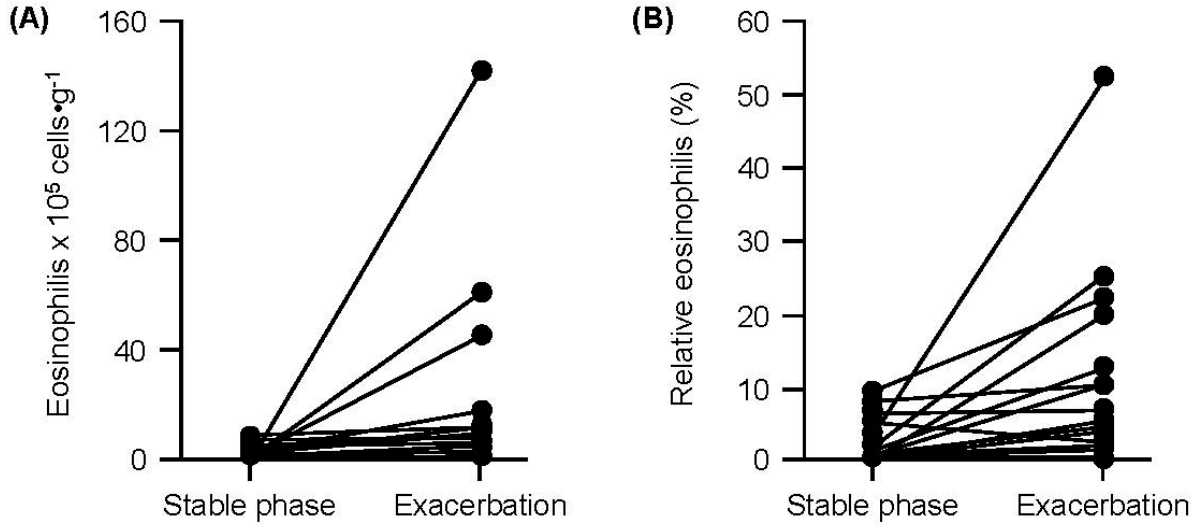


Figure 2. Moderate or severe exacerbation rates (per patient per year) for patients treated with fluticasone furoate plus vilanterol versus vilanterol alone by (a) eosinophil count <2% and ≥2%, and (b) four eosinophil cut-off strata (Pascoe 2015).

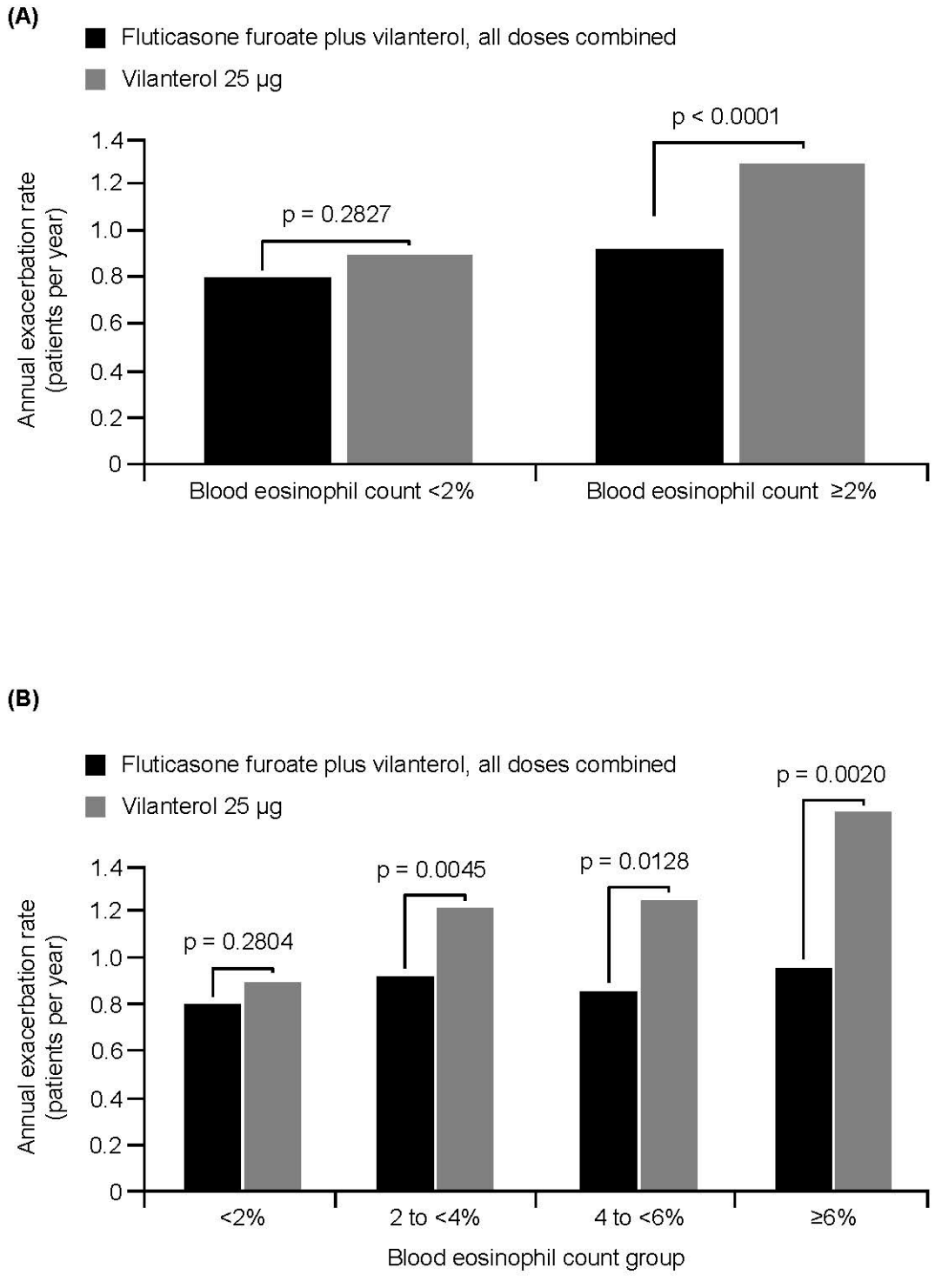


Figure 3. Modelled data on exacerbation frequency with LABA versus ICS/LABA by eosinophil count for (a) fluticasone furoate/vilanterol or vilanterol (Siddiqui 2016), and (b) beclomethasone/formoterol or formoterol (Siddiqui 2015).

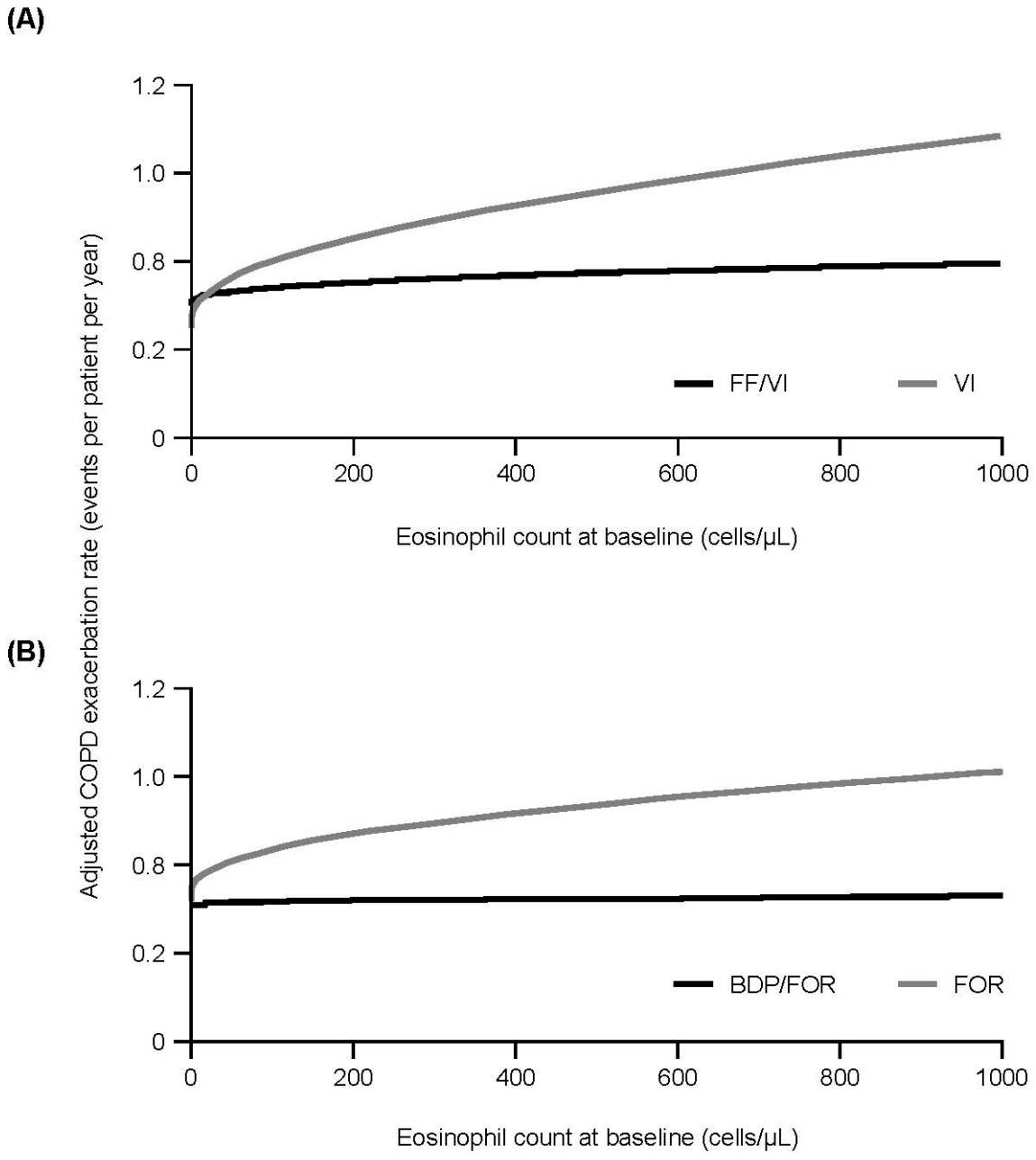


Figure 4. Adjusted rate ratios (and 95% confidence intervals) for moderate-to-severe COPD exacerbations on BDP/FF/GB vs tiotropium by baseline eosinophil count (TRINITY) (Vestbo 2017).

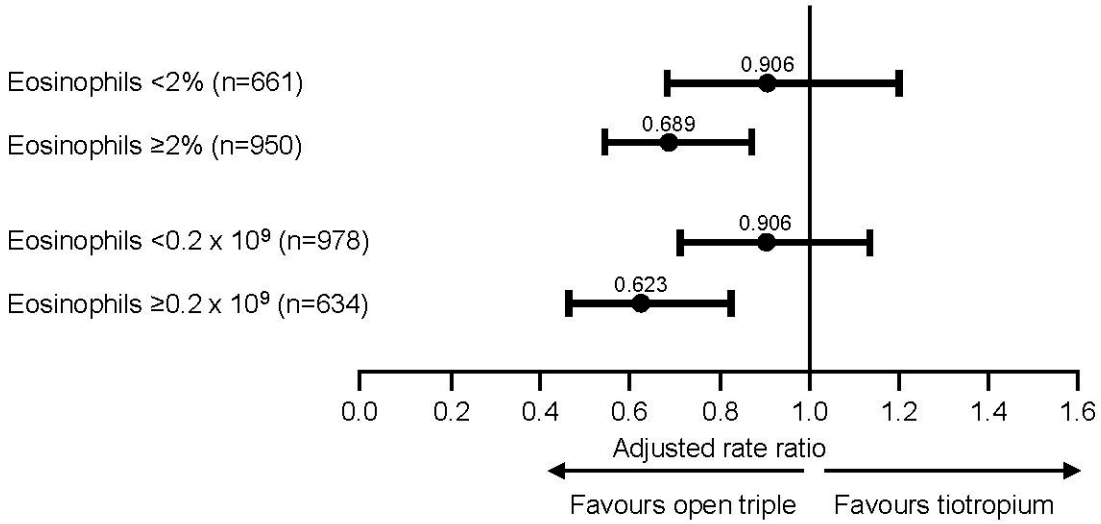


Figure 5. Decline in post-bronchodilator FEV_1 by blood eosinophil count (ISOLDE) (Barnes 2016).

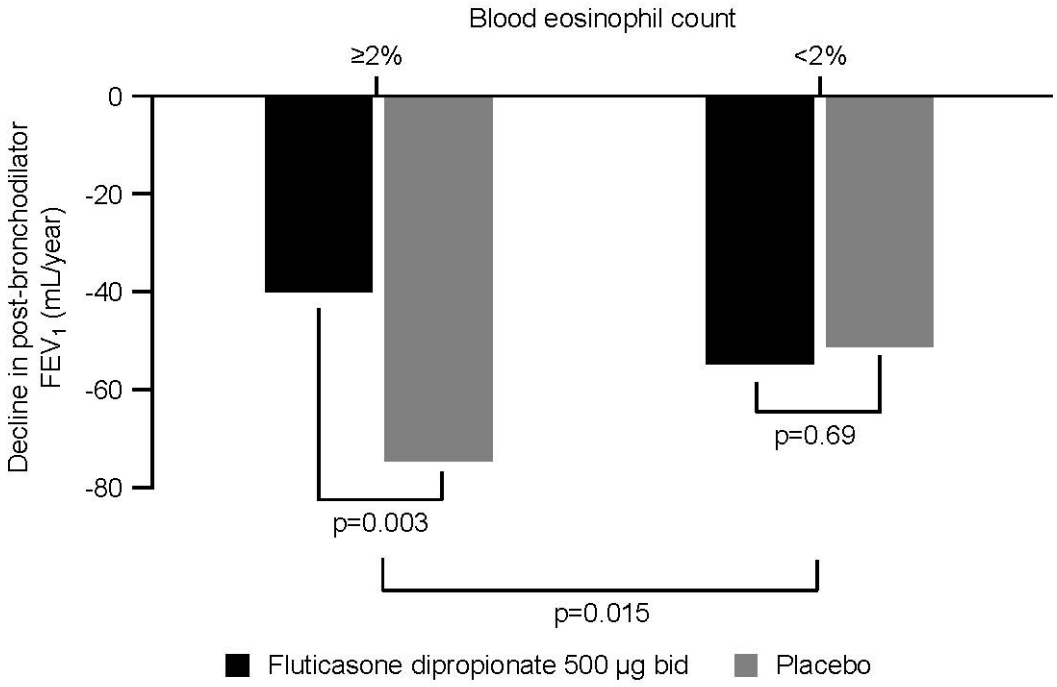
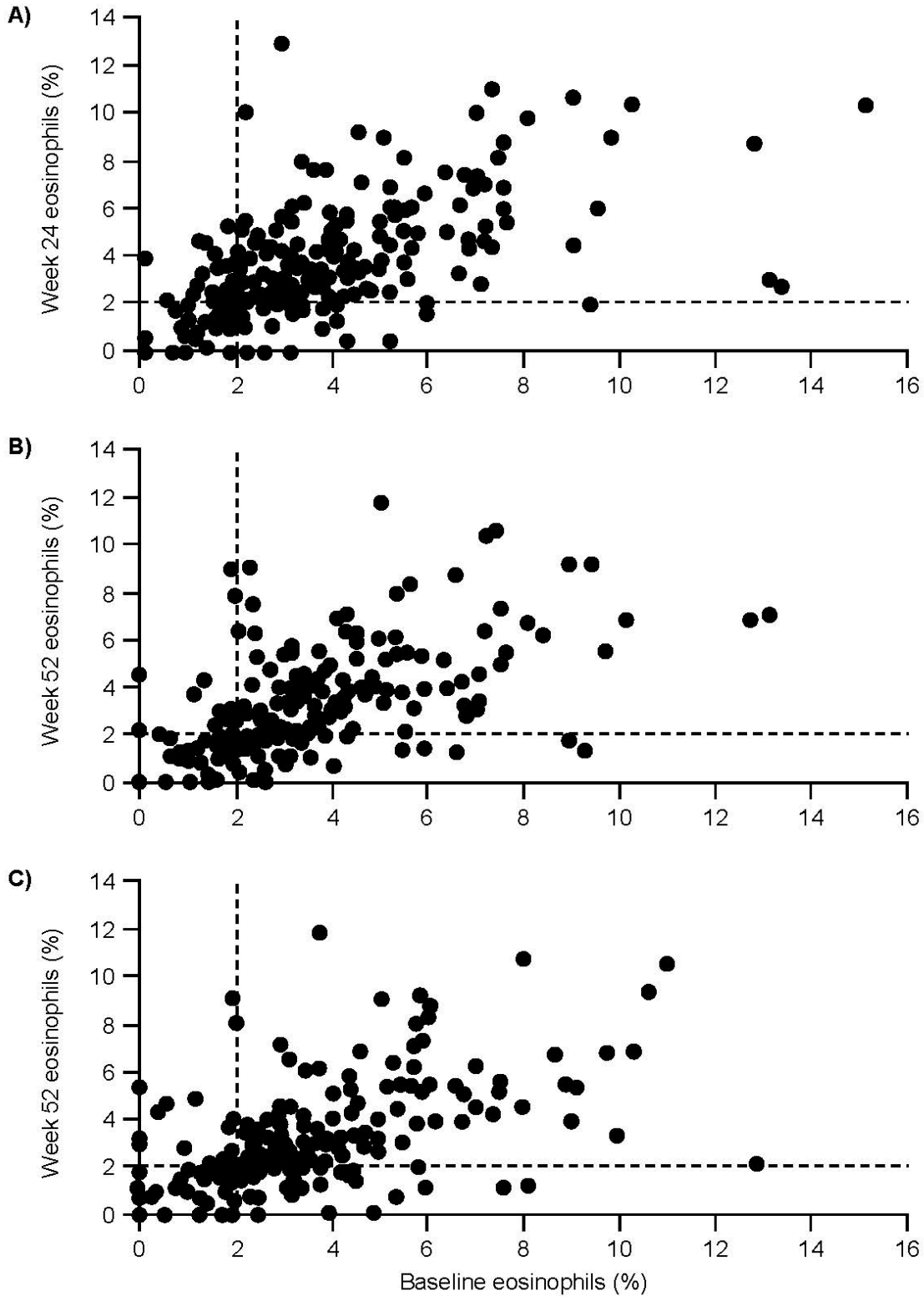


Figure 6. Scatter plots of (a) 24-week post-baseline blood eosinophils versus baseline blood eosinophils, (b) 52-week post-baseline blood eosinophils versus baseline blood eosinophils, and (c) 24-week post-baseline blood eosinophils versus 52-week post-baseline blood eosinophils, in the placebo group of TRISTAN (SFCB3024) (Pavord 2016).



Dashed lines represents 2% eosinophils.

Table

Trial / reference	N	Study treatment	Patient steroid use	Higher eosinophil level cut off	Effect in patients with higher eosinophil levels
Effect on exacerbations					
Follow-up study using OPCRD (Kerkhof 2015, database analysis)	16565	NA	Steroid naïve	≥500 cells/μL	Blood eosinophilia (≥500 cells/μL) is an independent predictor of exacerbation risk (odds ratio 1.43, $p=7x10^{-7}$ [non-smokers]; 0.89, p=0.31 [current smokers])
ECLIPSE (Singh 2014, observational study)	1483	NA	90% receiving ICS	≥2% blood eosinophils	No difference in exacerbation rates (1.06 per PY [≥2%] vs 1.07 [<2%]; p=0.277)
SPIROMICS (Weir 2014, observational study)	1091	NA	NR	≥3% blood eosinophils	Lower exacerbation frequency (0.36 [>3%] vs 0.28 [1–2.99%] vs 0.57 [<0.99%]; p=0.009)
The Copenhagen General Population Study (Vedel-Krogh 2016, observational study)	203	NA	Steroid naïve	340 cells/μL	≥340 cells/μL: 3.21-fold increased risk of severe exacerbations ≥2%: 1.85-fold increased risk of severe exacerbations
Two randomised trials; post-hoc analysis (Pascoe 2015, RCT)	3177	ICS (FF/VI vs VI)	History of steroid use; no ICS ≤30 days prior to screening	≥2% or ≥150 cells/μL	Exacerbation frequency reduced in eosinophil ≥2% subgroup by 29% with ICS use (FF/VI 0.91 mean per PY vs VI 1.28 per PY, p<0.0001) compared with a 10% reduction in the <2% subgroup (FF/VI 0.79 per PY vs VI 0.89 per PY, p=0.2827)

TRINITY (Vestbo 2017, RCT)	2691	BDP/FF/tiotropium BDP/FF/GB vs tiotropium vs	NR	≥2%	Significant decrease in exacerbations BDP/FF/GB vs tiotropium in ≥2% (30%) vs <2% subgroups (7–8%)
Post-hoc analysis (Cheng 2016)	248	High dose (1000 µg/day) vs medium dose (500 µg/day) Fluticasone	History of steroid use; no systemic use 1 month prior to screening	≥3%	Patients receiving high dose fluticasone had reduced incidence of exacerbations in ≥3% subgroup vs <3% subgroup (13.5% vs 22.6%, p<0.05); similar results observed in medium dose group ≥3% vs <3% (14.7% vs 28.7, p<0.01)
Effect on lung function					
INSPIRE, TRISTAN and SCO30002 (Pavord 2016, systematic review)	3045	FP/SAL vs tiotropium, FP, SAL or placebo	NR	≥2%	No difference in treatment effect on FEV_1 by eosinophil ≥2% vs <2%
Hokkaido COPD cohort study, prospective (Suzuki 2015, observational study)	279	NA	NR	Not stated	Slower annual decline in FEV_1 (p=0.002) associated with higher eosinophil count
Two randomised trials; post-hoc analysis (Pascoe 2015, RCT)	3177	ICS (FF/VI vs VI)	Steroid naïve	≥2% or ≥150 cells/µL	Improvement in FEV_1 with FF/VI vs VI not associated with blood eosinophil level (mean improvement 49 mL ≥2% vs 27 mL <2%; no interaction between treatment and eosinophil count, p=0.7873)
FORWARD (post-hoc analysis) (Siddiqui 2015, RCT)	1184	BDP/FF vs FF	NR	≥279.8 cells /µL	Treatment difference in favour of BDP/FF for adjusted mean change in FEV_1 were highest in the top eosinophil level quartile (0.102L in quartile ≥279.8 cells/µL, p=0.001) lost in middle quartiles (0.023L in quartile 181.6–<279.8 cells/µL, p=0.456; 0.052L in quartile 110.4–<181.6 cells/µL,

					p=0.090) and retained in lowest quartile (0.083L in quartile 0–<110.4 cells/µL, p=0.006)
ISOLDE post-hoc analysis (Barnes 2016, RCT)	738	FP vs placebo	NR	≥2%	Slower rate of decline of lung function with FP vs placebo in ≥2% group (-40.6 mL/year vs -74.5 mL/year); not observed in the <2% group (-54.2 mL/year vs -51.3 mL/year, p=0.068)
General treatment response with oral corticosteroids					
(Bafadhel 2014, systematic review)	243	Prednisolone vs placebo	NR	≥2%	Significantly reduced treatment failure rates with prednisolone vs placebo in ≥2% subgroup (66% vs 11%); not seen in <2% subgroup (26% vs 20%, p=0.035)
Retrospective observational case-control study (Serafino-Agrusa 2016, retrospective analysis)	132	Prednisolone	All patients treated with IV steroids	≥2%	Improved response to OCS in ≥2% vs <2% subgroup (total dosage: 19.2 vs 35.7mg/day; p=0.012) and shorter hospital stays (8.9 vs 11.3 days; p=0.028)
London COPD cohort (Donaldson 2015, observational study)	184	Prednisolone or placebo	NR	≥2%	Shorter mean exacerbation duration (13.5 days [≥2%] vs 11 days [<2%]; p=0.001)
Russel 2016 (observational study)	549	OCS (not specified)	NR	>2%	Shorter mean length of hospital stay in >2% vs ≤2% subgroup (4.1 vs 6.5 days, p=0.046)
Crossover trial (Pizzichini 1998, trial)	18	Prednisone and placebo	NR	≥3%	Improved dyspnoea with prednisone over placebo in ≥3% vs <3% subgroup: mean paired difference 0.8 (p=0.008) vs 0.3 (p=0.5); improved QoL in ≥3% vs <3% subgroup: mean paired difference 1.96 (p=0.01) vs 0.9 (p=0.5)

(Bafadhel 2016, RCT)	243	Prednisolone	Prednisolone-treated	≥200 cells/µL and/or ≥2%	Shorter mean hospital stays in prednisolone-treated patients with ≥200 cells/µL vs <200 cells/µL (5 vs 6.5 days; p=0.015)
General treatment response with inhaled corticosteroids					
INSPIRE; TRISTAN (Pavord 2016, systematic review)	719; 1049	FP/SAL vs tiotropium or placebo	NR	≥2% blood eosinophils	Significant reductions in exacerbation rates in ≥2% subgroup with FP/SAL vs tiotropium (rate ratio 0.75, p=0.006) and vs placebo (rate ratio 0.63, p=<0.001); No significant differences in <2% subgroup
(Siva 2007, RCT)	82	Treatment according to BTS guidelines or ICS	Both (continued ICS if already receiving them)	>3%	Overall severe exacerbations reduced in sputum group vs the BTS treatment group (0.2 per PY vs 0.5 per PY; 62% mean reduction, p=0.037); most benefit occurred in >3% eosinophil group
FORWARD post-hoc analysis (Siddiqui 2015, RCT)	1184	BDP/FF vs FF	NR	≥279.8 cells/µL	Significant adjusted treatment difference in COPD exacerbation rate in ≥279.8 cells/µL quartile (46% reduction in exacerbation rate of BDP/FF vs FF) and 181.6–<279.8 cells/µL quartile (28% reduction of BDP/FF vs FF, p=0.041); not observed in lower eosinophil level quartiles
WISDOM post-hoc analysis (Watz 2016, RCT)	2296	FP/SAL/tiotropium followed by continued or reduced ICS	NR	>5% blood eosinophils	Frequency of exacerbations on cessation vs continuation of ICS treatment increased with increasing eosinophil counts (rate ratio 1.22 [≥2%], 1.63 [≥4%], 1.82 [≥5%])
FLAME post-hoc analysis (Roche 2017, RCT)	3362	Indacaterol/ glycopyrronium vs FP/SAL	NR	>5% blood eosinophils	Treatment efficacy was superior with indacaterol/ glycopyrronium vs FP/SAL for prevention of exacerbations in the <2%, ≥2%, <3%, <5%, and <150 cells/µl eosinophil count subgroups; at no cutoff was FP/SAL superior to indacaterol/glycopyrronium

LANTERN; ILLUMINATE, post-hoc analysis (Wedzicha 2015, RCT pooled analysis)	1263	FP/SAL vs QVA149	NR	>300 cells/µL	Greater reduction in exacerbations following ICS-containing treatment compared with bronchodilators; in <2% subgroup QVA149 was more effective

BDP, beclomethasone diproprianate; BTS, British Thoracic Society; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FF, fluticasone furoate; FP, fluticasone propionate; GB, glycopyrronium bromide; ICS, inhaled corticosteroid; IV, intravenous; NR, not reported; OPCRD, Optimum Patient Care Research Database; PY, patient-year; RCT, randomised controlled trial; SAL, salmeterol; VI, vilanterol