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# Eosinophilia and Chronic Airway Diseases

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## ABSTRACT

Asthma has long been associated with an eosinophilic trait. Some early studies have shown a relationship between circulating eosinophil numbers and bronchial hyperresponsiveness. Soon after the use of bronchoscopy and induced sputum as a research tool in mild-to-moderate asthma, it was shown that airway eosinophilia, demonstrated by bronchoalveolar lavage, bronchial biopsies, and sputum cell counts, is related to disease severity. Sputum studies have established the concept of asthma inflammatory phenotypes, with approximately 50% of patients having significant airway eosinophilia. Airway eosinophilia is not sufficient to cause asthma, but is a risk factor for poorly controlled disease. Chronic obstructive pulmonary disease, a disease typically associated with prominent neutrophilic inflammation, sometimes also exhibits a significant eosinophilic trait, even if it is less frequent than in asthma and not associated with disease severity. However, both in asthma and chronic obstructive pulmonary disease, eosinophilic inflammation predicts a good response to inhaled or oral corticoids and to anti-interleukin-5.

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## INTRODUCTION

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by a history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time and in intensity, together with variable expiratory airflow limitation[1]. Eosinophilia driven by CD4-derived Th2 cytokines has been considered as a conspicuous trait of allergic asthma as is the case for other atopic diseases like rhinitis or dermatitis[2]. The link between systemic and airway eosinophilia and clinical expression of asthma, irrespective of its allergic component, has been established over the last two decades. More recently it has also been highlighted that eosinophilic inflammation may exist in some patients with chronic obstructive pulmonary disease (COPD). In both asthma and COPD, the eosinophilic trait seems to confer sensitivity to corticoids and may carry some prognostic value regarding disease control. Here we review the incidence and role of eosinophilia in the two main chronic airway diseases and how eosinophils may impact the management of the disease.

## ASTHMA

### Several facets of eosinophilic inflammation

#### *Blood eosinophils*

The relationship between peripheral blood eosinophilia and bronchial hyperresponsiveness has been shown in several studies[3-5]. In an allergy study, baseline circulation eosinophil was found to be inversely correlated to methacholine bronchial hyperresponsiveness. After allergenic challenge, there was a rise in blood eosinophil in those patients developing late bronchospasm, an observation absent in those exclusively developing an early phase[3]. In another study including 87 asthmatics, the authors found bronchial hyper-reactivity related to peripheral blood eosinophil counts but not to total serum immunoglobulin E (IgE) nor to mite-specific IgE[4]. The relationship between blood eosinophil and bronchial hyperresponsiveness was found irrespective of the atopic status[5].

#### *Bronchoalveolar lavage and bronchial biopsy eosinophils*

The application of bronchoscopy as a research tool in mild-to-moderate asthma in the late 1980s confirmed eosinophilic airway infiltration even in the mildest forms of the disease[6]. Wardlaw et al.[7] demonstrated a relationship between PC20 methacholine (concentration of inhaled methacholine causing a 20% fall in forced expiratory volume in 1 second [$FEV_1$]) and bronchoalveolar lavage (BAL) eosinophils and their activation product major basic protein (MBP). Djukanovic et al.[8] showed an increased number of eosinophils in bronchial biopsies associated with signs of mast cell degranulation. Soon after, in a series of 43 asthmatics, Bousquet et al.[9] demonstrated that peripheral blood, BAL, and intraepithelial eosinophils related to asthma severity, though a bronchial biopsy study showed that some severe asthmatics may be characterized by increased neutrophilic infiltration without evidence of eosinophils[10].

### *Sputum eosinophils*

In the early 1990s the technique of induced sputum was shown to be a non-invasive alternative to bronchoscopy for investigating airway inflammation[11]. Sputum eosinophils were shown to correlate reasonably well with bronchial biopsy and BAL eosinophils[12]. The technique rapidly became popular in several centres and allowed sampling larger series of patients than previously done with bronchoscopy. In keeping with what had been shown with blood and BAL eosinophils, bronchial hyperresponsiveness was found to correlate with sputum eosinophils. In series of 118 mild-to-moderate corticosteroid-naive asthmatics, sputum eosinophils accounted for 16% of the variation in the concentration of methacholine, causing a fall in $FEV_1$ of 20% (PC20M)[13]. The relationship was shown to be even more convincing with indirect constricting agents, such as adenosine, than with direct agents like methacholine[14]. The asthma severity assessed by a composite of symptoms and lung function appeared to be related to massive airway infiltration with both eosinophils and/or neutrophils, thereby confirming biopsy studies[15,16]. In a very large cohort of asthmatics, we found that sputum eosinophilia was specifically associated with a low $FEV_1$/FVC ratio (forced expiratory volume in 1 second/forced vital capacity), while sputum neutrophil was related to high functional residual capacity[17].

## Taking into account eosinophilic inflammation in diagnosis of asthma

Using sputum eosinophil count > 1% as an aid to diagnose asthma in mild-to-moderate patients with normal baseline lung function was shown to have excellent performance, classifying just second to methacholine challenge with a diagnostic accuracy of 74%[18]. Induced sputum, which requires local technical expertise, cannot, however, be applied on a large scale, which would be necessary for a diagnostic tool of a common disease such as asthma. Fractional exhaled nitric oxide (FeNO), a totally non-invasive and user-friendly technique, was shown to reflect the extent of airway eosinophilic inflammation in asthma[19]. Not surprisingly, FeNO and sputum eosinophils were shown to perform equally to diagnose asthma in mild-to-moderate patients with preserved baseline airway calibre. In a group of corticoid-naive patients complaining of chronic respiratory symptoms, sputum eosinophils and FeNO were shown to be superior to the recording of peak expiratory variability or the response to inhaled b2 agonists to confirm asthma diagnosis. In these circumstances, the best threshold values seem to be 3% for sputum eosinophils and 20 parts per billion (ppb) for FeNO, with both thresholds having a 92% negative predictive value[20]. In steroid-naive patients with preserved baseline lung function and not showing significant reversibility to $\beta_2$ agonist, we have found that a threshold of 34 ppb FeNO had an 88% positive predictive value to diagnose asthma based on a positive methacholine challenge (PC20 < 16 mg/ml)[21].

## Emergence of the concept of asthma inflammatory phenotype

Green et al.[22] found that approximately 50% of asthmatics encountered in daily practice in a secondary care centre in the UK had a

sputum eosinophil count > 1.9%, irrespective of their treatment. We found that that 69% of mild atopic corticosteroid-naive patients displayed sputum eosinophils > 2.4%[13]. Gibson et al.[23] drew attention to the fact that rather, some asthmatics show prominent neutrophilic inflammation. Induced sputum has been pivotal in gaining insight in the concept of asthma inflammatory phenotypes. Thanks to large series of healthy subjects, it has been possible to determine reference normal values for sputum cell counts[24,25]. In a seminal paper, Simpson et al.[26] proposed to classify the asthmatics according to the type of granulocyte in the sputum. They distinguished four categories of asthmatics: (i) eosinophilic, (ii) neutrophilic, (iii) mixed granulocytic, and (iv) pauci-granulocytic asthma. Based on studies conducted in healthy subjects, there is a general agreement to state that an abnormal sputum eosinophil count is a count > 1-3%. Choosing a cut-off at 3% had the advantage of being outside the "grey zone". Several large cohort studies including more than 100 patients have reported the proportion of eosinophilic asthmatics. Though differences may appear between the studies based on the disease severity, treatment with inhaled corticosteroids (ICS), and the proportion of atopic patients in the different asthma cohorts, it is reasonable to conclude that eosinophilic asthma represents approximately half of the patients and that this figure is possibly increasing with disease severity[13,17,22,27-33] (Table 1). There is more controversy regarding the neutrophilic threshold that defines a neutrophilic asthma phenotype. Thresholds ranging from 49 to 93% have been proposed by different research groups. What is well established is that neutrophil count, in contrast to eosinophil count, increases with age[34] and cumulative smoking history[28]. Our threshold value for sputum neutrophil counts based on large series of healthy subjects, whose mean age is around 40 years, is as high as 76%. In a very large cohort of asthmatics seen at a university hospital, we found that eosinophilic and pauci-granulocytic were the two most frequent categories accounting for 42 and 40% of the patients, respectively[17]. Building further on the eosinophilic inflammation, we have recently proposed a new classification of asthma based on the concordant versus discordant presence of raised eosinophils in blood versus sputum. Setting abnormal threshold values of sputum and blood eosinophils at 3% and 400 blood eosinophils/µl, respectively, we have distinguished four classes of asthmatics in patients seen in daily practice. The first class comprises the patients with < 3% sputum eosinophil and < 400 blood eosinophils/µl and accounts for 49% of the patients. The second class comprises patients with raised sputum eosinophil counts but normal blood eosinophil counts and represents 25% of the patients. The third class represents a small group of patients with high blood eosinophil counts but sputum eosinophil counts < 3%, while the fourth class features those patients who combine high sputum and blood eosinophil counts and accounts for 19% of the patients. The patients from the latter group are clearly the most severe with the greatest lung function impairments and the highest exacerbation rate[35].

The stability of inflammatory phenotype remains a key issue when it comes to mounting a treatment strategy based on a one time-point assessment of airway inflammation. Repeating sputum induction within one week has shown good reproducibility in sputum

**Table 1.** Proportion of eosinophilic asthma in large cohort of adult asthmatics (n > 100) with varying disease severity

| Ref. | (n) | ICS | $FEV_1$ (% pred) | Asthma severity | Sputum eos (%) | Sputum eosinophil threshold to define eosinophilic phenotype | Proportion of asthmatics with raised sputum eosinophil |
|---|---|---|---|---|---|---|---|
| Louis R et al.[13] Allergy. 2002 | 118 | None | Mean (SD) 95 (12) | Mild-to-moderate essentially atopic | Median (range) 4.8 (0-75) | 2% | 69% |
| Green R et al.[22] Thorax. 2002 | 259 | 41% treated 114 145 | Mean (SE) 86 (1.4) 86 (1.6) | Mild-to-moderate Atopic Non-atopic | Median (IQR) 2.5 (7) 2 (13) | 1.9% | 52% for the whole group |
| D'silva et al.[27] Can Respir J. 2006 | 664 175 | Yes Yes | Mean (SD) 88 (19) Mean (SD) 58 (17) | Mild-to-moderate Severe | ND ND | 1.1% 1.1% | 42% 45% |
| Hastie A et al.[29] J Allergy Clin Immunol. 2010 | 175 | ND | ND | All disease severity | ND | 2% | 35% |
| Heaney L et al.[30] Thorax. 2010 | 123 | All | Mean (SD) 65 (24) | Severe | Median (IQR) 3 (0.25-11.25) | 3% | 50% |
| Mc Grath et al.[31] Am J Respir Crit Care Med. 2012 | 350 645 | None All | Mean (SD) 84 (13) Mean (SD) 83 (15) | Mild-to-moderate Mild-to-moderate | ND ND | 2% 2% | 36% 17% |
| Schleich F et al.[17] BMC Pulm Med. 2013 | 508 | 70% treated Yes No | Mean (SD) 84 (19) | All disease severity | Median (range) 2 (0-94) | 3% | 44% for the whole group 41% 45% |
| Zhang X et al.[33] Clin Exp Allergy. 2014 | 164 | All | Mean (SD) 74 (20) | All disease severity | ND | 3% | 43% |
| Wagener A et al. [32] Thorax. 2015 | 110 | All | Mean (SD) 101 (17) | Mild-to-moderate | Median (IQR) 0.6 (0.1-3.6) | 3% | 27% |
| Shaw D et al.[59] Eur Respir J. 2015 | 28 53 | All | Mean (SEM) 67 (1) | Severe non-smoking Severe smoking or ex-smoking | Median (IQR) 2.7 (0-19) 4.1 (1-14) | 1.9% 1.9% | 58% 60% |
| Demarche S et al.[28] BMC Pulm Med. 2016 | 833 464 344 | 62% treated | ND | All disease severity Atopic Non-atopic | Median (IQR) 2.8 (0.2-13.8) 1.6 (1.4-4) | 3% 1% | 46% (for the whole group) 58% (for the whole group) |

* Eos: eosinophils; $FEV_1$: forced expiratory volume in 1 second; ICS: inhaled corticosteroid; IQR: interquartile range; ND: no data; pred: predicted; SD: standard deviation; SE: standard error; SEM: standard error of the mean.

eosinophil and neutrophil count, though the latter may show an increase at the second induction when repeated within 24 hours after the first. However, the long-term stability of inflammatory phenotype over time has not been extensively studied. There is one report from the Netherlands of patients with prominent sputum eosinophilia sampled five years apart[36]. Another study from USA, including asthmatics engaged in a drug trial, showed a

slight majority of patients displaying persistent non-eosinophilic phenotype, while there are patients in whom sputum eosinophilia may be fluctuating over time[31].

## Approaching the eosinophilic phenotype

There have been several attempts to approach the eosinophilic phenotype with a user-friendly biomarker. To date both FeNO and blood eosinophil counts have proved to be the most satisfactory biomarkers in that respect[17,32]. More recently, several studies have measured volatile organic compounds in the exhaled breath[37]. This approach has the potential to identify and discriminate between eosinophilic and neutrophilic inflammation both *in vitro*[38] and *in vivo*[39], a separation not possible using FeNO or blood markers. To date, however, no clear breath prints specific of different phenotypes have been established due to limited series and poor reproducibility[37].

## Mechanisms leading to airway eosinophilia in asthma

Mechanisms leading to airway eosinophilia are numerous and complex (Fig. 1). There is convincing evidence from the literature that mast cell activation upon allergen exposure in sensitized asthmatics results in an eosinophilic influx in the airways. The model of bronchial allergenic challenge has provided much information in this respect[40]. A dramatic increase in BAL or sputum eosinophils occurs 4-6 hours after allergen exposure, which is concomitant of late bronchospasm. This is thought to be linked to the release of Th2 cytokines from CD4 lymphocytes, epithelial cells, and mast cells following IgE receptor cross linking at cell surface. We showed a sharp increase in interleukin (IL)-4 from sputum cell culture supernatant six hours after allergen exposure, highlighting the amplification of the Th2 response following allergen exposure in sensitised subjects[41]. In stable asthmatics, a relationship between sputum tryptase level, a biomarker of mast cell degranulation, and sputum eosinophils was demonstrated[42]. Further supporting the link between IgE-mediated pathway and eosinophilia, we found that asthmatics in whom sputum IgE was detectable had greater sputum eosinophil counts compared to those without detectable sputum IgE[43]. Cysteinyl-leucotrienes, which are released upon IgE mast cell activation, contribute to sputum eosinophil chemotactic activity in asthmatics[44]. In a large cohort of more than 500 patients, we found that total serum IgE, FeNO, and blood eosinophils were independently associated with sputum eosinophils[17]. The IgE-mediated sensitisation to animal dander has recently been shown to be particularly associated with the eosinophilic phenotype. However, sputum eosinophilia may be present in asthma irrespective of the atopic status[28]. Though it has been shown that non-atopic asthmatics may sometimes have airway IgE production directed towards some aeroallergens[45], there is currently great interest in epithelial cells and innate lymphoid cells as key players in driving eosinophilic inflammation in non-atopic subjects[46]. Epithelial cells may release IL-8, which, once bound to IgA, has chemotactic activity for eosinophils. The amounts of IL-8/IgA were found to be correlated with sputum eosinophils in asthmatics[47]. Epithelial cells may also release thymic stromal

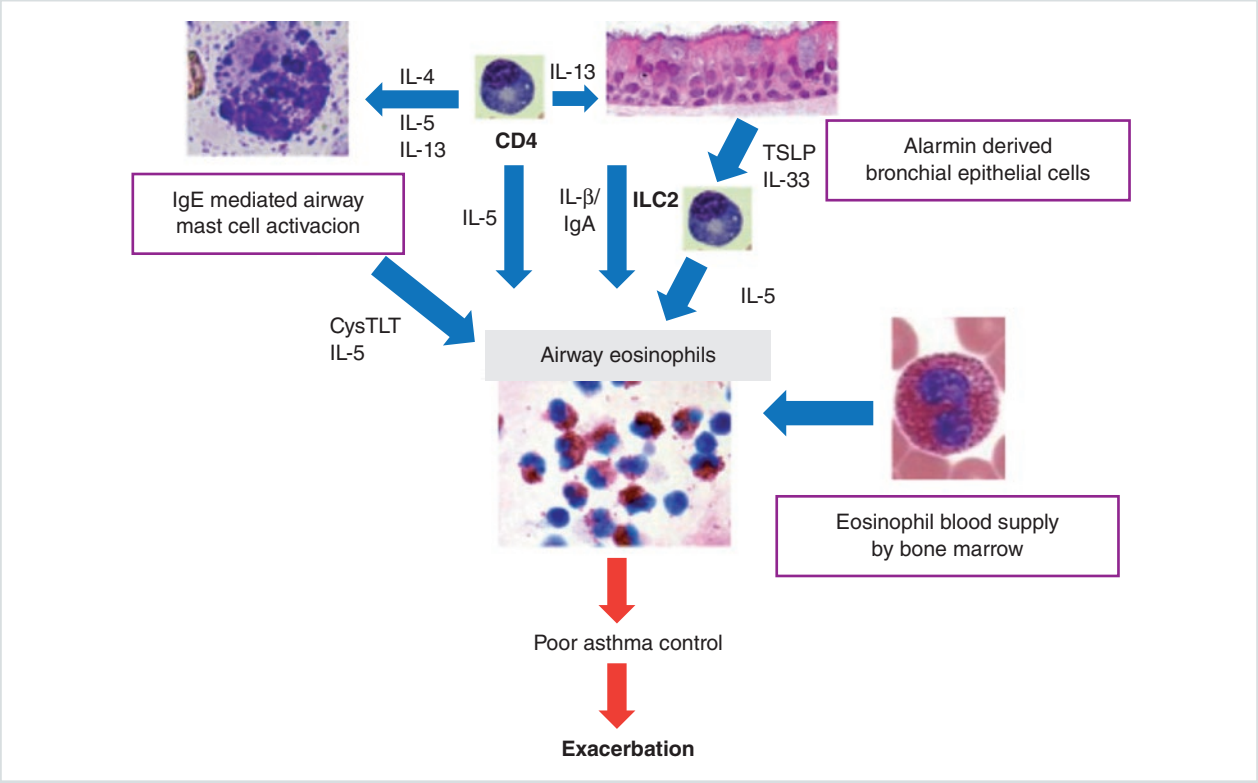


**Figure 1.** Causes and consequences of airway eosinophilia in asthma. Several potential mechanisms leading to airway eosinophilia. Immunoglobulin-E-mediated mechanism is likely to be of great importance in atopic asthma, while the epithelium-driven mechanism may be key in non-atopic asthma and perhaps also in some chronic obstructive pulmonary disease. The blood compartment, supplied by bone marrow activity, is also essential in providing a pool of cells to be recruited in the airways. High airway eosinophilia is a risk factor for poor asthma control and exacerbation.
CystLT: cysteinyl leukotriene; IgE: immunoglobulin E; IL: interleukin; ILC2: type 2 innate lymphoid cell. TSLP: thymic stromal lymphopoietin.

lymphopoietin (TSLP) and IL-33, which stimulate innate lymphoid cells to release cytokines like IL-5[46]. Whichever the type of lymphocyte involved in this immunological process, eosinophilic asthmatics display greater sputum lymphocyte and epithelial cell counts, supporting the concept of a close interaction between lymphocytes and eosinophils leading to epithelial injury and shedding[28]. What seems clear is that eosinophilic asthma appears to be determined by different molecular mechanisms and gene expression as compared to other asthma phenotypes.

Eosinophilic asthmatics display raised IL-5 and IL-13 levels in their sputum supernatant[43] and a peculiar gene signature in sputum cells[48] compared to healthy subjects. Interestingly, sputum cells from eosinophilic asthmatics cultured for 24 hours were shown to produce greater levels of IL-4 but less tumour necrosis factor (TNF)-α compared to those of healthy subjects[49]. This finding might partly explain why treatment with anti-TNF-α has been rather disappointing in most of the studies conducted in asthma[50], whereas it has become an established treatment in other

inflammatory diseases like rheumatoid arthritis or Crohn's disease. Neutrophilic asthma is driven by other molecular mechanisms. It seems to be more related to innate immune reaction towards infectious and irritant agents, leading to inflammasome activation[51] and some microRNA have also recently been shown to contribute to this phenotype[52].

## How inflammatory phenotype relates to severe asthma

We have shown that uncontrolled asthma is associated with raised airway eosinophilia[53] and this is particularly true when raised blood eosinophil count > 400 cells/µl combines with elevated sputum eosinophils > 3%, a situation associated with poor asthma control and greater exacerbation rate[35]. In a recent retrospective study from the UK including more than 100,000 patients, it clearly appeared that having blood eosinophil counts > 400 cells/µl, which occurs in 16% of patients, is a major risk factor for uncontrolled asthma and exacerbations[54]. Since the early publications pointing out massive granulocytic airway infiltration in severely uncontrolled asthma (symptoms and lung function)[15,16], the definition of severe asthma has been firmly established: consensus American Thoracic Society (ATS) and the European Respiratory Society (ERS). Simply said, a severe asthmatic is a patient who remains uncontrolled despite a combination of high dose of ICS associated with long acting b2 agonist, or one who needs to receive such a treatment to keep control[55]. Based on this concept, there have been now several large series of severe asthmatics investigated using induced sputum to characterise the inflammatory phenotypes. The majority of asthmatic patients seen in secondary care in a UK centre had significant sputum eosinophilia[56]. Similarly, the majority of refractory asthmatics included in a UK registry had sputum eosinophils ≥ 3%[30]. The same was found in the Belgian severe asthma registry including more than 300 severe asthmatics, with 55% of patients being qualified as eosinophilic based on sputum eosinophil counts ≥ 3%, while only 20% had significantly raised sputum neutrophil count > 76%[57]. The importance of sputum eosinophilia was slightly less in the US Severe Asthma Research Program (SARP) cohort, with median value of sputum eosinophil count ranging between 1 and 2% in the most severe clusters[58]. The recent European U-BIOPRED adult severe asthma cohort, including more than 400 severe asthmatic patients, has shown that the majority of severe asthmatics had sputum eosinophils > 3%. Interestingly, those with a current or a past history of smoking did not have less sputum eosinophils, clearly indicating that smoking history in asthmatics does not preclude the presence of eosinophilic inflammation[59]. In the same study, there is clearly no link between asthma severity and sputum neutrophil counts. Therefore, it appears that the majority of severe asthmatics display significant eosinophilic rather than neutrophilic inflammation. All these data point to a residual airway eosinophilic inflammation that is relatively resistant to ICS and in some cases to oral corticoids. It does not mean, however, that the patients are completely insensitive to corticoids because Brinke et al.[60] demonstrated some clinical improvement and reduction in eosinophilic inflammation in refractory eosinophilic asthmatics when they were intramuscular injected with triamcinolone. Nevertheless, controlling eosinophilic inflammation

in these patients can only be achieved at the expense of serious side effects that outweigh clinical benefits. It is important, however, to bear in mind that some severe asthmatics failed to exhibit any sign of eosinophilic inflammation and that other factors may drive disease severity; these can be obesity[56] or psychosocial disorders[61].

## Taking into account eosinophilic inflammation in the management strategy of asthma

Early bronchial biopsy studies had already shown consistent decrease in bronchial biopsy eosinophils after a few weeks treatment with ICS. The change in mucosal eosinophilia correlated with a change in bronchial hyperresponsiveness[62]. This initial observation has been largely confirmed by studies using induced sputum to assess airway eosinophilic inflammation[63]. In mild-to-moderate corticosteroid-naive patients, intervention studies with ICS using induced sputum have usually revealed that a sputum eosinophil count < 1-3% at the initiation of treatment was predictive of a poor response to ICS over a period of 4-8 weeks in terms of lung function and patient perspective outcomes (Table 2)[64-68]. Due to technical difficulty, it is not reasonable to assume that sputum cell count could be obtained in every single asthmatic before initiating or adjusting maintenance treatment. Therefore, studies have been conducted with FeNO as a surrogate marker of sputum eosinophils. Thresholds ranging from 33 to 47 ppb were shown to be predictive of response to ICS[67,69], which fits the finding that a threshold of 41 ppb is the best compromise to identify a corticosteroid-naive asthmatic with a sputum eosinophil count ≥ 3% (Fig. 2). Application of FeNO in primary care has recently proved to be useful to manage mild-to-moderate asthma in a cost effective way as it allows to step up ICS in those who are really in need (FeNO > 50 ppb) while down titrating them when FeNO is < 25 ppb, suggesting an absence of residual corticosensitive airway inflammation[70].

If using induced sputum is not realistic on a large scale, this investigation seems to be perfectly justified at expert centres dealing with severe asthma. One of the pivotal studies showing the interest in using induced sputum in managing severe asthma was published more than 10 years ago by Green et al[71]. They conducted a study to compare two strategies for adjusting the dose of ICS in severe asthmatics who required on average two courses of oral corticoids in the year prior to randomisation. The authors showed that adjusting the dose of ICS and oral corticoids according to sputum eosinophil count in order to maintain it below 3% resulted in less exacerbation and hospitalisation. Therefore, the authors claimed that ICS and oral corticoids titration in severe asthmatics should be decided based on sputum eosinophil counts rather than on symptoms and lung function. A few years later a similar sputum strategy was shown to be essentially useful in severe asthmatics but not so pertinent in mild-to-moderate asthmatics. In addition, the sputum-guided strategy only reduced eosinophilic exacerbation, which did not account for more than 50% of all exacerbations seen in asthmatics[72].

The emergence of anti-IL-5 has strengthened the view that eosinophils play a major role in some severe asthmatics. Indeed both

**TABLE 2.** Prospective trials comparing the effect of inhaled corticosteroids in steroid-naive asthmatics classified into eosinophilic asthma and non-eosinophilic asthma phenotypes (with data on sputum eosinophils and at least one clinical outcome)

| Ref. | Patients | Sputum eos (%) | (n) | Base-line $FEV_1$ (% pred) | ICS, dose and duration | Placebo | Sputum eos (%) without ICS* | Sputum eos (%) with ICS* | Clinical outcomes Intragroup comparison# | Clinical outcomes Intergroup comparison |
|---|---|---|---|---|---|---|---|---|---|---|
| Bacci et al.[66] 2006 | Moderate asthmatics | ≤ 3% | 17 | 90.2 ± 17.3[a] | BDP 500 µg bid 4 weeks | / | 1 (0-2.7)[b] | 0.6 (0-11.9) | ↓ rescue $\beta_2$-agonist use, trend for ↓ symptom score, no significant effect on lung function parameters | Higher PEF and lower symptom score after 4 weeks of treatment in patients with EA |
| | | > 3% | 50 | 87.9 ± 15.4[a] | | / | 18.2 (3.1-80.1)[b] | 1.4 (0-13.8)† | ↑$FEV_1$, ↑$PD_{20}$M, ↑morning PEF, ↓PEF amplitude % mean, ↓% of days with abnormal PEF amplitude % mean, ↓symptom score, ↓rescue $\beta_2$-agonist use | |
| Berry et al.[65] 2007 | Asthmatics with a Juniper asthma control score > 1.57 | < 1.9%‡ | 10 | 88 (4.9)[c] | MF 400 µg once daily 8 weeks | Yes (cross-over study) | 0.4 (0.2)[d] | 0.5 (0.1) | No significant effect on Juniper asthma QoL score and $PC_{20}$M | More improvement in $PC_{20}$M and Juniper asthma QoL score in EA (versus placebo) |
| | | > 1.9% | 6 | 90.3 (6.2)[c] | | | 9.9 (0.1)[d] | 2.3 (0.2)† | ↑$PC_{20}$M, ↑Juniper asthma QoL score | |
| Cowan et al.[67] 2010 | Moderate asthma | < 2% | 28 | 88 ± 16[a] | FP 1000 µg daily 28 days | / | 0.5 (0.4-0.8)[e] | 0.6 (0.3-1.0) | ? | More improvement in ACQ, ACT, AQLQ, $PC_{20}$AMP in EA versus NEA |
| | | ≥ 2% | 60 | 88 ± 16[a] | | / | 17.9 (14.1-22.8)[e] | 3.6 (2.3-5.8)† | ? | |
| Godon et al.[68] 2002 | Mild uncontrolled asthmatics | < 1% | 14 | 79.6 ± 22.3[a] | FP 250 µg bid 1 month | / | 0.4 (1.0)[b] | 0.1 (1.1) | ↓symptom score, ↑QoL, ↓ $\beta_2$-agonist use, ↑$FEV_1$, ↑$PC_{20}$M | No statistically significant difference for clinical outcomes between EA and NEA |
| | | ≥ 1% | 32 | 80.4 ± 15.5[a] | | / | 9.1 (13.6)[b] | 1.0 (2.9)† | | |
| | | < 3% | 21 | 83.5 ± 21.1[a] | | / | 1.0 (1.6)[b] | 0.3 (1.3) | | |
| | | ≥ 3% | 25 | 77.3 ± 14.8[a] | | / | 12.1 (14.2)[b] | 1.0 (3.6)† | | |
| Pavord et al.[85] 1999 | Asthmatics | < 3% | 9 | 81.3[f] | Bud 400 µg bid 2 months | / | 0.7[g] | ? | No significant effect on $FEV_1$, symptom VAS, PEF amplitude % mean, $PC_{20}$M | More improvement in $PC_{20}$M and symptom VAS in EA versus NEA |
| | | ≥ 3% | 14 | 86.2[f] | | / | 11.0[g] | ? | ↓symptom VAS, ↓ PEF amplitude % mean, ↑$PC_{20}$M | |

*For uncontrolled studies, the percentage of sputum eosinophils without and with ICS refers to the percentage before and after treatment with ICS, respectively. For crossover studies, the percentage of sputum eosinophils without and with ICS refers to the end of treatment with placebo and the end of treatment with ICS, respectively. #For uncontrolled studies: comparison before-after treatment with ICS; for controlled studies: comparison of ICS versus placebo. †$p < 0.05$ versus sputum eosinophils without ICS. ‡On at least 2 occasions separated by 1 month.

[a]Mean ± SD. [b]Median (IQR). [c] Mean (SE). [d]Geometric mean (log SE). [e]Geometric mean (95% CI). [f]Mean. [g]Geometric mean.

ACQ: Asthma Control Questionnaire; ACT: Asthma Control Test; AQLQ: Asthma Quality of Life Questionnaire; BDP: beclomethasone dipropionate; bid: twice daily; Bud: budesonide; EA: eosinophilic asthma; eos: eosinophils; $FEV_1$: forced expiratory volume in 1 second; FP: fluticasone propionate; ICS: inhaled corticosteroid; IQR: interquartile range; MF: mometasone furoate; NEA: non-eosinophilic asthma; $PC_{20}$M: provocative concentration of methacholine causing a 20% fall in $FEV_1$; $PC_{20}$AMP: provocative concentration of adenosine monophosphate causing a 20% fall in $FEV_1$; $PD_{20}$M: provocative dose of methacholine causing a 20% fall in $FEV_1$; PEF: peak expiratory flow; QoL: quality of life.; SD: standard deviation; SE: standard error.

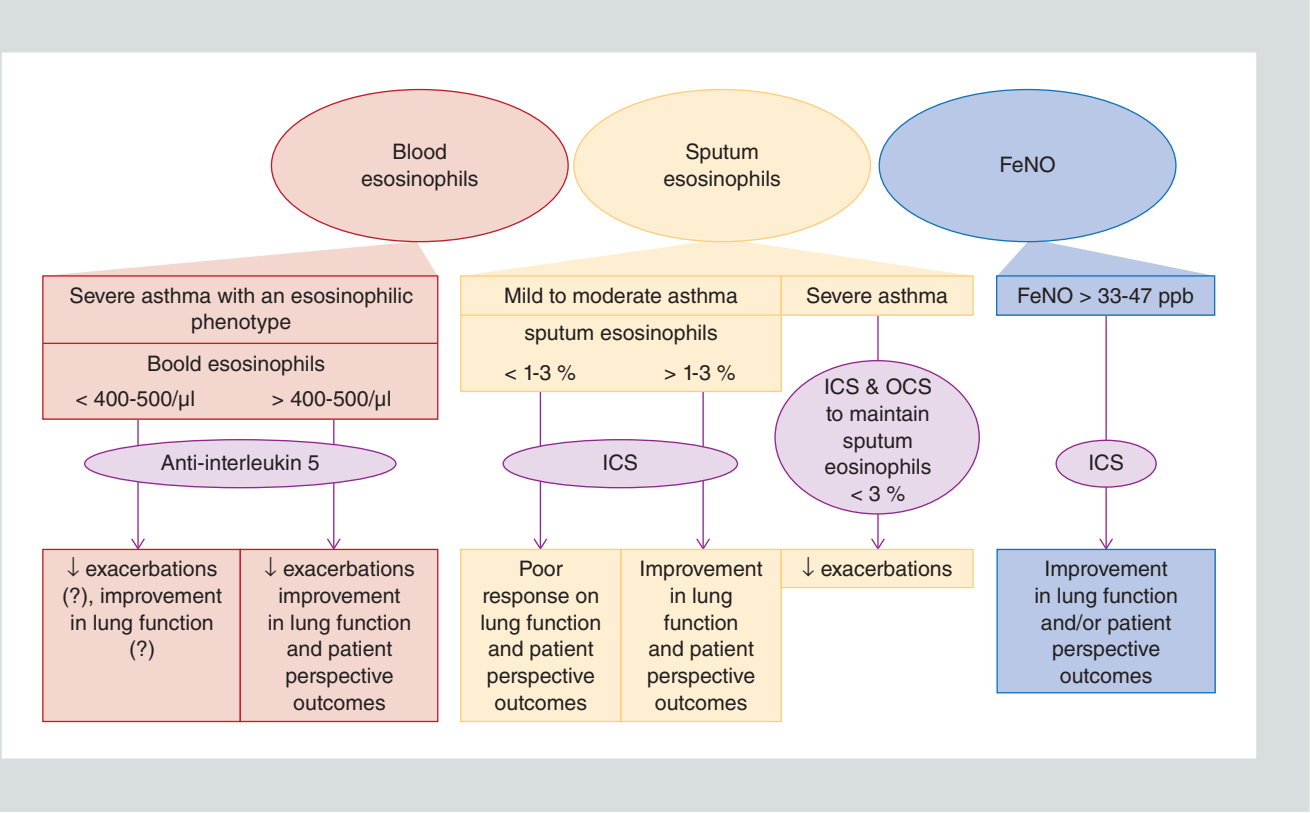


**Figure 2.** Predictive values of the eosinophilic trait (including surrogate fractional exhaled nitric oxide) in response to corticosteroids and anti-interleukin-5 in asthmatics.
FeNO: fractional exhaled nitric oxide; ICS: inhaled corticosteroid; OCS: oral corticosteroid.

mepolizumab[73] and reslizumab[74] have been shown to dramatically reduce the number of exacerbations in asthmatics who keep an eosinophilic phenotype despite high-dose ICS and, sometimes, oral corticoids (Fig. 2). The size effect is proportional to baseline blood eosinophil count and particularly marked when blood eosinophil counts exceed 400-500 cells/µl, a clinical condition in which anti-IL-5 may also improve day-to-day asthma control[74,75]. Interestingly, sputum eosinophil count is less predictive of the effect of anti-IL-5, whereas it helps to adjust ICS dose as demonstrated by Green et al.[71] and Jayaram et al.[72] in their studies. Mepolizumab was shown to rapidly and deeply deplete the blood compartment from eosinophils. Effects on bone marrow and bronchial content in eosinophils, though significant, were less marked[76]. This finding suggests that depleting the circulating pool of eosinophils is of great importance to prevent exacerbation in the severe patients who maintain high blood eosinophil counts.

## Eosinophilic chronic bronchitis

The fact that asthma is more than just airway eosinophilic inflammation was demonstrated by the description of an entity called "eosinophilic chronic bronchitis". These patients have symptoms of productive cough, and sometimes wheeze, often exacerbated during the night, but they do not have bronchial hyperresponsiveness and consequently do not

really complain of breathlessness[77]. Brightling et al.[78] showed that the histopathologic difference between asthma and eosinophilic chronic bronchitis was actually the presence of mast cells infiltrating the airway smooth muscle in asthmatics not found in those displaying airway eosinophilia without bronchial hyperresponsiveness. This finding emphasizes the importance of the interaction between mast cells and smooth muscle to have the full-blown asthma phenotype in which bronchospasm is critical. Not surprisingly, the symptoms of patients with chronic eosinophilic bronchitis are well controlled by ICS.

## CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease is a chronic airway disease due to repeated exposure to smoke and toxic particles that results in progressive and irreversible airway flow rate limitation. Though COPD has mainly been seen as neutrophilic airway disease[79,80], it has become clear that a proportion of COPD patients actually display raised eosinophil counts (≥ 3%) in sputum sampled in stable state. It seems that this proportion ranges between 30 and 40% according to the studies. Therefore, eosinophilic inflammation cannot be neglected in this disease entity.

In contrast to asthma, eosinophil counts in sputum have never been considered as an aid to the diagnosis of COPD, which is essentially based on a spirometric criterion. The eosinophilic component, however, raises some important issues in the management of the disease.

## Eosinophilic chronic obstructive pulmonary disease

In a series of 83 patients, Brightling et al.[81] found that one third of stable COPD patients had sputum eosinophil count > 4.5%. Furthermore, Leigh et al.[82] found that 37% of stable COPD patients had sputum eosinophils > 3%. We have recently found a similar figure in a series of 58 out of 155 patients (37%) displaying sputum eosinophil counts ≥ 3%[83]. In our experience, airway eosinophilia in COPD does not seem to be linked with any specific trait of the disease. In particular, there is no relation with atopy, smoking history, or lung function impairment. A report from the ECLIPSE study has even indicated that the disease might be less severe as assessed by the BODE index when blood eosinophil count is > 2%[84]. In the same report, only 4% of patients had repeated sputum eosinophil counts > 3%. Thus, the proportion of regular eosinophilic COPD seems to be much less than that of occasional ones. Some authors suggested that eosinophilic COPD might actually be the long-term evolution of the aforementioned chronic eosinophilic bronchitis[85].

There has been recent renewed interest in eosinophilic inflammation during a COPD exacerbation. Yet, one of the first reports that drew attention to the eosinophilic component in COPD exacerbation was published more than 20 years ago by Saetta et al.[86]. Using bronchial biopsies, the authors showed that COPD had significant increases in mucosal eosinophilia during an exacerbation. More recently, Bafadhel et al.[87] has shown that 28% of COPD exacerbation featured blood eosinophil counts > 2%. Furthermore, in COPD patients admitted to hospital for an exacerbation,

eosinopenia < 50/µl was to shown be an independent risk factor of mortality together with acidosis, intense dyspnea, and atrial fibrillation[88].

## How to approach eosinophilic chronic obstructive pulmonary disease

Unlike what has been shown in asthma, FeNO is rarely elevated in COPD and is overall a poor predictor of sputum eosinophils in COPD. This is mainly due to cigarette smoking that dramatically reduces FeNO levels. By contrast, blood eosinophil counts do reasonably well in predicting sputum eosinophil count, with the best threshold identified at 160 cells/µl or 2%[89]. Interestingly, these thresholds are slightly lower than those in asthma, which could suggest that the driving force attracting the eosinophils from the circulating pool into the airways is actually stronger in COPD than in asthma.

## Mechanisms of eosinophilic inflammation in chronic obstructive pulmonary disease

Though mast cell activation through an IgE-mediated process has clearly been shown to be a major contributor to eosinophilic inflammation in asthma[2], the molecular mechanisms behind eosinophilia in COPD remain unclear. Atopy does not seem to play a major role, even if some eosinophilic COPD patients may show signs of mast cell activation in their airways as reflected by raised sputum tryptase levels[90]. As aforementioned, it is likely that potent eosinophilic chemotactic factors are released from epithelial cells as a consequence of chronic smoke exposure. Interleukin-8, a potent neutrophil chemotactic agent that is highly increased in airways of COPD[91], may become chemotactic for eosinophils when combined to IgA, the key immunoglobulin within the airways[92]. Papi et al.[93] found that viral infection with rhinovirus may actually lead to a recruitment of eosinophils in the airways of COPD patients, which may explain why some COPD exacerbations display eosinophilic inflammation. It also is conceivable that type 2 innate lymphoid cell (ILC2) activated by epithelial-derived IL-33 play a key role in these circumstances as it is anticipated in non-atopic asthma, but this has to be confirmed.

## Taking into account eosinophilic inflammation in the management strategy of chronic obstructive pulmonary disease

Brightling et al.[81] first demonstrated that eosinophilic COPD (sputum eosinophil count > 4.5%) displayed a greater functional and symptomatic response to a 15 day course of oral prednisolone compared to those without airway eosinophilia (< 1%). The same was shown with inhaled corticoids given for eight weeks[94]. The changes in outcomes were, however, much less pronounced than those seen in asthmatics. A retrospective analysis of ICS/LABA drug trials has recently shown that adding an ICS to a long-acting $\beta_2$-agonist (LABA) brings advantage in terms of reducing exacerbations only when the blood eosinophil count was > 2% at baseline[95]. Some studies investigated the sputum strategy in managing COPD. In a small monocentric study, Siva

et al.[96] showed that adjusting the dose ICS and oral corticoids to sputum eosinophil counts in COPD to maintain it below 3% resulted in less exacerbation and hospitalisation. This is of great importance given the potential poor outcome of a hospitalisation in COPD. The impact of anti-IL-5 on COPD exacerbations warrants further studies, even though a small monocentric study has suggested some benefit in the patients whose blood eosinophil count exceeds 200 cells/µl[97].

## CONCLUSION

Blood and airway eosinophilia is a common and important trait to look at in chronic airway inflammatory diseases. Although not enough to paint the complete disease picture in asthma, the eosinophilic trait is associated with a more severe disease in terms of lung function impairment, asthma control, and propensity of exacerbation (Fig. 1). Both in asthma and COPD, the eosinophilic trait predicts a good response to treatment with inhaled ICS and, sometimes, oral corticoids. In those patients who remain eosinophilic despite appropriate treatment with glucocorticoids, anti-IL-5 has been shown to be very effective in reducing exacerbation and improving asthma control (Fig. 2).

## REFERENCES


1. Global Initiative of Asthma, 2016. Available at: http://ginasthma.org/2016-gina-report-global-strategy-for-asthma-management-and-prevention/ [Accessed 14/04/2016].
2. Kay AB. Allergy and allergic diseases. First of two parts. N Engl J Med. 2001;344:30-7.
3. Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. Clin Allergy. 1985;15:411-8.
4. Iijima M, Adachi M, Kobayashi H, Takahashi T. [Relationship between airway hyperreactivity and various atopic factors in bronchial asthma]. Arerugi. 1985;34:226-33.
5. Taylor KJ, Luksza AR. Peripheral blood eosinophil counts and bronchial responsiveness. Thorax. 1987;42:452-6.
6. Djukanovic R, Roche WR, Wilson JW et al. Mucosal inflammation in asthma. Am Rev Respir Dis. 1990;142:434-57.
7. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. Am Rev Respir Dis. 1988;137:62-9.
8. Djukanovic R, Wilson JW, Britten KM et al. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. Am Rev Respir Dis. 1990;142:863-71.
9. Bousquet J, Chanez P, Lacoste JY et al. Eosinophilic inflammation in asthma. N Engl J Med. 1990;323:1033-9.
10. Wenzel SE, Schwartz LB, Langmack EL et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med. 1999;160:1001-8.
11. Pin I, Gibson PG, Kolendowicz R et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax. 1992;47:25-9.
12. Maestrelli P, Saetta M, Di Stefano A et al. Comparison of leukocyte counts in sputum, bronchial biopsies, and bronchoalveolar lavage. Am J Respir Crit Care Med. 1995;152:1926-31.
13. Louis R, Sele J, Henket M et al. Sputum eosinophil count in a large population of patients with mild to moderate steroid-naive asthma: distribution and relationship with methacholine bronchial hyperresponsiveness. Allergy. 2002;57:907-12.
14. Joos GF, O'Connor B, Anderson SD et al. Indirect airway challenges. Eur Respir J. 2003;21:1050-68.
15. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med. 2000;161:9-16.
16. Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. Am J Respir Crit Care Med. 1999;160:1532-9.
17. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. BMC Pulm Med. 2013;13:11.
18. Hunter CJ, Brightling CE, Woltmann G, Wardlaw AJ, Pavord ID. A comparison of the validity of different diagnostic tests in adults with asthma. Chest. 2002;121:1051-7.
19. Schleich FN, Seidel L, Sele J et al. Exhaled nitric oxide thresholds associated with a sputum eosinophil count >/=3% in a cohort of unselected patients with asthma. Thorax. 2010;65:1039-44.
20. Smith AD, Cowan JO, Filsell S et al. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. Am J Respir Crit Care Med. 2004;169:473-8.
21. Schleich FN, Asandei R, Manise M, Sele J, Seidel L, Louis R. Is FENO50 useful diagnostic tool in suspected asthma? Int J Clin Pract. 2012;66:158-65.
22. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax. 2002;57:875-9.
23. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest. 2001;119:1329-36.
24. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. Am J Respir Crit Care Med. 2000;161:475-8.
25. Spanevello A, Confalonieri M, Sulotto F et al. Induced sputum cellularity. Reference values and distribution in normal volunteers. Am J Respir Crit Care Med. 2000;162:1172-4.
26. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology. 2006;11:54-61.

27. D'Silva L, Hassan N, Wang HY et al. Heterogeneity of bronchitis in airway diseases in tertiary care clinical practice. Can Respir J. 2011;18:144-8.
28. Demarche S, Schleich F, Henket M, Paulus V, Van HT, Louis R. Detailed analysis of sputum and systemic inflammation in asthma phenotypes: are paucigranulocytic asthmatics really non-inflammatory? BMC Pulm Med. 2016;16:46.
29. Hastie AT, Moore WC, Meyers DA et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. J Allergy Clin Immunol. 2010;125:1028-36.
30. Heaney LG, Brightling CE, Menzies-Gow A, Stevenson M, Niven RM. Refractory asthma in the UK: cross-sectional findings from a UK multicentre registry. Thorax. 2010;65:787-94.
31. McGrath KW, Icitovic N, Boushey HA et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. Am J Respir Crit Care Med. 2012;185:612-9.
32. Wagener AH, de Nijs SB, Lutter R et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. Thorax. 2015;70:115-20.
33. Zhang XY, Simpson JL, Powell H et al. Full blood count parameters for the detection of asthma inflammatory phenotypes. Clin Exp Allergy. 2014;44:1137-45.
34. Thomas RA, Green RH, Brightling CE et al. The influence of age on induced sputum differential cell counts in normal subjects. Chest. 2004;126:1811-4.
35. Schleich FN, Chevremont A, Paulus V et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir J. 2014;44:97-108.
36. van Veen IH, ten BA, Gauw SA, Sterk PJ, Rabe KF, Bel EH. Consistency of sputum eosinophilia in difficult-to-treat asthma: a 5-year follow-up study. J Allergy Clin Immunol. 2009;124:615-7.
37. Fens N, van der Schee MP, Brinkman P, Sterk PJ. Exhaled breath analysis by electronic nose in airways disease. Established issues and key questions. Clin Exp Allergy. 2013;43:705-15.
38. Schleich FN, Dallinga JW, Henket M, Wouters EF, Louis R, Van Schooten FJ. Volatile organic compounds discriminate between eosinophilic and neutrophilic inflammation in vitro. J Breath Res. 2016;10:016006.
39. Plaza V, Crespo A, Giner J et al. Inflammatory asthma phenotype discrimination using an electronic nose breath analyzer. J Investig Allergol Clin Immunol. 2015;25:431-7.
40. O'Byrne PM, Gauvreau GM, Brannan JD. Provoked models of asthma: what have we learnt? Clin Exp Allergy. 2009;39:181-92.
41. Bettiol J, Sele J, Henket M et al. Cytokine production from sputum cells after allergenic challenge in IgE-mediated asthma. Allergy. 2002;57:1145-50.
42. Bettiol J, Radermecker M, Sele J, Henquet M, Cataldo D, Louis R. Airway mast-cell activation in asthmatics is associated with selective sputum eosinophilia. Allergy. 1999;54:1188-93.
43. Manise M, Holtappels G, Van CK, Schleich F, Bachert C, Louis R. Sputum IgE and cytokines in asthma: relationship with sputum cellular profile. PLoS One. 2013;8:e58388.
44. Hemelaers L, Henket M, Sele J, Bureau F, Louis R. Cysteinyl-leukotrienes contribute to sputum eosinophil chemotactic activity in asthmatics. Allergy. 2006;61:136-9.
45. Mouthuy J, Detry B, Sohy C, Pirson F, Pilette C. Presence in sputum of functional dust mite-specific IgE antibodies in intrinsic asthma. Am J Respir Crit Care Med. 2011;184:206-14.
46. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: Eosinophilic airway inflammation in nonallergic asthma. Nat Med. 2013;19:977-9.
47. Louis R, Shute J, Biagi S et al. Cell infiltration, ICAM-1 expression, and eosinophil chemotactic activity in asthmatic sputum. Am J Respir Crit Care Med. 1997;155:466-72.
48. Baines KJ, Simpson JL, Wood LG et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. J Allergy Clin Immunol. 2014;133:997-1007.
49. Quaedvlieg V, Henket M, Sele J, Louis R. Cytokine production from sputum cells in eosinophilic versus non-eosinophilic asthmatics. Clin Exp Immunol. 2006;143:161-6.
50. Schleich F, Louis R. [Targeted asthma therapies: confirmations, hopes, and disappointments]. Rev Med Liege. 2012;67:14-21.
51. Simpson JL, Phipps S, Baines KJ, Oreo KM, Gunawardhana L, Gibson PG. Elevated expression of the NLRP3 inflammasome in neutrophilic asthma. Eur Respir J. 2014;43:1067-76.
52. Maes T, Cobos FA, Schleich F et al. Asthma inflammatory phenotypes show differential microRNA expression in sputum. J Allergy Clin Immunol. 2016;137:1433-46.
53. Quaedvlieg V, Sele J, Henket M, Louis R. Association between asthma control and bronchial hyperresponsiveness and airways inflammation: a cross-sectional study in daily practice. Clin Exp Allergy. 2009;39:1822-9.
54. Price DB, Rigazio A, Campbell JD et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. Lancet Respir Med. 2015;3:849-58.
55. Chung KF, Wenzel SE, Brozek JL et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43:343-73.
56. Haldar P, Pavord ID, Shaw DE et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008;178:218-24.
57. Schleich F, Brusselle G, Louis R et al. Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR). Respir Med. 2014;108:1723-32.
58. Moore WC, Meyers DA, Wenzel SE et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181:315-23.
59. Shaw DE, Sousa AR, Fowler SJ et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. Eur Respir J. 2015;46:1308-21.
60. ten BA, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. "Refractory" eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. Am J Respir Crit Care Med. 2004;170:601-5.
61. ten BA, Ouwerkerk ME, Zwinderman AH, Spinhoven P, Bel EH. Psychopathology in patients with severe asthma is associated with increased health care utilization. Am J Respir Crit Care Med. 2001;163:1093-6.
62. Djukanovic R, Wilson JW, Britten KM et al. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. Am Rev Respir Dis. 1992;145:669-74.
63. Kips JC, Inman MD, Jayaram L et al. The use of induced sputum in clinical trials. Eur Respir J Suppl. 2002;37:47-50s.
64. Brightling CE. Clinical applications of induced sputum. Chest. 2006;129:1344-8.
65. Berry M, Morgan A, Shaw DE et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax. 2007;62:1043-9.
66. Bacci E, Cianchetti S, Bartoli M et al. Low sputum eosinophils predict the lack of response to beclomethasone in symptomatic asthmatic patients. Chest. 2006;129:565-72.
67. Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. Thorax. 2010;65:384-90.
68. Godon P, Boulet LP, Malo JL, Cartier A, Lemiere C. Assessment and evaluation of symptomatic steroid-naive asthmatics without sputum eosinophilia and their response to inhaled corticosteroids. Eur Respir J. 2002;20:1364-9.
69. Smith AD, Cowan JO, Brassett KP et al. Exhaled nitric oxide: a predictor of steroid response. Am J Respir Crit Care Med. 2005;172:453-9.
70. Honkoop PJ, Loijmans RJ, Termeer EH et al. Symptom- and fraction of exhaled nitric oxide-driven strategies for asthma control: A cluster-randomized trial in primary care. J Allergy Clin Immunol. 2015;135:682-8.
71. Green RH, Brightling CE, McKenna S et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet. 2002;360:1715-21.

72. Jayaram L, Pizzichini MM, Cook RJ et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. Eur Respir J. 2006;27:483-94.
73. Pavord ID, Korn S, Howarth P et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet. 2012;380:651-9.
74. Castro M, Mathur S, Hargreave F et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. Am J Respir Crit Care Med. 2011;184:1125-32.
75. Ortega HG, Liu MC, Pavord ID et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med. 2014;371:1198-207.
76. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. Am J Respir Crit Care Med. 2003;167:199-204.
77. Gibson PG, Dolovich J, Denburg J, Ramsdale EH, Hargreave FE. Chronic cough: eosinophilic bronchitis without asthma. Lancet. 1989;1:1346-8.
78. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med. 2002;346:1699-705.
79. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. Am J Respir Crit Care Med. 1997;155:449-53.
80. Moermans C, Heinen V, Nguyen M et al. Local and systemic cellular inflammation and cytokine release in chronic obstructive pulmonary disease. Cytokine. 2011;56:298-304.
81. Brightling CE, Monteiro W, Ward R et al. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. Lancet. 2000;356:1480-5.
82. 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.
83. Schleich F, Corhay JL, Louis R. Blood eosinophil count to predict bronchial eosinophilic inflammation in COPD. Eur Respir J. 2016;47:1562-4.
84. 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.
85. Brightling CE, Woltmann G, Wardlaw AJ, Pavord ID. Development of irreversible airflow obstruction in a patient with eosinophilic bronchitis without asthma. Eur Respir J. 1999;14:1228-30.
86. Saetta M, Di Stefano A, Maestrelli P et al. Airway eosinophilia and expression of interleukin-5 protein in asthma and in exacerbations of chronic bronchitis. Clin Exp Allergy. 1996;26:766-74.
87. Bafadhel M, McKenna S, Terry S et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. Am J Respir Crit Care Med. 2011;184:662-71.
88. Steer J, Gibson J, Bourke SC. The DECAF Score: predicting hospital mortality in exacerbations of chronic obstructive pulmonary disease. Thorax. 2012;67:970-6.
89. Schleich F, Corhay JL, Louis R. Blood eosinophil count to predict bronchial eosinophilic inflammation in COPD. Eur Respir J. 2016;47:1562-4.
90. Louis RE, Cataldo D, Buckley MG et al. Evidence of mast-cell activation in a subset of patients with eosinophilic chronic obstructive pulmonary disease. Eur Respir J. 2002;20:325-31.
91. Moermans C, Bonnet C, Willems E et al. Sputum cytokine levels in patients undergoing hematopoietic SCT and comparison with healthy subjects and COPD: a pilot study. Bone Marrow Transplant. 2014;49: 1382-8.
92. Shute JK, Lindley I, Peichl P, Holgate ST, Church MK, Djukanovic R. Mucosal IgA is an important moderator of eosinophil responses to tissue-derived chemoattractants. Int Arch Allergy Immunol. 1995;107:340-1.
93. Papi A, Bellettato CM, Braccioni F et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med. 2006;173:1114-21.
94. Brightling CE, McKenna S, Hargadon B et al. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. Thorax. 2005;60:193-8.
95. 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.
96. Siva R, Green RH, Brightling CE et al. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. Eur Respir J. 2007;29:906-13.
97. Brightling CE, Bleecker ER, Panettieri RA 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.