Is it time for a simplified method to evaluate airway eosinophilia?

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Shareable abstract (@ERSpublications)
Induced sputum is a useful methodology to evaluate airway inflammation. Unfortunately, it is time consuming and not available in many asthma centres. New procedures could be useful to obtain airway inflammatory data. https://bit.ly/3Huyols

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Evaluation of sputum cells is a standardised methodology useful to assess airway inflammation. When patients are able to expectorate, spontaneous sputum can be analysed [1], while, if this does not occur, sputum can be induced with the inhalation of hypertonic saline solution. Induction and processing of sputum samples are standardised methodologies and guidelines were published many years ago in order to ensure uniform application [2–4]. Sputum cell percentages are the most useful data, from the clinical point of view, that we can obtain from sputum samples. Reference values in healthy controls with different age ranges were published and reproducibility of the data, particularly of neutrophils and eosinophils, was demonstrated [5–8]. Aligning with the type of cells present in the airways, different inflammatory phenotypes have been distinguished: eosinophilic, neutrophilic, mixed granulocytic or paucigranulocytic [9, 10]. Sputum and blood eosinophilia are risk factors for developing persistent airflow limitation [11, 12]. A mixed granulocytic airway inflammatory pattern, characterised by elevated levels of both neutrophils and eosinophils, may exert a long-term impact on lung function, especially in the context of fixed airway obstruction [13–15].

The presence of sputum eosinophils, independently from symptoms, should prompt clinicians to increase inhaled corticosteroid dose in asthmatic patients [16]. Additionally, in symptomatic patients, inhaled corticosteroid treatment can be adjusted based on sputum eosinophils (>3%) both in the step-up and in the step-down of therapy [17]. The main utility to determine inflammatory phenotypes is evident in subjects with severe asthma: to define the type of inflammation and select new therapies [18]. Sputum eosinophils are the most characteristic cells present in the airways of asthmatic patients and they are a T2 biomarker [19]. Exhaled nitric oxide fraction and serum IgE are other biomarkers of a T2 phenotype, even if they are triggered by different immunological and inflammatory processes [20, 21]. Blood eosinophils are used as a surrogate of airway eosinophils, since they usually reflect eosinophil trafficking from the bone marrow to the airways [22]. Even when all these biomarkers are low, sputum analysis allows for the detection of eosinophils in the airways, making patients suitable for an anti-T2 treatment [23]. Characterisation of the inflammatory profile of severe asthmatic patients offers the opportunity to select monoclonal antibodies with greater precision and to monitor patients even in the case of non-responder subjects. The 2024 Global Initiative for Asthma report [24] recognises the usefulness of sputum cell analysis, and highlights the importance that this methodology is performed by expert personnel.

Unfortunately, sputum induction and processing are still limited to a few centres, both in Europe and elsewhere. Despite the numerous courses organised to train new individuals interested in adopting this methodology, most of them eventually give up due to organisational issues and technical difficulties. Considering that the induction phase of sputum involves a simple nebulisation of hypertonic saline solution, which can be performed in any respiratory physiology unit [3], the main obstacle appears to be

the availability of a dedicated laboratory for routine processing of sputum samples. Clinical analysis laboratories are often not available to handle this type of methodology, and the complexity of the processing phase may even discourage anatomical pathology laboratories. For these reasons, new approaches that favour implementation of this methodology are welcome.

In this issue of *ERJ Open Research*, Patel *et al*. [25] present compelling data on the feasibility of assessing eosinophilic airway inflammation by processing sputum samples similarly to tissue biopsies – fixing sputum plugs in 10% formalin and embedding them in paraffin (the Sputum-Minimising Processing, Maximising Clinical Outcomes "SSIMPLE" method). Paraffin blocks were sliced, stained and eosinophils counted at optical microscope. Sputum was obtained from patients with different airway diseases and asthmatic patients treated with oral corticosteroid drugs and/or monoclonal antibodies. Each sputum sample was treated with both dithiothreitol (DTT) as standard procedure of plug solubilisation and with the SSIMPLE method. The proposed procedure demonstrated an excellent inter-observer reproducibility (four blinded observers with different degree of expertise). Adequate number of cells was obtained in 96% of the analysed samples (>400 cells). Sputum from healthy volunteers was also analysed, and 64% of the samples yielded sufficient material to be evaluated using both the DTT and SSIMPLE methods. The Bland–Altman test showed a moderate agreement between the two sample types. Eosinophils in sputum samples analysed using the two methods showed a significant correlation, with no differences observed between spontaneous and induced sputum. A moderate agreement was confirmed by Bland–Altman analysis. Lower agreement was observed at higher eosinophil percentages, likely due to cell clustering in the SSIMPLE method. ROC analysis yielded an area under the curve of 0.957 ($p<0.0001$). Using a $\geqslant$2.6% eosinophil cut-off, the sensitivity and specificity were 85.4% and 93.0%, respectively. A further validation of the SSIMPLE method was achieved through anti-eosinophil peroxidase immunofluorescence staining, which demonstrated a correlation between the eosinophil counts in the samples and the staining intensity.

A strong correlation and moderate agreement (Bland–Altman analysis) between SSIMPLE and DTT method were also observed in samples from asthmatic subjects before and after treatment with oral corticosteroids or monoclonal antibodies, confirming the new method's sensitivity in detecting treatment-induced reductions in eosinophil counts.

The authors concluded that the SSIMPLE method is a valid alternative to detect eosinophils in sputum samples. One of the advantages of the method is the long-term storage at room temperature of the sample without compromising its quality and the possibility to integrate this methodology in a routine pathology department. Samples can also be sent to centralised laboratory with experience in the field. Furthermore, the method saves time, and, consequently, cost.

Difficulties in making sputum processing available in different centres have been reported for many years. Previous attempts to facilitate the procedure were proposed by other groups who treated sputum samples with dimethyl sulfoxide [26] or paraformaldehyde [27], but results were not completely satisfactory. We also tried to offer a rapid method to evaluate eosinophils in sputum samples smearing the selected sputum plugs without processing them. Correlation between DTT-treated and smeared samples was high for both eosinophils and neutrophils, however, reading of smeared samples is not as easy [28]. The SSIMPLE method could be a genuinely new prospect to increase the feasibility of sputum eosinophils evaluation. However, we have to take in consideration that, at least with haematoxylin and eosin staining, only eosinophils are easily recognisable; other cells, neutrophils, macrophages and lymphocytes are less recognisable and they were not analysed in the study. Information regarding these cells is less useful for clinical decisions, however, a high amount of neutrophils, particularly in repeated sputum samples, could be a sign of infection and/or bacterial colonisation of the airways [29]. The presence of cell clusters, in which individual cells are difficult to analyse, represents another limitation of the method.

Furthermore, DTT processing of sputum samples allows the acquisition of additional information, such as total cell counts. When elevated, cell count is usually linked to the presence of high neutrophil percentage and again a sign of infection. This information could be useful for the clinician in selecting the appropriate therapy [30]. On the other hand, high sputum neutrophils with normal total cell count could be a more instable condition associated with different triggers like air pollution, active smoking or comorbidities (gastro-oesophageal reflux disease, obesity, hypertension, *etc*.) [15].

Even cell viability, obtained with the DTT-processing method, is underrated information. A sample with high cell viability reflect a rapid cell recruitment into the airways standing for an active process. Low viable cell counts could be due to apoptotic eosinophils in patients treated with inhaled corticosteroids, thereby demonstrating patient compliance with the treatment. Low viability of sputum cells could also be

due to a high percentage of epithelial cells, which are frequently dead cells and are markers of damage to the epithelium. The DTT processing method also offers the capability to evaluate sputum soluble biomarkers [31, 32]. Even though to date no soluble biomarker has proved useful to influence clinical decisions, many cytokines, chemokines and other biomarkers in sputum highlight inflammatory mechanisms, which could be the target for new therapeutic drugs.

In conclusion, the SSIMPLE method presented in this issue of the *ERJ Open Research* [25] could be an exciting opportunity to make sputum eosinophil evaluation more accessible and feasible, particularly in less specialised centres. However, the DTT processing method remains the standard procedure to obtain more complete data both on cells and on sputum soluble biomarkers.

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References

1 Bhowmik A, Seemungal TA, Sapsford RJ, *et al.* Comparison of spontaneous and induced sputum for investigation of airway inflammation in chronic obstructive pulmonary disease. *Thorax* 1998; 53: 953–956.
2 Djukanovic R, Sterk PJ, Fahy JV, *et al.* Standardised methodology of sputum induction and processing. *Eur Respir J* 2002; 20, Suppl. 37: 1s–2s.
3 Pizzichini E, Pizzichini MM, Leigh R, *et al.* Safety of sputum induction. *Eur Respir J* 2002; 20, Suppl. 37: 9s–18s.
4 Efthimiadis A, Spanevello A, Hamid Q, *et al.* Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. *Eur Respir J* 2002; 20, Suppl. 37: 19s–23s.
5 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: 1172–1174.
6 Belda J, Leigh R, Parameswaran K, *et al.* Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000; 161: 475–478.
7 Pignatti P, Ragnoli B, Radaeli A, *et al.* Age-related increase of airway neutrophils in older healthy nonsmoking subjects. *Rejuvenation Res* 2011; 14: 365–370.
8 Spanevello A, Migliori GB, Sharara A, *et al.* Induced sputum to assess airway inflammation: a study of reproducibility. *Clin Exp Allergy* 1997; 27: 1138–1144.
9 Simpson JL, Scott R, Boyle MJ, *et al.* Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006; 11: 54–61.
10 Schleich FN, Manise M, Sele J, *et al.* Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. *BMC Pulm Med* 2013; 13: 11.
11 Hancox RJ, Pavord ID, Sears MR. Associations between blood eosinophils and decline in lung function among adults with and without asthma. *Eur Respir J* 2018; 51: 1702536.
12 Graff S, Demarche S, Henket M, *et al.* Increase in blood eosinophils during follow-up is associated with lung function decline in adult asthma. *Respir Med* 2019; 152: 60–66.
13 Louis RE, Schleich FN. Granulocytic airway inflammation and clinical asthma outcomes. *Am J Respir Crit Care Med* 2021; 203: 797–799.
14 Hastie AT, Mauger DT, Denlinger LC, *et al.* Mixed sputum granulocyte longitudinal impact on lung function in the severe asthma research program. *Am J Respir Crit Care Med* 2021; 203: 882–892.
15 Pignatti P, Visca D, Zappa M, *et al.* Analysis of patients with asthma and mixed granulocytic inflammatory pattern in sputum. *Respiration* 2022; 101: 1121–1130.
16 Green RH, Brightling CE, McKenna S, *et al.* Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; 360: 1715–1721.
17 Petsky HL, Cates CJ, Kew KM, *et al.* Tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils): a systematic review and meta-analysis. *Thorax* 2018; 73: 1110–1119.
18 Walsh CJ, Zaihra T, Benedetti A, *et al.* Exacerbation risk in severe asthma is stratified by inflammatory phenotype using longitudinal measures of sputum eosinophils. *Clin Exp Allergy* 2016; 46: 1291–1302.
19 Woodruff PG, Modrek B, Choy DF, *et al.* T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009; 180: 388–395.
20 Haccuria A, Michils A, Michiels S, *et al.* Exhaled nitric oxide: a biomarker integrating both lung function and airway inflammation changes. *J Allergy Clin Immunol* 2014; 134: 554–559.
21 Shrestha SK, Drews A, Sharma L, *et al.* Relationship between total serum immunoglobulin E levels, fractional exhaled breath nitric oxide levels and absolute blood eosinophil counts in atopic and non-atopic asthma: a controlled comparative study. *J Breath Res* 2018; 12: 026009.
22 Abdala-Valencia H, Coden ME, Chiarella SE, *et al.* Shaping eosinophil identity in the tissue contexts of development, homeostasis, and disease. *J Leukoc Biol* 2018; 104: 95–108.

23 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.
24 Global Initiative for Asthma. 2025 GINA report, global strategy for asthma management and prevention (2025 update). Date last updated: 24 July 2025. https://ginasthma.org/2025-gina-strategy-report/
25 Patel Z, Suray Tan N, Huynh A, *et al.* A novel method to assess airway eosinophilia utilising sputum plugs. *ERJ Open Res* 2026; 12: 00604-2025.
26 Holz O, Mücke M, Zarza P, *et al.* Freezing of homogenized sputum samples for intermittent storage. *Clin Exp Allergy* 2001; 31: 1328–1331.
27 Kelly MM, Hargreave FE, Cox G. A method to preserve sputum for delayed examination. *Eur Respir J* 2003; 22: 996–1000.
28 Pignatti P, Visca D, Leoni V, *et al.* Airway inflammatory phenotypes: making sputum cell evaluation more accessible for clinical use. *Clin Respir J* 2018; 12: 1989–1990.
29 Nair P, Surette MG, Virchow JC. Neutrophilic asthma: misconception or misnomer? *Lancet Respir Med* 2021; 9: 441–443.
30 Svenningsen S, Nair P. Asthma endotypes and an overview of targeted therapy for asthma. *Front Med (Lausanne)* 2017; 26: 158.
31 Kelly MM, Keatings V, Leigh R, *et al.* Analysis of fluidphase mediators. *Eur Respir J* 2002; 20, Suppl. 37: 24s–39s.
32 Dente FL, Carnevali S, Bartoli ML, *et al.* Profiles of proinflammatory cytokines in sputum from different groups of severe asthmatic patients. *Ann Allergy Asthma Immunol* 2006; 97: 312–320.