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Induced sputum cell counts: their usefulness in clinical practice

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Induced sputum cell counts: their usefulness in clinical practice. L. Jayaram, K. Parameswaran, M.R. Sears, F.E. Hargreave. ©ERS Journals Ltd 2000.

ABSTRACT: Airway inflammation is fundamental to the aetiology and persistence of asthma and other airway conditions. The presence and type of airway inflammation can be difficult to detect clinically, delaying the introduction of appropriate treatment.

Induced sputum cell counts are a relatively noninvasive, safe and reliable method of identifying airway inflammation. They can accurately discriminate eosinophilic airway inflammation from noneosinophilic airway inflammation, and help guide therapy. Eosinophilic airway inflammation is steroid responsive whilst noneosinophilic (usually neutrophilic) inflammation generally is not.

Macrophages containing haemosiderin can be useful in detecting left ventricular dysfunction and macrophages containing lipid are suggestive of oropharyngeal reflux with microaspiration, both of which can complicate or confuse assessment of airway disease.

To date, studies using induced sputum are primarily observational. Management studies based on examination of induced sputum are now needed to validate the clinical utility of this test.

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Sputum is defined here as secretions from the airways of the lungs, although some refer to it as the expectorate of sputum plus saliva. Its constituents include mucus and cells. The cell composition has been known for over 100 yrs to be altered in asthma, showing an increase in eosinophils [1]. Initially, sputum cells were examined on stained smears and there was a brief period of their use in clinical practice in the 1950s, 1960s and 1970s [2]. However, the sputum could not always be expectorated and only one smear tended to be examined in which the cells were irregularly distributed and difficult to recognize in the mucus. The results therefore were not reliable and this use of sputum fell into disrepute. More recently, however, the success of expectoration of sputum was increased by induction with an aerosol of hypertonic saline and the methods of examination were refined, evaluated and applied to investigate the pathogenesis, pathophysiology, and treatment of asthma and other airway conditions. In this article, asthma will be defined physiologically as variable airflow limitation, and bronchitis as an increase in sputum inflammatory cells. The rationale for examining sputum cell counts in clinical practice, the process of sputum induction and examination relevant to this, and the evidence that sputum cell counts have uses in clinical diagnosis and treatment will all be discussed.

For editorial comments see page 1.

Rationale for sputum examination

Airway inflammation is central to the pathogenesis, exacerbation and persistence of asthma and other airway conditions [3]. Ongoing inflammation can cause irreversible structural changes such as epithelial damage, subepithelial basement membrane thickening, increased vascularization, myofibroblast proliferation and hypertrophy and hyperplasia of smooth muscle [3–7]. Prevention and treatment of airway inflammation is therefore the mainstay of management [8].

The nature and extent of inflammation has, until recently, been assessed using direct, invasive bronchoscopy with bronchial washings or biopsy or bronchoalveolar lavage (BAL), or by indirect measurements of symptoms, spirometry, peak expiratory flow, tests of airway hyperresponsiveness or peripheral blood inflammatory markers [9]. Bronchoscopy with tissue diagnosis, while providing the gold standard assessment of airway inflammation, is limited by discomfort to the patient, risks of the procedure and expense. The indirect measurements relate variably with each other and reflect more than airway inflammation, for which they are nonspecific [10]. As a result, not surprisingly, they may not correlate with direct measurements of airway inflammation.

In the last 10 yrs (1989–1999) examination of induced sputum has evolved as a direct, relatively noninvasive, valid and repeatable method of measuring airway inflammation [11–13]. Inflammatory findings correlate best with bronchial washings and more variably, but reasonably, with bronchial biopsies and BAL [14–18]. These relationships are understandable since they reflect differences in different airway compartments; induced sputum and bronchial washings reflect secretions from the more central airway lumen, BAL from the peripheral lumen and bronchial biopsies from the more central airway walls. Sputum eosinophils are more sensitive and specific for the presence of asthma than blood eosinophils or serum eosinophilic cationic protein (ECP) [19] or the more recently introduced analysis of nonrespiratory gases in exhaled air such as nitric oxide (NO) [20]. Sputum eosinophils are more responsive to change, increasing ahead of clinical variables [21] and exhaled NO during exacerbations [22], and respond more slowly to treatment [23]. It would therefore not be surprising if clinicians were poor at judging whether airway inflammatory was present surprising or not [24].

Sputum induction and examination

The methods of sputum induction have been reviewed in detail elsewhere [25, 26]. The induction is carried out with an aerosol of hypertonic saline, *e.g.* for three periods of 5 or 7 min. After each inhalation, the patient is asked to cough to try to expectorate sputum into a universal container. Safety is of prime concern. A β_2-agonist is inhaled first to inhibit any bronchoconstriction caused by inhalation of the saline aerosol and the forced expiratory volume in one second (FEV_1) is monitored before and after each inhalation. In addition, if the baseline value is <70%, the inhalations are begun with normal saline and inhaled for shorter times [23]: if the FEV_1 falls during the induction by 10–19% any further inhalations are given cautiously, and if it falls by 20%, the inhalations are discontinued and a bronchodilator given. The procedure can be performed safely, even in patients with exacerbations of asthma or moderate chronic airflow limitation providing appropriate precautions are taken [23, 27–29]. Measures are also taken during the procedure to prevent contamination with post nasal drip and to limit contamination with saliva by blowing the nose, rinsing the mouth with water and swallowing the water before expectoration. The expectorated specimen is a mixture of sputum and saliva. One group has tried to limit salivary contamination by attempting to collect sputum and saliva separately [30]. The procedure can be successful in >80% of adults and older children who cannot produce sputum spontaneously [11].

Accurate sputum analysis requires that the specimen is examined fresh within ~2 h. Two methods have been described with subsequent modifications of each. In one, salivary squamous cell contamination can be reduced further by pouring the expectorate into a Petri dish and selecting all of the more opaque or dense portions; as little as 50 mg is sufficient (fig. 1) [11, 25, 31]. In the other, the whole expectorate of sputum plus saliva is processed [30, 32]. In both methods the specimen is treated with dithiothreitol 0.1% to break up the mucus and disperse the cells. The cell suspension can then be filtered to remove debris and portions of remaining mucus. The nonsquamous cell count and cell viability (with trypan blue) are determined in a haemocytometer. Cytospins are made and stained and a differential cell count is performed. The cytospins concentrate the cells onto a small portion of the slide and so speed counting.

Observations of sputum cell counts relevant to clinical practice

A number of observations suggest that sputum cell counts have a place in clinical practice.

Types and causes of inflammation

In healthy subjects, cell counts show that macrophages and neutrophils predominate and eosinophils and lymphocytes are few (table 1) [33]. Abnormalities in differential cell counts re-emphasize the occurrence of different types of inflammation and their different causes (table 2), Airway inflammation is not airway inflammation is not airway inflammation!

An increase in sputum eosinophils is regarded as characteristic of asthma and can be induced by inhaled allergen [34, 35] or chemical sensitizers [36, 37] in sensitized subjects and by a reduction in steroid treatment in steroid-dependent asthma [21, 38, 39]. Serial measurements during periods at work and periods away from work can be used in the investigation of occupational asthma (fig. 2) [40]. Support for the diagnosis is provided by an increase in eosinophils at work followed by a fall away from work.

Sputum cell counts were responsible for the recognition of eosinophilic bronchitis without asthma [41, 42]. This condition was observed in patients with a chronic cough who had normal spirometry and normal airway responsiveness to methacholine and adenosine monophosphate. The sputum contained an increase in eosinophils, and this

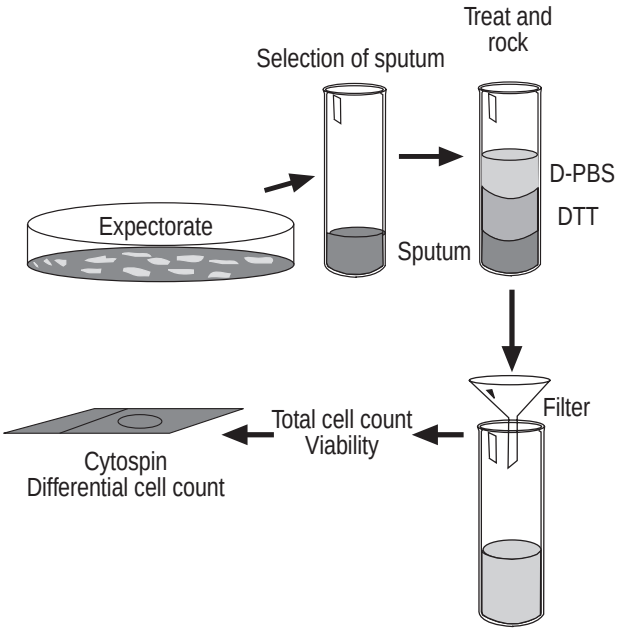

Fig. 1. – Method for sputum processing. D-PBS: Dulbecco's phosphate-buffered saline; DTT: dithiothreitol.

Table 1. – Differential cell counts in induced sputum from normal adults

Sputum cell counts	Median	IQR	Percentiles	
			10th	90th
Macrophages %	60.8	28.9	33.0	86.1
Neutrophils %	36.7	29.5	11.0	64.4
Eosinophils %	0.00	0.30	0.00	1.10
Lymphocytes %	0.50	1.80	0.01	2.60
Bronchial epithelial cells %	0.30	1.30	0.00	4.40
Metachromatic cells %	0.00	0.00	0.00	0.04

IQR: interquartile range.

plus the chronic cough were reversed by corticosteroid treatment. Subsequently, the condition was observed in 10–15% of patients presenting to tertiary care centres with a chronic cough [43, 44]. Like eosinophilic bronchitis with asthma, the condition was observed after exposure to allergens or chemical sensitizers in sensitized subjects [45], in atopic or nonatopic subjects and in smokers or nonsmokers. It can be transient [46], or persistent unless suppressed by regular steroid treatment [46]. If untreated, variable airflow limitation with airway hyperresponsiveness (asthma) [42, 46] or progressive airflow limitation [47] without airway hyperresponsiveness can develop. Early detection therefore may be important.

Several causes of sputum neutrophilia are known including cigarette smoking (especially when this is associated with chronic airflow limitation) [13, 48, 49], pollutants such as ozone [50–52], endotoxin [53] and infection [54]. The intensity of the neutrophilia is indicated by the total cell count and is most pronounced in bronchial infection. Noneosinophilic (usually neutrophilic) exacerbations of asthma are described but their cause and incidence has not been systematically examined [39, 54]. A likely cause is viral infection [55]. Occupational neutrophilic asthma has also been described [56].

Table 2. – Summary of the clinical utility of induced sputum examination

Cell	Causes of increase in cell type
Eosinophilic	Uncontrolled asthma
	Eosinophilic bronchitis without asthma
	Allergen and chemical sensitizer exposure
	Steroid responsive chronic airflow limitation
Neutrophilic	Cigarette smoking
	Pollutants *e.g.* ozone
	Endotoxin
	Infection
	Steroid resistant asthma
Lymphocytic	Sarcoidosis
	Chlamydia pneumoniae

Inflammation and treatment

The differentiation of inflammation into eosinophilic and noneosinophilic is important with respect to treatment. In patients presenting with chronic cough, asthma or chronic airflow limitation, the presence of sputum eosinophilia usually predicts a clinical response to steroid treatment [23, 57–62] while the lack of this usually indicates resistance to added steroid treatment [23, 62, 63]. An adequate steroid dose reduces sputum eosinophils into the normal range.

In chronic cough with sputum eosinophilia, inhaled steroids are usually effective but prednisone may be required transiently [46] or regularly [47]. In contrast, chronic cough without sputum eosinophilia does not benefit from corticosteroids [64].

Persistent sputum eosinophilia in patients with "difficult asthma" should raise the possibility of under treatment or noncompliance with corticosteroid medication. Noncompliance can be confirmed by direct questioning, review of pharmacy records or by administration of medication under direct supervision, with subsequent reversal of the eosinophilia [65].

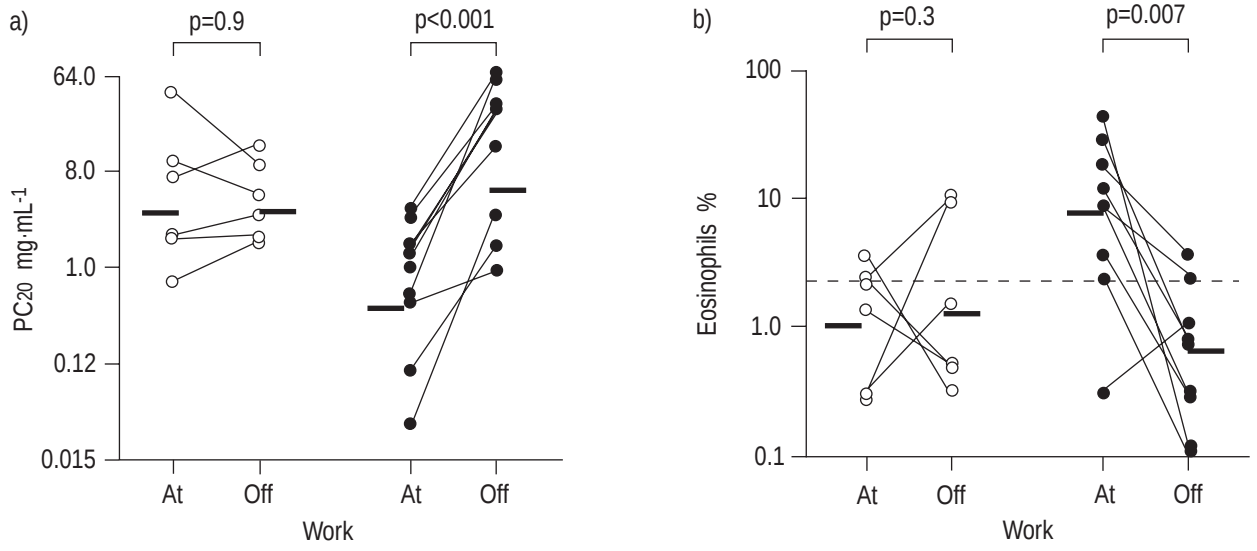

Fig. 2. – Changes in provocation concentration of methacholine to cause a fall in forced expiratory volume in one second of 20% (PC_{20}) (a) and sputum eosinophils (b) between periods at work and away from work in patients with occupational asthma (●) and nonoccupational asthma (○). Horizontal bars illustrate the median eosinophil percentage and the geometric mean PC_{20} and the dashed horizontal line 2SD of the mean in healthy adults, *i.e.* the upper limit of normal. The patients with occupational asthma, who were diagnosed clinically by a four-fold increase in PC_{20} after four weeks away from work, also had a fall in eosinophils. Adapted from [40].

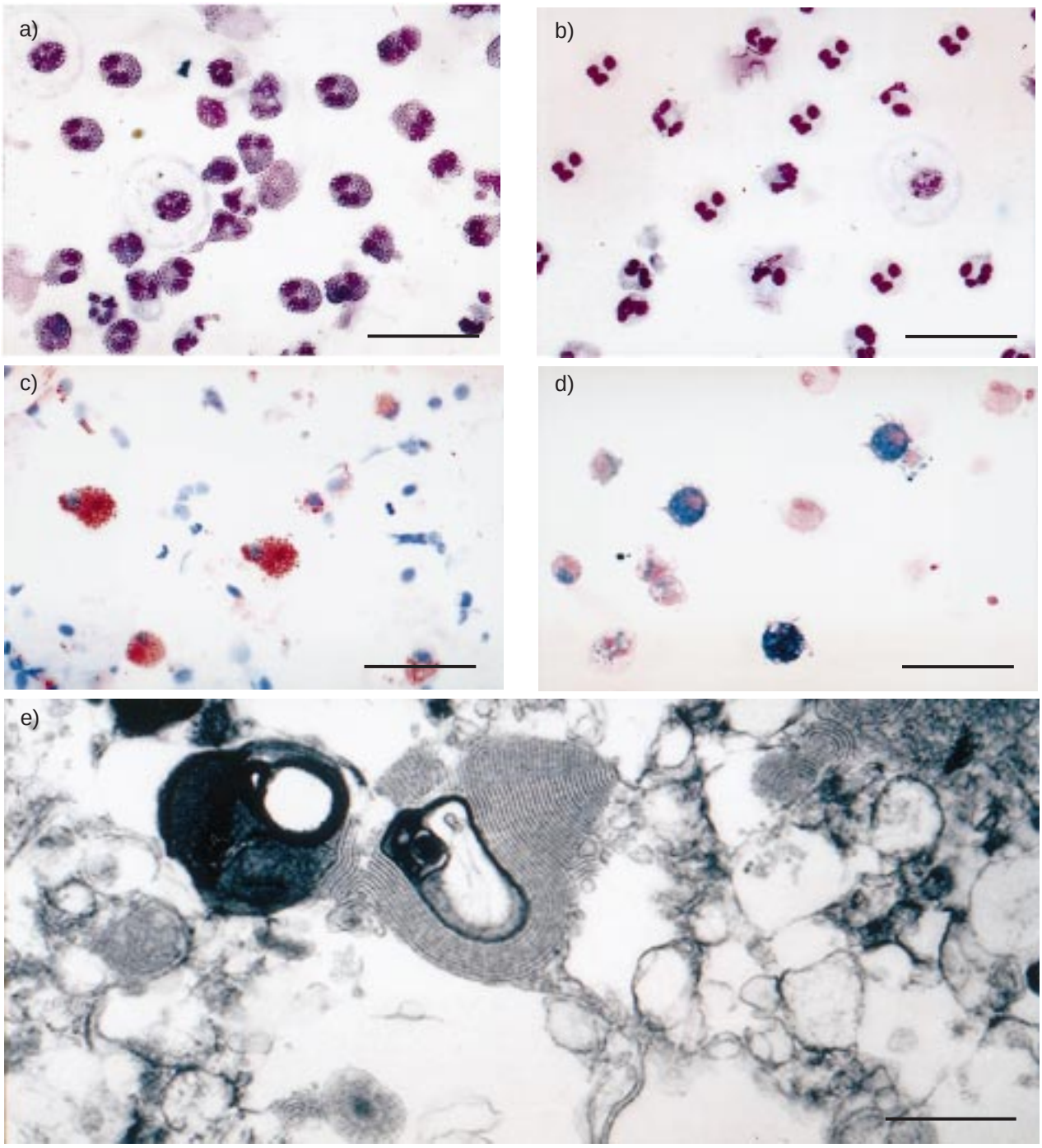

Fig. 3. – Sputum eosinophilia (a), neutrophilia (b), lipid-laden macrophages (c), haemosiderin-laden macrophages (d), and a high-magnification electron micrographic view of a lamellar inclusion body within the cytoplasm of a bronchial epithelial cell in Fabry′s Disease (e; from [76]). (Internal scale bars a–d=100 μm, e=0.5 μm).

Smoking related chronic airflow limitation is generally associated with a sputum neutrophilia that is not steroid responsive [48, 66]. Some patients, however, also have a concomitant sputum eosinophilia that is responsive to corticosteroid therapy. In a single blind, sequential cross-over trial of placebo and prednisone (30 mg·day^{-1}) each given for 2 weeks, in which sputum measurements were double blind, improvements in clinical and inflammatory variables with prednisone were observed only in those patients with a sputum eosinophilia of at least 3% [67]. This group had a significant and clinically important improvement in the dyspnoea domain of quality of life, associated with a reduction in sputum eosinophils, ECP and fibrinogen. The study was not adequately powered to detect a change in FEV_1.

Sputum cell counts can help to identify appropriate add-on therapy in asthma. Although long acting β-agonists significantly improve symptoms and pulmonary function, they

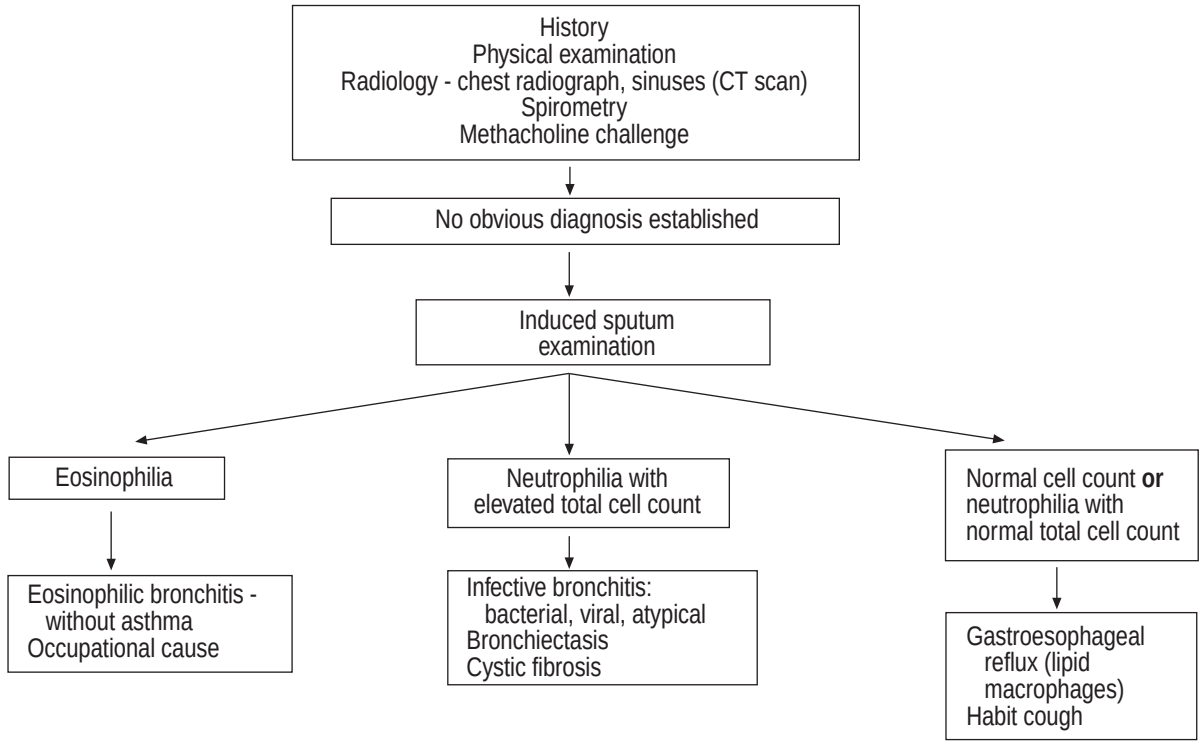

Fig. 4. – Algorithm for assessment of chronic cough using induced sputum examination. CT: computed tomography.

do not reduce sputum eosinophilia [58] and indeed can mask this. McIvor *et al*. [68] demonstrated that salmeterol controlled symptoms and lung function in asthmatic subjects undergoing corticosteroid withdrawal allowing greater sputum eosinophilia to develop before a clinically recognizable exacerbation. On the other hand, long acting β-agonists improve asthma control in symptomatic patients with normal cell counts on corticosteroid therapy [69].

Leukotriene antagonists have demonstrated anti-inflammatory properties in bronchoscopy based studies and this has been confirmed using induced sputum examination [70–72]. In a double blind, randomized, controlled, parallel group study over 4 weeks, montelukast improved clinical outcomes and reduced the sputum eosinophil count; during placebo treatment sputum eosinophils increased [72].

Other cellular abnormalities

The examination of specific inclusions in induced sputum macrophages provides a window to oropharyngeal reflux with presumed microaspiration or to left ventricular dysfunction. The demonstration of lipid in macrophages can be an indicator of aspiration of gastric contents and the examination of BAL for lipid laden macrophages has been suggested as a useful diagnostic test for aspiration [73, 74]. Recently, the examination of induced sputum for lipid laden macrophages was shown to have high sensitivity (90%) and specificity (89%) for oropharyngeal reflux measured by 24-h dual channel oesophageal pH monitoring, for an alveolar macrophage lipid laden index of 7.0 [75] (fig. 3c). The lipid laden index is a composite score derived from counting 100 consecutive macrophages, grading the intracellular lipid from 0 (no lipid droplets) to 4 (many intracellular oil-red-O stained lipid droplets obscuring the nucleus), the grades for each cell then summed to give a lipid index ranging 0–400.

The presence of macrophages containing haemosiderin in induced sputum (fig. 3d) can be a marker of left ventricular dysfunction in breathless patients suspected of having cardiac or respiratory disease. Counts of haemosiderin containing macrophages >2% had a sensitivity of 80% and a specificity of 94% and a positive predictive value of 96% for the presence of left ventricular dysfunction confirmed by 2D echocardiographic criteria [77]. The value of these haemosiderin macrophage counts needs to be investigated in patients with chronic airflow limitation and dyspnoea where the question of concomitant cardiac disease with left ventricular dysfunction is considered.

Induced sputum examination has successfully confirmed lung involvement in Fabry′s disease, a storage disorder, where typical lamellar inclusion bodies within bronchial epithelial cells were demonstrated in sputum (fig. 3e) [76]. In untreated patients with newly diagnosed stage I and II sarcoidosis compared with normal subjects, induced sputum examination was shown to be an alternative to bronchoscopy with bronchial washings or BAL to demonstrate a lymphocytosis [78]. Induced sputum examination was initially introduced to diagnose lung malignancy, but this use has now chiefly been taken over by bronchoscopy. In one study, in which it was compared with bronchoscopy, these tests identified primary lung cancer in 84% and 92% of cases respectively [79].

How to use sputum examination in clinical practice

From the preceding section, it is evident that sputum examination does have a place in clinical practice. Apart from the traditional uses for diagnosis of infection and malignancy, sputum examination can identify the presence, type and intensity of airway inflammation as well as drawing attention to the presence of micro aspiration, left ventricular dysfunction or lung involvement in metabolic storage disease.

In chronic cough, measurements are needed to be confident about the diagnosis of eosinophilic bronchitis without asthma (fig. 4). Serial measurements, during periods at work and periods away from work, are needed to objectively document an occupational cause for it [40]. When an eosinophilic bronchitis is not present, the sputum cell counts can point towards other causes. An intense neutrophilia with elevation of total cell count and the proportion of neutrophils, would suggest an infective bronchitis [55], bronchiectasis [80] or another cause for this such as cystic fibrosis. On the other hand, if the sputum cell counts are normal or if there is a neutrophilia with a normal total cell count, other conditions such as gastroesophageal reflux [75], habit cough need to be considered.

The place of sputum cell counts to monitor anti-inflammatory and other treatment in asthma requires prospective investigation. However, like other measurements, it would not be surprising if physicians were not good at recognizing the presence or type of inflammatory cell infiltration [24]. The authors have found sputum cell counts to be useful in assessing and managing difficult asthma (fig. 5). In this situation, sputum eosinophilia should draw attention to possible noncompliance [65], a common allergen or occupational [40] cause, or inadequate steroid treatment [39]. Alternatively, there maybe no eosinophilia but a neutrophilia, raising the possibility of misdiagnosis, associated disease [80], or steroid-resistant asthma [81]. If the patient is already on steroid treatment, these findings would raise the possibility that the steroid dose can be reduced or that additional appropriate treatment might be added. For example, added treatment might include an antibiotic for infection, or a long-acting β_2-agonist for variable airflow limitation.

Sputum cell counts also have a place in the treatment of chronic airflow limitation associated with cigarette smoking or emphysema. They can be useful at the time of initial assessment or during exacerbations. Sputum eosinophilia predicts a response to prednisone [67]. An intense neutrophilia would suggest infection that might benefit from antibiotic treatment. As in asthma, the role of sputum cell counts in monitoring various treatment options needs investigation.

Promise and problems

While sputum cell counts hold much promise, their implementation in clinical practice poses problems. The promise is that objective measurements will aid diagnosis and treatment, may identify more patients with disease at an earlier stage, and benefit both patients and physicians. The problems relate to the safety of sputum induction, the need to examine the sputum while it is fresh, the need for trained staff with regular quality control and the time and cost of the procedures. At present, reliable measurements are only available to clinical practice in a few centres where they are established in research. In the authors′ unit (St. Joseph′s Hospital, Hamilton, Ontario, Canada), sputum induction is performed in the pulmonary function laboratory by pulmonary function technologists. The procedure takes up to 30 min and the precautions are similar to a

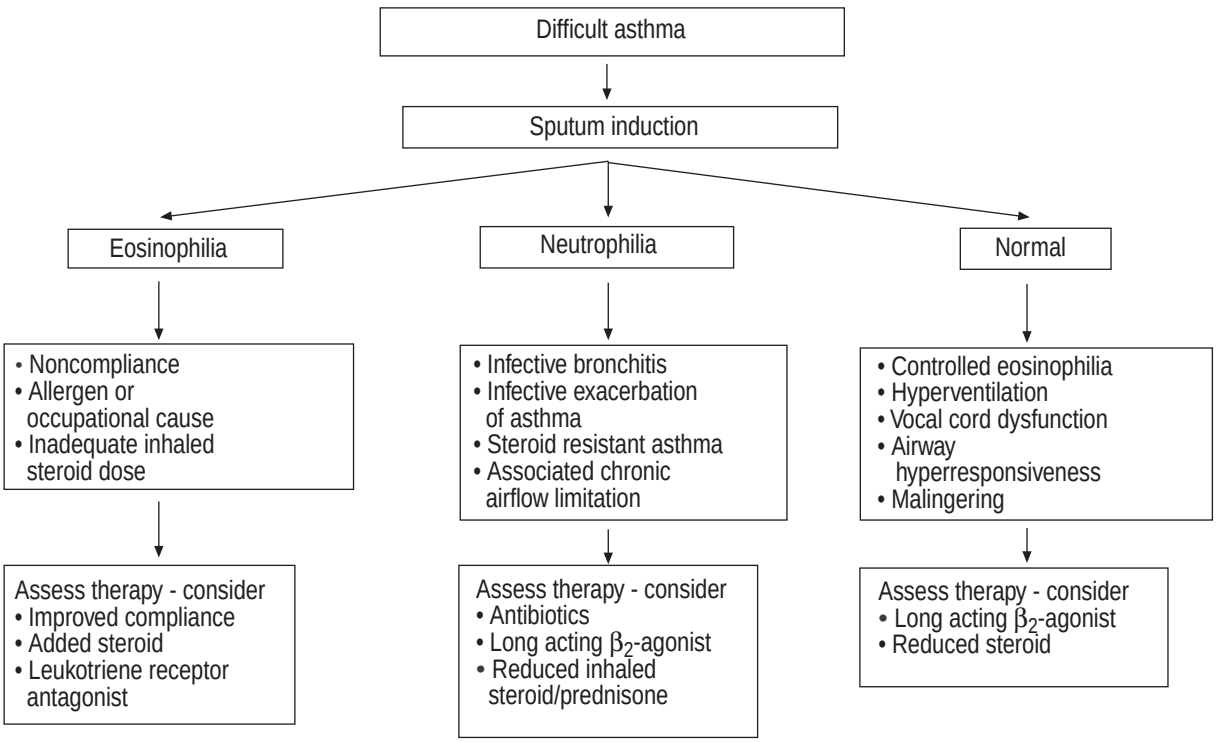

Fig. 5. – Algorithm for assessment of difficult to control asthma using examination of induced sputum.

methacholine inhalation test. In the authors′ unit the processing and examination of sputum is performed in the research laboratory by haematologically trained registered technologists. The procedures for total and differential counts take just over an hour. With present methods therefore, the measurements will only be available in specialist centres and this is appropriate until further research identifies their role in management and ways of making them more widely available are established.

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