

EXPERT
REVIEWS

Biomarkers of eosinophilic inflammation in asthma

Expert Rev. Respir. Med. 8(2), 143–150 (2014)

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Eosinophils are mediators of allergic inflammation and are implicated in the pathogenesis of numerous conditions including asthma, parasitic infections, neoplasms, hyper-eosinophilic syndromes, vasculitic disorders, and organ-specific conditions. Assessing eosinophilic inflammation is therefore important in establishing a diagnosis, in monitoring and assessing response to treatment, and in testing novel therapeutics. Clinical markers of atopy and eosinophilic inflammation include indirect tests such as lung function, exhaled breath condensate analysis, fractional exhaled nitric oxide, serum immunoglobulin E levels and serum periostin. Direct measures, which quantify but do not anatomically localise inflammation include blood eosinophil counts, serum or plasma eosinophil cationic protein and sputum eosinophil levels. Cytology from bronchoalveolar lavage and histology from endobronchial and transbronchial biopsies are better at localising inflammation but are more invasive. Novel approaches using radiolabelled eosinophils with single-photon emission computed tomography, offer the prospect of non-invasive methods to localise eosinophilic inflammation.

KEYWORDS: allergy • biomarkers • biopsy • eosinophils • inflammation • nitric oxide • radioligands • SPECT-CT • sputum

The eosinophil & its role in inflammation

Eosinophils are granulocytes formed in the bone marrow from common hematopoietic stem cells and multi-lineage progenitors under the control of the transcription factors PU.1, GATA and C/EBP. Under physiological conditions, they partition in the circulation between marginated and more freely circulating pools, where they account for 1–3% of leukocytes and have an intravascular lifespan of approximately 25 h [1]. Morphologically, they are recognized by their distinctive bi-lobed nucleus and a cytoplasm rich in granules containing lipid mediators and cationic proteins including eosinophil-derived neurotoxin (EDN), major basic protein (MBP), eosinophil peroxidase and eosinophil cationic protein (ECP). They are recruited to areas of inflammation by cytokines such as IL-5 and members of the eotaxin family of chemokines; there they degranulate in response to appropriate stimuli and release their toxic granule contents by exocytosis, piecemeal degranulation and cytolysis.

Although part of the innate immune system, eosinophils are known to have complex interactions with other leukocytes and are able to modulate the functions of lymphocytes, mast

cells, macrophages, dendritic cells and neutrophils [2]. Rosenberg *et al.* [2] have reviewed the role of eosinophils in anti-viral host defense, their anti-bacterial functions through the production of extracellular trap-like structures and their role in parasitic infections. Several excellent articles have also addressed the role of eosinophils in parasitic infections [3,4].

Despite literature suggesting a possible protective/beneficial role of eosinophils in settings such as muscle regeneration, mucosal homeostasis, *Trichinella spiralis* larvae survival and cancer progression, a much larger body of evidence implicates eosinophils in the pathogenesis of a wide range of diseases.

It is becoming increasingly apparent that asthma is a heterogeneous disorder characterized by different phenotypes or 'endotypes' [5]. Identifying a TH2-driven, eosinophilic phenotype is therefore important in guiding the management of these patients. Eosinophilic inflammation is a hallmark of allergic inflammation and eosinophilic asthma; in the airway and nose, these cells migrate in response to a variety of cytokines and chemokines including IL-5, CCL3, CCL5 and eotaxin-1 (CCL11), which regulate the adherence/migration, priming/activation, terminal differentiation and

survival of these cells. Eosinophils contribute to the pathophysiology of asthma through interactions with cells of the adaptive immune system and release of their toxic granules leading to airway hyper-responsiveness and bronchoconstriction [6]. Eosinophils are also implicated in airway remodeling through mediators such as TGF- β [7], and reducing their numbers in mild asthma using anti-IL-5 treatment is associated with a reduction of extracellular matrix proteins in the airway [8]. Assessment of eosinophil function suggests the presence of primed and activated cells in asthma. Eosinophils from asthmatic patients produce more oxygen metabolites in response to platelet activating factor [9] and immunohistochemical analysis of ECP indicates degranulated eosinophils beneath the basement membrane and among epithelial cells [10]. Similarly, MBP and EDN are significantly elevated in the bronchoalveolar lavage (BAL) fluid of symptomatic asthmatics when compared with asymptomatic patients [11].

A peripheral blood eosinophilia is also frequently present in parasitic infection, certain neoplastic disorders, adrenal insufficiency and organ-specific diseases affecting the lung, for example, allergic bronchopulmonary aspergillosis and eosinophilic pneumonia; the gastrointestinal tract, for example, inflammatory bowel disease, eosinophilic esophagitis and radiation enteritis; the skin, for example, eczema and cellulitis and certain vasculitides including eosinophilic granulomatosis with polyangiitis (EGPA). Tissue eosinophil infiltration is also a feature of IgG₄-related disease [12]. When no obvious secondary cause is found, eosinophilia is thought to be idiopathic or part of the hyper-eosinophilic syndromes. Given the pathological consequences of eosinophilic inflammation in these common diseases, the ability to assess the degree of eosinophilic inflammation is essential to aid diagnosis, tailor treatment, assess treatment response and to provide a tool for further research.

Current assessment methods

Indirect assessments

Lung function

A very indirect readout of airway inflammation (often eosinophilic in the context of allergic asthma) is the presence of air-flow obstruction measured through peak expiratory flow and spirometry, which are often used in clinical trials as asthma outcome measures when testing novel therapeutics. Both measures are widely available and used in the diagnosis and monitoring of asthma. Although physiological parameters may be able to quantify the degree of airway obstruction present, and peak flow variability may indicate poor asthma control, such monitoring is very dependent on patient technique and compliance, and one off normal results do not exclude asthma.

The forced expiratory volume in 1 s (FEV1) has been demonstrated to correlate with both asthma severity and peripheral blood eosinophilia [10]. Asthma treatment can lead to improvements in lung function in some [13–18] but not all cases [19–21], despite drug-induced reductions in airway and blood eosinophil numbers. Similarly, FEV1 and peak flow variability do not often correlate with more direct measures of eosinophilic inflammation such as the number of bronchial mucosal

eosinophils [22], or the degree of eosinophilic inflammation evident in induced sputum [23]. Indeed, a dissociation has been demonstrated between airway inflammation and hyper-responsiveness in allergic asthma [23].

Such studies indicate that PEF/spirometry measurements in isolation are not an accurate reflection of the extent of eosinophilic inflammation *in vivo* and are clearly affected by multiple co-existing disease processes.

Exhaled breath condensate

An extensive review of exhaled breath condensate (EBC) as a non-invasive way of sampling the lining fluid of the airways and lung has been published by the American Thoracic Society/European Respiratory Society Task Force [24]. The collection of condensate is influenced by numerous factors, and compounds present in the ambient air can form part of, or can react with, compounds in the EBC. The breathing pattern, age of the subject, smoking status, extent of salivary contamination, presence of systemic disease and the subject's current medication all influence constituents of the EBC [24]. The main component of EBC is condensed water vapor with detectable amounts of adenosine, thromboxane, ammonia and hydrogen peroxide. EBC assays for cytokines, leukotrienes and prostaglandins have also been reported [25,26].

Although EBC is a safe method of collecting respiratory fluids, and the volumes collected reproducible during tidal breathing, the levels of mediators detected are highly variable and subject to significant intra-subject variability. To date, only one study has shown a correlation between the percentage of eosinophils in induced sputum (across a spectrum of lung diseases) and levels of hydrogen peroxide in EBC [27]. At present therefore, the collection and analysis of EBC remains a largely experimental technique to measure airway inflammation and EBC markers of eosinophil-specific airways inflammation are lacking.

Fractional exhaled nitric oxide

Measurement of fractional exhaled nitric oxide (FE_{NO}) is a non-invasive, reproducible, safe and quantitative albeit indirect measurement of airway inflammation. NO is produced in the human lung and this test is in standard clinical use to aid the diagnosis and monitoring of asthma. An elevated level of >50 parts per billion (ppb) in symptomatic adults can support the diagnosis of eosinophilic asthma, and predicts response to corticosteroid treatment, which reduces the FE_{NO} level; levels of <25 ppb make the diagnosis of atopic, eosinophilic asthma unlikely and suggest that corticosteroids are unlikely to work [28]. A persistently elevated FE_{NO} can be indicative of ongoing exposure to allergen. It can, however, also reflect poor inhaler technique or non-adherence to treatment, and directly observed inhaled corticosteroid treatment can lead to a greater reduction in FE_{NO} in patients with difficult asthma [29].

The use of FE_{NO} levels to assess novel therapeutics and guide asthma management has led to conflicting results. Anti-IL-4 [15] and anti-IL-13 [16] trials have demonstrated a reduction in FE_{NO} levels, whereas mepolizumab, a humanized monoclonal

antibody (mAb) against IL-5 did not, despite reductions in blood and sputum eosinophil levels [20]. When Smith *et al.* compared the use of FE_{NO} levels to tailor asthma management with conventional guidelines, they demonstrated a 46% reduction in exacerbations (which did not reach statistical significance) and a significant reduction in the dose of inhaled corticosteroids [30]. By contrast, other studies have not demonstrated a reduction in either exacerbations or inhaled corticosteroid dose compared with conventional asthma management [31,32], and similar strategies have not been found useful in children [33].

Although studies have suggested a direct association between FE_{NO} and eosinophil numbers in BAL, bronchial biopsies and induced sputum [26,28], this index does not correlate with other conventional methods of asthma control such as symptom scores, lung function or peak flow variability [22]. Despite associations with eosinophil numbers in sputum, the sensitivity of an elevated FE_{NO} level of >30 ppb to detect a sputum eosinophil count of >2% is 65%, specificity 67% and positive predictive value (PPV) 54% [34].

A limitation of using FE_{NO} is the skewed distribution of normal values with the upper limit overlapping with the range seen in asthmatic populations [28]. Furthermore, there may be day-to-day variability in the results caused by the measurement technique, exhalation flow rate and nasal NO contamination; FE_{NO} may also vary with age (particularly important in children), atopy, sex, weight and height [28].

Serum IgE levels

Serum IgE levels, allergy skin test reactivity and peripheral blood eosinophilia are tightly correlated [35], but reflect atopic status rather than eosinophilic inflammation. Likewise, in certain individuals these compartments clearly dissociate. However, IgE-dependent activation of mast cells and basophils results in the production of cytokines such as IL-4 and IL-5, which are important in the recruitment of eosinophils. Eosinophils also express both high- and low-affinity IgFcε receptors. Hence, measuring total serum IgE levels does offer an indirect proxy of IgE- and thereby eosinophil-related disease.

The total IgE level is associated with asthma, and allergen-specific IgE tests are able to identify sensitization to specific allergens [36]. High levels of allergen-specific IgE are associated with current wheeze in children and may also predict persistent wheeze [37]. A fall in serum IgE levels has been demonstrated in patients treated with omalizumab, a mAb against IgE [18] and with anti-IL-4 [15] and anti-IL-13 [16] treatment.

Compared with skin prick tests (SPT), measurement of IgE is time-consuming and expensive, and normal values vary according to age and gender [38]. It has also been suggested that SPT has a superior PPV in diagnosing current atopic asthma [39]. Although the measurement of total serum IgE is reproducible, as with SPT, it is not a sensitive predictor of clinical outcome [26].

Serum periostin

Periostin is a gene induced in bronchial epithelial cells by IL-13, a TH2 cytokine; it is associated with asthma and high expression

suggests responsiveness to corticosteroid treatment, which in turn reduces gene expression [40]. Serum periostin protein levels in patients with severe asthma have been demonstrated to be a better predictor of airway eosinophilia (sputum and BAL eosinophils) when compared with IgE levels, blood eosinophil numbers and FE_{NO} [41]. Consequently, periostin has been used as a systemic biomarker of airway eosinophilia in the Phase II trial of lebrikizumab, a humanized mAb against IL-13 [16].

Direct assessments

Peripheral blood eosinophilia

The peripheral blood eosinophil count is a widely available test with minimal risk or inconvenience to the patient. It may be the first indication of the presence of a pathological eosinophil-related process. In health, the absolute eosinophil count (AEC) should be $<0.5 \times 10^9/l$ with eosinophilia being classified as mild (AEC: $0.5\text{--}1.5 \times 10^9/l$), moderate (AEC: $1.5\text{--}5 \times 10^9/l$) or severe (AEC: $>5 \times 10^9/l$) [42]. Peripheral eosinophilia has been long associated with atopic asthma and is greater in those with more severe disease [43]. It is associated with elevated FE_{NO}, bronchial hyper-reactivity, higher symptom scores and a reduction in FEV1 [26]. The sensitivity of an elevated blood eosinophil count of $>300/\mu l$ to detect a sputum eosinophil count of >2% is 59%, specificity 65% and a PPV of 50% [34]. It is therefore a poor predictor of sputum eosinophilia despite statistically significant associations [34].

Eosinophilia is also present in other pulmonary hyper-eosinophilic syndromes such as chronic eosinophilic pneumonia, EGPA and allergic bronchopulmonary aspergillosis, where the counts may be markedly elevated.

The ability of glucocorticoids to induce eosinophil apoptosis is well established [44] and reduced blood eosinophils are seen with this, as well as anti-IgE [45] treatment. The ability of novel therapeutics to reduce peripheral eosinophil counts in asthma has also been demonstrated in anti-IL-5 trials [14,17,19–21] but not in the anti-IL-4 [15] or anti-IL-13 [16] trials.

There are, however, limitations in using the peripheral eosinophil count to assess eosinophilic inflammation. Taken in isolation, the test is non-specific, is overtly not able to localize inflammation and as demonstrated in patients receiving anti-IL-5 mAb treatment, may not be a true reflection of tissue eosinophilia [19]. Additional information is also required to differentiate between the many reactive, neoplastic or idiopathic causes of eosinophilia. Furthermore, counts may be entirely normal despite quite severe disease [42]. The diurnal variation in the peripheral eosinophil count, which may reflect at least in part movement of cells between the marginated and peripheral pools [1], and variations in serum cortisol level [46], also suggests that the reproducibility of the test will be somewhat dependent on the time of testing.

Eosinophil cationic protein

Many studies have assayed eosinophil granule proteins as biomarkers of eosinophilic inflammation, although as yet this is not in routine clinical use. ECP may be measured in sputum,

serum, plasma and BAL [47]. Although it has been shown to correlate with airway inflammation and asthma severity, ECP levels are non-specific and do not correlate with airway hyper-responsiveness [47]. Tests also involve lengthy laboratory handling and blood results vary according to whether the serum or plasma is sampled. The inadvertent *ex vivo* release of ECP from eosinophils, which is time and temperature dependent, may also complicate the interpretation of the results [48].

Sputum

An elevated differential eosinophil count in induced sputum is a sensitive but non-specific marker of airway eosinophilia. An elevated sputum eosinophil count of >2% or FE_{NO} >25 ppb is seen in 70–80% of patients with untreated asthma [49] and is a marker of steroid responsiveness. Normal sputum eosinophil counts suggest an alternative diagnosis or the presence of a non-eosinophilic asthma phenotype and predict a lack of steroid response.

Tailoring anti-inflammatory asthma treatment to the sputum eosinophil count rather than traditional British Thoracic Society Guidelines based on symptoms, rescue β_2 -adrenoreceptor agonist use and PEF, can lead to fewer severe exacerbations in adult patients with moderate-to-severe asthma [50]. By contrast, managing sputum eosinophil numbers in children with severe asthma does not reduce asthma exacerbations or improve control when compared with conventional symptom-based management [51].

Sputum eosinophilia has been associated with airway obstruction and hyper-responsiveness [52], and correlates with elevated FE_{NO} and BAL eosinophilia but has a poor PPV for mucosal eosinophilia [53]. Treatment with corticosteroids reduces sputum eosinophil numbers [54] and the technique is widely used as an outcome measure in trials of novel therapeutics. Treatment with mepolizumab [17,20] and reslizumab [14], another anti-IL-5 therapy, reduces sputum eosinophil levels.

Although the sputum eosinophil count is a reproducible and valid test [26], not every patient can tolerate the induction of sputum, which may cause bronchoconstriction; this test is therefore rarely performed in the clinical setting. The technique involves time-consuming and skilled processing, and the quality of the sputum is dependent on both patient and user factors.

Bronchoalveolar lavage

Flexible fiberoptic bronchoscopy and BAL is a more invasive procedure but one that is in widespread clinical use and allows cytology and biopsy of the large and small airways. The BAL total and differential cell count is used to aid the diagnosis of a variety of inflammatory and infectious disorders. A normal BAL cell differential is expected to show >85% alveolar macrophages, 10–15% lymphocytes, $\leq 3\%$ neutrophils, $\leq 1\%$ eosinophils and $\leq 5\%$ squamous or ciliated/columnar epithelial cells [55]. Eosinophils in the BAL fluid are present in asthma [56] and have been shown to correlate with disease severity [57]. They are also found in eosinophilic pneumonia [58], EGPA and in patients with interstitial lung disease, AIDS-associated pneumonia and drug-induced eosinophilia [59]. In asthma, sputum eosinophilia and FE_{NO} correlate well with the presence of eosinophils in the

BAL fluid [53]. Reductions in BAL fluid eosinophils have been demonstrated when testing novel therapeutics [19,20].

Endobronchial or transbronchial biopsy

Despite the utility of assessing airway eosinophilia through FE_{NO}, sputum and BAL eosinophils, an endobronchial or transbronchial biopsy performed during bronchoscopy is arguably the most direct measure of small airways inflammation that is in clinical use. It has been suggested that the transmigration of eosinophils from the tissue into the airway can occur in the absence of epithelial injury and may represent resolution of, rather than active inflammation [60]; sputum and BAL eosinophilia may not therefore always reflect whether the inflammatory response is evolving or resolving and is a less than ideal marker of tissue eosinophilia.

In the initial assessment of the effects of an anti-IL-5 mAb, there was no effect on airway hyper-responsiveness despite the reduction of peripheral and sputum eosinophils [21]. This led initially to debate regarding the role of eosinophils in asthma. However, a subsequent clinical trial of the same drug demonstrated a reduction in peripheral eosinophils by 100%, airway eosinophils by 55% and bone marrow eosinophils by 52%, but no reduction of bronchial mucosal staining of eosinophil MBP [19]. Tissue eosinophils may therefore still be propagating inflammation despite a reduction in peripheral blood and airway lumen eosinophilia. Knowledge of the inflammatory status of the tissue is therefore essential before conclusions are drawn regarding the efficacy of drugs.

Although transbronchial biopsy can provide direct assessment of tissue inflammation, this approach has significant disadvantages. It is an invasive procedure, can trigger clinically significant exacerbations and is associated with complications including bleeding (mild–moderate bleeding of 0.8% [61]) and pneumothorax (in 1–6% of cases [61]). It is also contraindicated in certain patients and although the site of biopsy can be guided by computed tomography (CT), biopsies are small and hence can be non-representative and non-diagnostic.

Expert commentary

The ability to accurately assess eosinophilic inflammation with a region of interest (typically the lung) is vital not only for the diagnosis and monitoring of disorders characterized by such inflammation, but also for proof of efficacy studies for novel therapeutics aimed at reducing tissue eosinophilia. If indirect measures of eosinophilic inflammation are used, which do not reflect tissue eosinophilia, it is feasible that the efficacy of therapeutics is either over- or underestimated. The more commonly used and relevant markers of eosinophilic inflammation include the peripheral eosinophil count, FE_{NO} levels, sputum and BAL eosinophil counts, bronchial biopsies and more recently serum periostin.

The peripheral eosinophil count is quick and easy to test, but is non-specific when elevated, is a poor predictor of sputum eosinophilia and may be normal despite the presence of tissue eosinophilia.

With greater emphasis placed on accurate phenotyping of asthmatic patients, there is a move toward tailoring therapy according

to quantifiable airway inflammation rather than standard guidelines (often based on spirometry), so-called inflammometry. This has most notably been done using the FE_{NO} and sputum eosinophil count. Inducing and processing sputum, however, makes this a difficult test relative to FE_{NO} to implement in routine clinical practice. Furthermore, the strategy of using inflammometry to guide treatment has only been successful in adults when using sputum counts, not the FE_{NO} levels. Although both sputum eosinophil counts and FE_{NO} are used as outcome measures of clinical trials, they have their limitations, and serum periostin may be a better biomarker of airway eosinophilia.

Despite the utility of the above biomarkers, only cytology from BAL and particularly histology from endobronchial and transbronchial biopsies can anatomically localize the site eosinophilic inflammation. In fact, only tissue biopsy can accurately inform of the presence of parenchymal eosinophilia, although this is invasive and carries risk. A biopsy also gives no information on cell flux through the tissue compartment. The need therefore, for a less invasive method to localize parenchymal eosinophilic inflammation in the lung remains.

Five-year view

The field is likely to evolve significantly in the next 5 years with the evolution of novel blood, airway, tissue and genetic biomarkers. The development of newer imaging techniques is also likely to provide less invasive means of assessing eosinophilic inflammation. Serum EDN has been shown to reflect disease activity in children with asthma [62]. It can also be measured in the urine where elevated levels in wheezy children can predict the development of asthma [48]. However, it is important to note that serum EDN is an identical protein to liver ribonuclease [63] and hence elevated levels of EDN may be non-specific.

Studies have also investigated the association between single nucleotide polymorphisms of eosinophil proteins and diseases [48]. Results, however, are variable and associations may be disease- and/or ethnicity-specific. The eosinophil protein content of airway macrophages that have ingested apoptotic eosinophils is increased in patients with moderate-to-severe asthma and after corticosteroid therapy [64]. In recent years, imaging modalities such as CT and MRI, with or without hyperpolarized helium, have evolved to assess lung structure and function (e.g., ventilation and perfusion) in asthma [65]. However, literature on the use of imaging techniques to assess eosinophilic inflammation in the lung has been limited. ^{18}F -Fluorodeoxyglucose (^{18}F -FDG) uptake, quantified by PET, has been used to

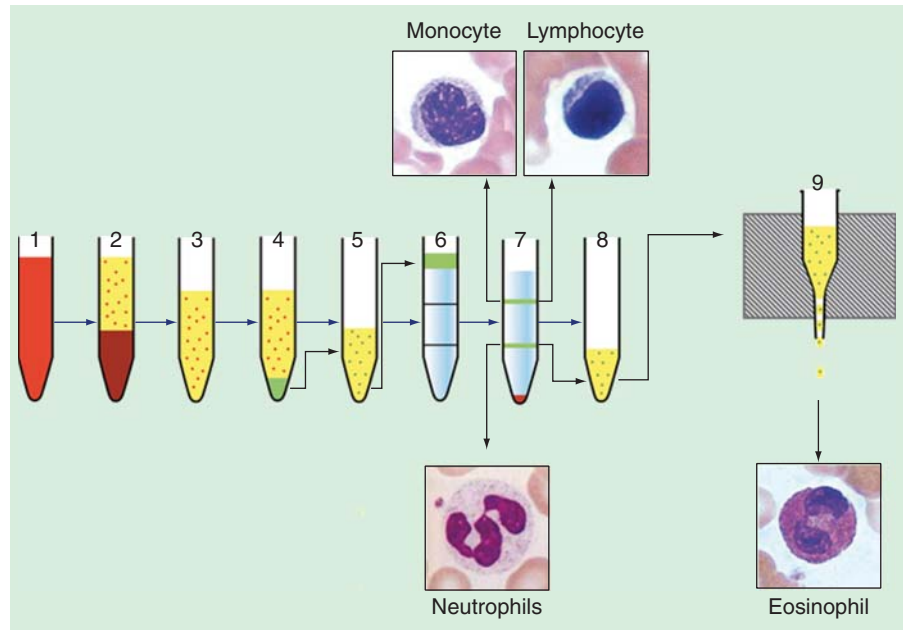


Figure 1. Eosinophil isolation from whole blood. (1) Hetastarch is added to whole blood and left for 30–60 min. (2) Erythrocytes sediment at the base of the tube and a platelet-rich, leukocyte-rich plasma supernatant is formed. (3) The platelet-rich, leukocyte-rich supernatant is carefully removed and then centrifuged to generate (4) a platelet-rich plasma supernatant and leukocyte pellet at the base of the tube (shown in green). (5) The supernatant is removed and the leukocyte pellet is suspended in plasma. (6) The leukocytes are overlaid onto discontinuous Percoll gradients and centrifuged. (7) Granulocytes settle between two gradients and are carefully removed and (8) resuspended in plasma before being incubated with anti-CD16 immunomagnetic which bind to neutrophils. (9) The granulocytes are passed through a magnetic column which retains the CD16⁺ neutrophils. (10) Eosinophils, which do not bind anti-CD16 immunomagnetic antibody pass through the magnetic column and are collected. Adapted from [71].

demonstrate an increase in signal in asthmatics who have undergone bronchoscopic allergen challenge [66]. Although this may represent an emerging biomarker of eosinophilic inflammation, other studies using ^{18}F -FDG-PET have not demonstrated a difference in ^{18}F -FDG uptake between asthmatics and healthy volunteers [65]. The contribution of other cells, particularly neutrophils to the ^{18}F -FDG signal should also be considered.

More recently, the use of radiolabeled autologous eosinophils has been used to determine the circulating kinetics and distribution of the cells in humans [1]. Granulocytes are isolated from whole blood following the removal of erythrocytes by centrifugation and the use of discontinuous Percoll gradients (FIGURE 1). Eosinophils are subsequently isolated by negative selection using clinical grade anti-CD16 immunomagnetic beads [67]. The cells are then radiolabeled before being injected back into the donor. The technique allows the isolation of pure eosinophils that behave physiologically when re-injected [1]. The use of radiolabeled leukocytes coupled to planar gamma camera imaging has been used to image infection and inflammation for decades [68], but only generate 2D images. Single-photon emission computed tomography (SPECT) (FIGURE 2) allows 3D imaging of the distribution of radioactivity *in vivo* and has been used to quantify the migration of labeled neutrophils into



Figure 2. Single-photon emission computed tomography scanner.

regions of the lung of patients with chronic obstructive pulmonary disease [69]. Although the technique remains experimental, radiolabeled eosinophils combined with SPECT-CT imaging has recently been shown to localize focal eosinophilic inflammation within the lung nodules and foot ulcers of a patient with anti-neutrophil cytoplasmic antibody-positive vasculitis [70]. The method showed uptake of radiolabeled eosinophils in

some but not all of his lung nodules, indicating the capacity of the technique, unlike CT, to differentiate between areas of active and quiescent inflammation.

The use of radiolabeled eosinophils with SPECT demonstrates the potential of this technique to accurately localize eosinophilic inflammation *in vivo*, quickly, non-invasively and without the complications and contraindications associated with invasive sampling and biopsy. The development of such imaging modalities over the next 5 years offers the prospect of a novel and tolerable test that reflects tissue eosinophilia, and is applicable not only to the diagnosis and management of a spectrum of eosinophilic disorders, but has significant applications to research and the testing of novel therapeutics.

Financial & competing interests disclosure

The work from the Chilvers laboratory is supported by Asthma UK (08/11); the Medical Research Council (grant number MR/J00345X/1); the Wellcome Trust (grant number 098351/Z/12/Z); Biotechnology and Biological Sciences Research Council (grant number BB/H531100/1); and Cambridge National Institute for Health Research Biomedical Research Centre. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Eosinophils are key mediators of allergic inflammation and play a pathogenic role in numerous conditions including parasitic infections, neoplasms, hyper-eosinophilic syndromes, vasculitic disorders and organ-specific conditions affecting the lung, nose, skin and gastrointestinal tract.
- Assessing eosinophilic inflammation is essential for the diagnosis and monitoring of diseases, and for testing the efficacy of novel therapeutics.
- Indirect markers of atopic status, eosinophilic inflammation and asthma include, serum IgE levels, lung function tests, blood and sputum eosinophilia, exhaled breath condensates and exhaled nitric oxide.
- Bronchoalveolar lavage samples the airway directly but may not accurately reflect tissue eosinophilia.
- Endobronchial and transbronchial biopsy reflects tissue eosinophilia, but this test is invasive and associated with complications.
- Novel eosinophil biomarkers include assaying eosinophil granule proteins and searching for clinically relevant polymorphisms of regulatory pathways.
- Radiolabeled eosinophils coupled with single-photon emission computed tomography offers the prospect of a non-invasive test that localizes focal eosinophilic inflammation *in vivo*.

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