The eosinophil in COPD: Just another biomarker?

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Summary

The eosinophil is an innate immune cell and under certain conditions can be recruited to the lung, where it can play an as yet undefined role in health and disease. In chronic obstructive pulmonary disease (COPD) the eosinophil has been found in the airway, tissue and circulation both at stable state and during exacerbations. The utility of the eosinophil to be a valid biomarker require it to have relevance to important clinical outcomes. Recently, epidemiological observations and post-hoc analyses have shown the circulating eosinophil in COPD, is associated with risk of exacerbations, mortality, and response to both inhaled and systemic corticosteroids. Further studies are warranted to explore the mechanism of disease in COPD in addition to defining optimum levels of risk. In this *Lancet Respiratory Medicine* review, the role of the eosinophil as a biomarker and a mediator of disease in COPD is explored.

Introduction

'Wonder is the beginning of wisdom' Socrates c. 450BC

From its formation and production in the bone marrow, the circulating eosinophil, largely resides in the gastrointestinal tract and the thymus. Eosinophils under certain responses, can be recruited to tissue where, secretion of chemokines, cytokines and cytotoxic granular eosinophil products can facilitate an inflammatory reaction, where these cells are widely believed to play an as yet undefined role in the host immune response[1]. Recent advances in genetics, molecular biology and *in-vivo* experiments utilising eosinophil knock-out murine models (TgPHIL mouse[2]) have informed greatly the inherent complexity of this cell to both the stimulus required and the response that they then enact[3]. Widely held to play a role in host defence against helminth infection and contributing to allergic conditions including asthma[3], the recent finding of elevated circulating eosinophils in chronic obstructive pulmonary disease (COPD)[4,5] and the identification that this leads to predict corticosteroid responsiveness both at stable state with inhaled corticosteroids[6-9] and during acute exacerbations[5] has led to further question the part played by this granulocyte in chronic lung disease. In this *Lancet Respiratory Medicine* review, the role of the eosinophil as a biomarker and a mediator of disease in COPD is explored.

The biology of the eosinophil

Eosinophils, were first described by Paul Ehrlich in 1879, following the discovery of cells containing granules which have an affinity for the eosin stain. Usually, representing less than 5% of total leukocytes, the eosinophil spends less than 18 hours in the circulation[3], before residing largely in the small intestine of the gastrointestinal tract[10] and the thymic medulla[11]. Human eosinophils contain four basic proteins stored in secondary granules (major basic protein, eosinophil peroxidase, eosinophil cationic protein and eosinophil-derived neurotoxin), which have been shown to exhibit toxicity to numerous cell types including bronchial epithelial cells[12], cerebellar Purkinje cells[13], cardiac muscle[14] and endothelial cells[15]. The presence of degranulated eosinophils is believed to be associated with pathogenesis of disease[16], whether as a causal or associated effect is yet to be determined. Serum ECP is elevated in the acute phase of a myocardial infarct[17]; whilst in asthma both elevated numbers of eosinophils and degranulation are persistently found in histological lung specimens from patients with fatal asthma[18,19]. Ligand cell surface receptors support eosinophil cell function and relatively unique to eosinophil biology is the expression of the CC-chemokine receptor 3 (CCR3) and the interleukin-5 receptor alpha subunit (IL5Rα)[3]. IL5 is integral to the production, maturation and survival of the eosinophil. Additional

chemoattraction is provided by eotaxin (CCL11), a potent CC chemokine ligand. In the lung, locally secreted IL4 and IL13 are responsible for increased endothelial adhesiveness[20] and secretion of CCL11 by bronchial epithelial cells[21], support further recruitment of eosinophils to tissue. Enhanced tissue survival of the eosinophil is mediated by the action of IL5 and granulocyte-macrophage colony stimulating factor (GM-CSF). Eosinophils promote humoral immunity with priming of B-cells[3] and play a central part in Type-2 (T2) immunity[22], with the capacity to 'present' antigen to CD4+ T-cells and secretion of eosinophil granular contents containing T2 mediators, including IL4, IL5 and IL13[3], with tight regulation of Th1 and Th2 immunity[23]. Helminth infections such as Schistosoma mansoni elicit a profound T2 mediated reaction leading to accumulation of tissue eosinophils[24]. ECP has an affinity for bacterial lipopolysaccharide[25], but the anti-bacterial role of eosinophils remains unclear.

As can be seen, since their discovery and our advancement in molecular biology, much is now known about the structure and function of the eosinophil. Factors that initiate a complex eosinophil response in COPD remain elusive, however observations in asthma would suggest that the eosinophil is more than a bystander and thus may play an important role in COPD.

Observations suggesting a possible pathogenic role of the eosinophil in COPD

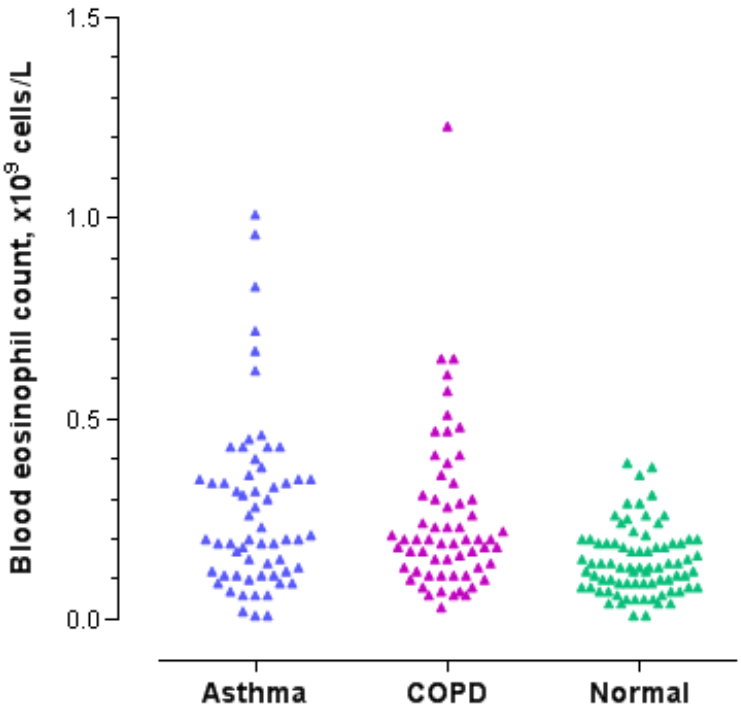
Lipid bodies found in eosinophils are the site of production of cysteinyl leukotrienes, which are key mediators in bronchial smooth muscle contractility[26]. In the COPD mucosa, cysteinyl leukotrienes receptors are increased at stable state and more so during an exacerbation[27]. Furthermore, in the murine model, the use of the CysLT1R antagonist montelukast, reduces inflammation and attenuates emphysematous change[28]. Furthermore, in COPD there is differential expression of the airway microbiome in eosinophilic and non-eosinophilic COPD both at stable state[29] and during acute exacerbations[30]. Eosinophil activation has been found to be mediated by non-typeable Haemophilus influenzae via beta-glucan receptors, expressed on the eosinophil cell surface[31] whilst H. influenzae is known to be a potent inducer of pro-inflammatory cytokines including IL1β from epithelial cells[32]. Recent *in vitro* experiments have shown that IL1β activates innate lymphoid cells (ILC), with the conversion of ILC2, a potent producer of the T2 cytokines IL5 and IL13 to ILC1 by the action of IL12, a process reversed by IL4[33]. This would suggest that dysregulation of this complex homeostatic immunity is likely to feature in the pathogenesis of chronic lung diseases including COPD, but further mechanistic insight, into whether the eosinophil is a key effector cell in the dysregulation is needed.

The eosinophil as a relevant and valid biomarker in COPD

The World Health Organization defines a biomarker as 'any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease'[34]. Thus, for a biomarker to be a useful surrogate endpoint, there is an ultimate requirement for it to be relevant and valid, both in relating to important clinical outcomes and demonstrating effectiveness as a surrogate. In airways disease, it has been long noted that the presence of airway eosinophils are pathognomonic of asthma[35]; associated with asthma severity[35] and mortality[36]. Elevated eosinophil numbers have been found in the blood, sputum, bronchoalveolar lavage and bronchial tissue of patients with asthma. Furthermore a reduction of airway eosinophil numbers with inhaled or oral corticosteroids, is associated with improved clinical outcomes, impacting on exacerbation frequency[37], symptoms and lung function[38]. In COPD, during stable disease eosinophils have been detected in varying degrees from the central and peripheral airways from both sputum and bronchoalveolar lavage in addition to bronchial biopsies[19,39]. Pooled data from asthma[37] and COPD[40] subjects entered into single-centre clinical trials have demonstrated that the sputum eosinophil numbers are similar[41]. This is also the case for bronchial tissue, but in asthma the eosinophils are degranulated in contrast to non-degranulated eosinophils in COPD[19]. Circulating peripheral blood eosinophils levels have been found to be similar between asthma and COPD patients, where the median level of circulating eosinophils is approximately 200 cells/μL, (**figure 1**). A mediator analysis of subjects with asthma and COPD, demonstrated very little difference in cytokine expression between disease groups, including dominant T2 mediators IL4 and IL5, whether they were assigned to having eosinophilic inflammation or not[42].

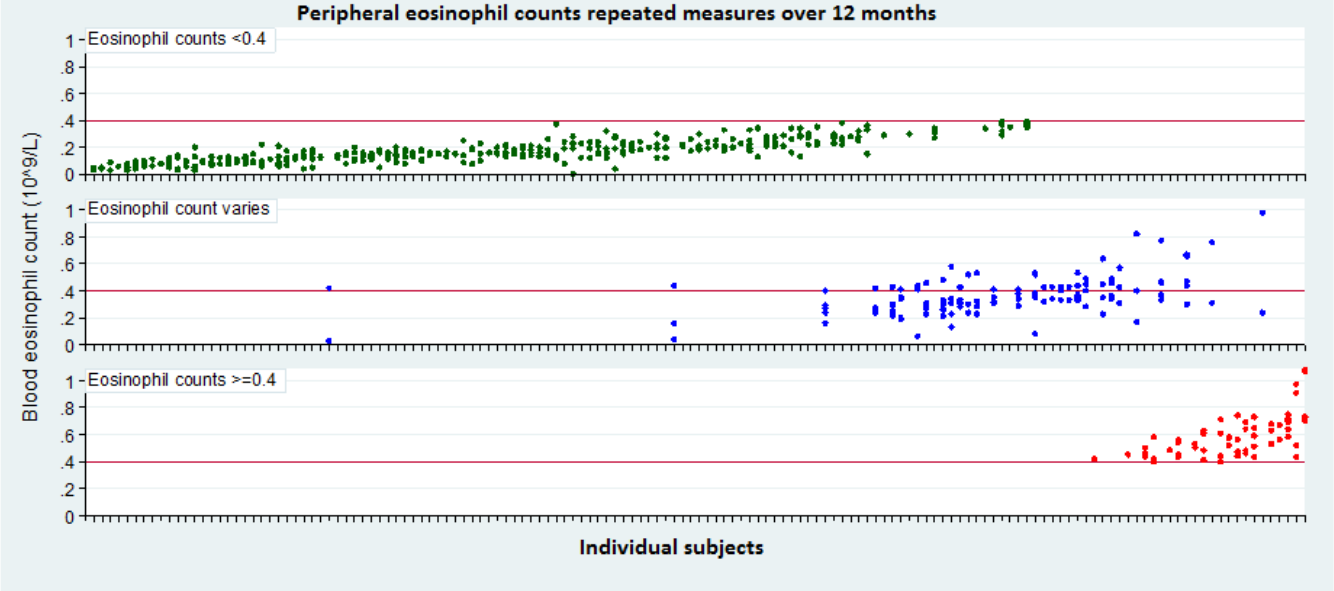
Circulating peripheral blood eosinophil levels, in single centre studies[43,44], in contrast to multi-centre studies[45,46], have been shown to correlate with sputum eosinophils. A circulating eosinophil count of 265 cells/μL has a sensitivity of 72% to identify a sputum eosinophilia of greater than 3%[43] at stable state; whilst at exacerbations a 2% relative circulating eosinophil count has a sensitivity and specificity of 90% and 60% in detecting an sputum eosinophilia during an acute exacerbation[4].

Figure 1. Peripheral blood eosinophil counts in asthma, chronic obstructive pulmonary disease and healthy controls. Data derived from Bafadhel et al [42] and Anand et al [47]



For a biomarker to be relevant and valid, although not in the original biomarker definitions described above by the WHO[34], its presence needs to be stable or repeatable over time. This is essential when considering life-long or expensive treatment options based upon the presence or level of the relevant biomarker i.e. in the use of inhaled corticosteroids or monoclonal antibodies respectively. The repeatability of sputum eosinophil counts has been shown to be moderate with an intra-class coefficient of 0.63 over a short time frame (2 weeks)[48] and 0.49 over a longer time period (12 weeks)[49]. The repeatability of the peripheral blood eosinophil count has been explored less frequently. In a sub-group analysis of the ECLIPSE[50] population, subjects were classified above and below 2% the relative circulating eosinophil count, over 3 years with yearly sampling, it was found that 51% of the COPD population remained either above or below 2% at each measure[45]. Repeated measures, 3 monthly over a 12-month minimum duration, from the Leicester MRC COPD cohort[4,5], have shown that the intra-class coefficient is 0.79 over 3 months, with approximately 65% of the COPD population remaining persistently above or below 400 cells/µL (**figure 2**)[51].

Figure 2. Repeated peripheral blood eosinophil counts over 12 months. Individual subjects presented along x-axis. Top panel presents, COPD subjects with eosinophil counts always less than 400 cells/µL (55%); middle panel presents COPD subjects with eosinophil counts varying above and below 400 cells/µl (35%); and lower panel presents COPD subjects with eosinophil counts always above 400 cells/µl (10%). Data derived from Bafadhel et al[4,5]. Reproduced with permission from E Millett.



Epidemiological association studies have shown relevance and validity of the circulating eosinophil as a biomarker in COPD, but often the reported eosinophil levels are within the normal reference ranges for many haematology laboratories. The WHO biomarker definition[34] does not make inference on whether a biomarker needs to be in a range that is outside reference for a normal control population or uniquely detected in one disease area; which poses further questions as to whether validity needs to include abnormal detection. It is worth noting, that the usual eosinophil (and full blood count) reference ranges for haematology laboratories are calibrated from recruiting healthy controls, which includes healthy controls with a history of atopy. Up to 40% of the general population have been found to have atopy[52]. In a small calibration study[47], the circulating eosinophil count mean and upper limit of normal (95% upper limit) reference range for all healthy controls was found to be 190 (420) cells/µL. Repeated reference laboratory calibration, in a separate healthy control population, where atopic (self-reported or elevated IgE) controls were excluded, demonstrated that the mean (95% upper limit) circulating eosinophil count was 150 (270) cells/µL. These findings would suggest that the relevance and validity of the circulating eosinophil in COPD may be well within the current conventional normal range; but above the reference limits if atopy, known to increase circulating eosinophils, is excluded. This needs to be considered when reviewing blood eosinophil levels to make treatment decisions. It may be necessary that in future specific reference ranges, for both atopic and non-atopic controls are reported.

The circulating eosinophil to identify risk in chronic obstructive pulmonary disease

The natural history of COPD is of progressive airflow obstruction and lung function decline. Episodes of acute deterioration are characterised by periods of worsening in both respiratory function and symptoms. These episodes, termed exacerbations, are associated with significant symptoms[53], accelerated lung function decline[54], an increased risk of further exacerbations[50] and increased mortality[55]. The current consensus agreement to define an exacerbation of COPD is largely symptom driven[56-58] and is likely to have impaired the discovery of a single objective biomarker of an exacerbation[59]. Defining the heterogeneity of the exacerbation utilising biology and mathematical cluster techniques has proven to identify a T2 high driven exacerbation[4]; an event clinically indistinguishable, but associated with higher levels of IL5 and CCL11 and corresponding sputum and circulating eosinophils. This is consistent with the previous identification of elevated numbers of eosinophils in BAL and tissue during an exacerbation of COPD[60,61]. Furthermore, the circulating eosinophil count has been determined to have high sensitivity and specificity in determining the risk of a sputum eosinophilic exacerbation[4]. In a single centre randomised clinical trial[5] (RCT), the circulating eosinophil count (relative count of 2%) at the time of an exacerbation, was used to direct systemic corticosteroid therapy. The study demonstrated non-inferiority to standard therapy and also that eosinophilic moderate exacerbations of COPD were associated with hastened symptomatic recovery and fewer treatment failures (defined as re-treatment, hospitalisation or death) than non-eosinophilic exacerbations of COPD treated with corticosteroids (2% treatment failure rate compared to 15% treatment failure rate). In data collected and pooled from 3 original prednisolone/placebo exacerbation studies[62], similar findings were observed, with greatest risk of treatment failure in eosinophilic exacerbations treated without systemic corticosteroids (66% treatment failures). The 2% cut off relative circulating eosinophils at the time of an exacerbation has also been found to be associated with a higher risk of all cause[63] and COPD related re-admission[63,64] of up to 4 times [OR 3.59, 1.67 to 7.82] with increased length of admission[65]. Furthermore, the presence of a low eosinophil count during an exacerbation of COPD identifies the risk of worsened outcomes. In several retrospective studies, a circulating eosinophil count below 50 cells/μL at the time of an exacerbation, has been found to be associated with a prolonged length of hospital stay, of approximately 3 days[66] and an almost 3 fold [OR 2.76, 95%CI 1.58 to 4.83] increased risk of mortality[67].

The relationship of the circulating eosinophil to other important clinical outcomes has been demonstrated in epidemiological studies[68-72]. In the longest and largest of these, conducted in three districts in the Netherlands over 30 years, Hospers et al determined that an eosinophilia (defined as a peripheral blood eosinophil count greater than 275 cells/μL) was associated with an increased risk of all-cause mortality, independent of age, gender, smoking habits and lung function[71] and associated with an increased risk of cardiovascular mortality[70]. From the same population studied, COPD specific mortality was increased (odds ratio 4.8, 95%CI 1.9 to 11.9) in the presence of a peripheral eosinophil count above 275 cells/μL[68]. More recently, further epidemiological analyses from the Copenhagen General Population Study showed that the circulating peripheral blood eosinophil level,>340 cells/μL, was associated with an increased risk of both moderate and a 2-fold risk of severe exacerbations[72].

These findings demonstrate that the circulating eosinophil at stable state can be used to predict risk of mortality and risk of exacerbations; whilst at the onset of an exacerbation identify those at risk of worsened outcomes; including risk of re-admission, length of hospital stay, response to corticosteroids and mortality. Stratification of single or combined risk of death, decline and exacerbations, modelled on circulating eosinophils, disease severity and response to treatment, would be required to determine the utility of this as a relevant and valid biomarker in COPD risk identification.

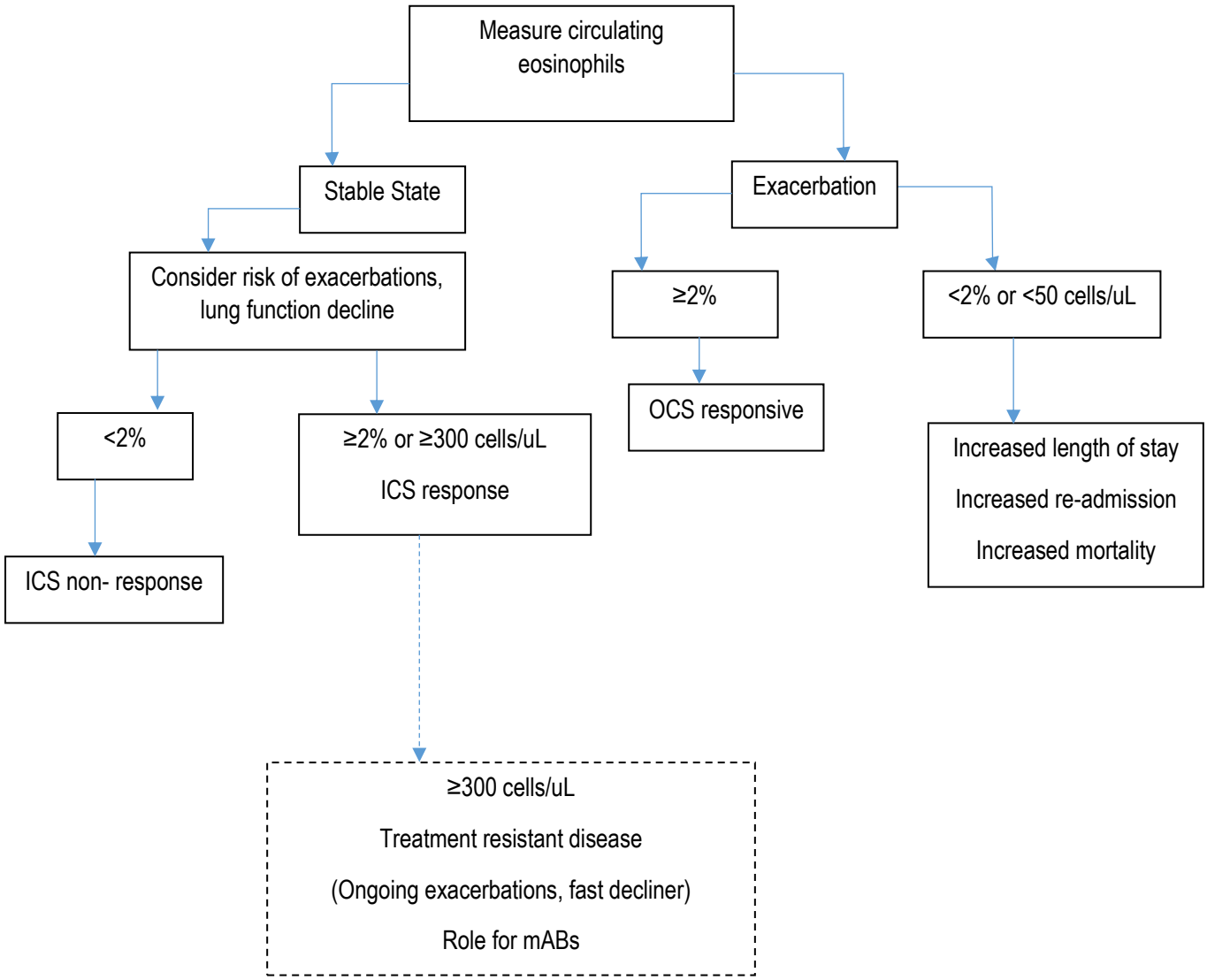
The use of the circulating eosinophil to reduce risk in chronic obstructive pulmonary disease

One of the earliest demonstrations that airway eosinophils conferred corticosteroid responsiveness was first described in subjects with chronic bronchitis in 1978 by Shim et al, in a small crossover prednisolone and placebo clinical trial[73]. Subjects seen to have elevated eosinophil numbers or ‘eosinophil clumps’ were more likely to have a favourable response of lung function, symptoms and exercise capacity to corticosteroids than those without. This finding has been repeatedly and consistently shown with airway eosinophils, of levels greater than 3% in COPD at stable state over the last two decades[40,74-76]; in addition to the demonstration that the normalisation of sputum eosinophils in both asthma[37] and COPD[77] leads to a reduction in moderate and severe exacerbations.

The use of inhaled corticosteroids (ICS) in COPD to improve outcomes has become widespread[78], with studies examining the hypotheses that ICS can reduce lung function decline, exacerbations and mortality[79-87]. In the cases of ISOLDE[79], TORCH[87], INSPIRE[80] and SUMMIT[84], the studies were negative for their primary outcomes. However, these clinical trials did not study either airway or circulating eosinophil characteristics prospectively. Furthermore, the use of ICS in COPD is associated with an increased risk of pneumonia[86-90]; with current ICS use being found to be associated with a 69% increased risk of severe pneumonia in COPD (RR 1.69, 95%CI 1.63 to 1.75)[89]. The recent WISDOM (Withdrawal of Inhaled Steroids during Optimized bronchodilator Management) trial[91] was the first to examine the effect of ICS withdrawal and thus dual bronchodilator therapy (LAMA and LABA), versus 'free' triple therapy of ICS, LAMA and LABA. The study found that the time to first moderate or severe exacerbation was similar in the two study groups, reaching its primary outcome of non-inferiority, but associated with an increase loss of lung function at the end of the study period in the ICS withdrawal group. Due to the initial failure of the ICS studies to demonstrate benefit and the safety concerns about long term ICS use, many of these studies have now been re-analysed exploring the circulating eosinophil to identify ICS treatment responsiveness **(table 1)**.

However, some caution is needed in the interpretation of these findings. The nature of post-hoc analyses have limitations and appropriately designed prospective studies are required to test the hypothesis that the circulating eosinophil identify benefit of ICS in patients with COPD; and these studies are anxiously awaited. Currently, there is no consensus as to whether the absolute or relative eosinophil count should be used; the relative circulating eosinophil count of 2% has been the only level prospectively identified and studied at the time of an exacerbation. Moreover, a relative count during an exacerbation may be relevant when information regarding the remainder of the leukocytes would be applicable; whilst the absolute count is likely to be pertinent at stable state. In addition to this, there is currently no agreed consensus as to the level of circulating eosinophil for which benefit for important clinical outcomes occur and the discrete eosinophil analysis in the post-hoc analyses have confounded that. A more distinct approach would be to apply continuous analyses to derive optimum levels of circulating eosinophils for ranks of ICS benefit. It is plausible to consider that there may be different levels of circulating eosinophils for ICS benefit to impact on lung function decline versus exacerbations and these approaches are important to review **(figure 3).**

Figure 3. Proposed schema illustrating how circulating eosinophils can be used to identify and reduce risk in COPD. Prospective studies and modelling of eosinophils are required to define optimum eosinophils to reduce identifiable risk. ICS inhaled corticosteroids: OCS oral corticosteroids: mABs monoclonal antibodies



Nonetheless the results of these post-hoc analyses have all demonstrated several key findings; i) COPD patients with elevated circulating eosinophils have more exacerbations; ii) the benefit of ICS in reducing exacerbations occurs in patients with an elevated eosinophil count, whether this is the primary or secondary outcome studied; iii) lung function decline and symptom ICS response are greatest with increasing eosinophil counts; iv) in all of these studies, despite treatment there is a basal exacerbation rate of approximately 0.75 exacerbations per year and provide further insight into identifying the COPD ICS responsive patient.

Conclusion

The eosinophil is likely to play an important, yet undefined role, in host-response in patients with COPD. Repeated observations, from large epidemiological studies and post-hoc analyses from clinical trial datasets and have shown that this signal is robust in identifying risk of exacerbations and mortality and reducing risk in identifying both inhaled and systemic corticosteroids. As a biomarker, the eosinophil is relevant and valid, with wide-ranging accessibility, repeatability and responsiveness. Future scientific exploration in addition to national and international guidance, has a duty of care to over 250 million people with COPD worldwide to ensure that eosinophil mechanisms in the pathogenesis of COPD are studied and that measurement of the circulating eosinophil is integral to management of patients with COPD.

Search strategy and selection criteria

References for this review were identified through searches of PubMed for articles published from January 1971 to November 2016, by the use of the terms, "COPD", "eosinophils", "exacerbations", "phenotypes", "corticosteroids" and "randomised clinical trials". Relevant articles published between 2000 and 2016 were identified through searches in Google Scholar and Conference Abstracts. Articles from these searches were reviewed. Articles published in English were included.

Table 1. Eosinophil post-hoc analyses of primary outcomes from original ICS in COPD RCTs

Study name	Study design	Primary outcome	Eosinophil cut off	Post-hoc analysis findings
ISOLDE[79]	Double-blind parallel-group, placebo controlled; FP (500 mcg BD) vs. Placebo	Rate of FEV_1 decline	2% at baseline	<2%: similar rate of decline between placebo and FP (-2.9ml/yr); ≥2%: decrease rate of decline with FP compared to placebo (33.9ml/yr). Reduction of exacerbations (secondary outcome)[92].
INSPIRE[80]	Double-blind, double dummy parallel group; FP/SAL (500/50mcg BD) vs. Tio (18mcg OD)	Exacerbation rate	2% at baseline	≥2%: 25% reduction in exacerbation rate with FP/SAL compared to Tio[9]
TRISTAN[82]	Double-blind parallel group placebo controlled; SAL (50mcg BD) vs. FP (500mcg BD) vs. FP/SAL (500/50mcg BD) vs. placebo	Pre-treatment FEV_1	2% at baseline	No difference in FEV_1[9]. Reduction of exacerbations (secondary outcome)
HZC102870/1[86]	Double-blind; FF/V (200/25mcg OD) vs. FF/V (100/25mcg OD) vs. FF/V (50/25mcg OD) vs. V (25mcg OD)	Exacerbation rate	2% at baseline	<2%: at all doses of FF 10% reduction in exacerbations; ≥2%: at all doses of FF 29% reduction in exacerbations; 42% reduction in exacerbations with FF/V at all doses compared to V alone if eosinophils >6%[6]
FORWARD[83]	Double-blind; BDP/FOR (100/6mcg 2BD) vs. FOR (12mcg BD)	Trough FEV_1 at 12 weeks & exacerbation rate	Quartiles	Significant difference in exacerbation rate reduction of BDP/FOR vs. FOR in eosinophils > 181.6 cells/uL. Highest quartile (>279.8 eosinophils/uL) associated with 46% reduction[7].
LANTERN[93]/ ILLUMINATE[94]	Double-blind double-dummy parallel study; IND/GLY (110/50mcg OD) vs. FP/SAL (500/50mcg BD)	Trough FEV_1 at 26 weeks/ AUC FEV_1 in 12hrs	300 cells/μL	Pooled analysis presented as a poster at ERS[95]. Trend to improvement with FP/SAL compared to IND/GLY in reducing exacerbations (secondary analysis).
WISDOM[91]	Double-blind parallel group; TIO (18 mcg OD) + FP/SAL (500/50mcg BD) vs. TIO (18mcg OD) + SAL (50mcg BD) (ICS withdrawal over 12 weeks)	Time to first moderate/ severe exacerbation	300 cells/μL	Following ICS withdrawal, risk of moderate/severe exacerbations increases when eosinophils >300 cells/μL (equivalent to 3% in study)[8]

ISOLDE Inhaled Steroids in Obstructive Lung Disease in Europe; INSPIRE Investigating New Standards for Prophylaxis in Reducing Exacerbations; TRISTAN Trial of Inhaled Steroids and long acting β2 agonists; FORWARD Foster 48-week Trial to Reduce Exacerbations in COPD; WISDOM Withdrawal of Inhaled Steroids during Optimised bronchodilator Management. FP Fluticasone propionate; SAL Salmeterol; FF Fluticasone Furate; V Vilanterol; BDP Beclomethasone Dipropionate; Tio Tiotropium; FOR Formoterol; IND Indacaterol; GLY Glycopyrronium; FEV_1 Forced expiratory volume in 1 second; AUC area under the curve

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