Induced sputum for the investigation of airway inflammation: Evidence for its clinical application

Frederick E Hargreave MD FRCP FRCPC
Asthma Research Group, Department of Medicine, St Joseph’s Hospital and
McMaster University, Hamilton, Ontario

Airway inflammation is considered to be the primary cause of airway diseases. Its prevention and reversal are the primary aims of treatment. Measurement of the inflammation is now possible relatively noninvasively and reliably by using induced sputum cell counts. The differential count indicates the presence and type of the inflammation (eosinophilic or neutrophilic) and the total cell count the intensity. Sputum eosinophilia responds to treatment with corticosteroid, while there is increasing evidence that an isolated neutrophilia does not. Clinical judgement of airway inflammation is made difficult because of the different types of inflammation and their inconsistent correlation with the clinical features. Hence, reliable measurement of induced sputum cell counts may be useful to guide treatment in clinical practice. Consideration should now be given as to how to make it more available.

Key Words: Airway inflammation, Asthma treatment, Eosinophilic bronchitis, Induced sputum, Sputum cell counts

Correspondence and reprints: Dr FE Hargreave, Firestone Regional Chest and Allergy Unit, St Joseph’s Hospital, 50 Charlton Avenue East, Hamilton, Ontario L8N 4A6. Telephone 905-521-6000 ext 3714, fax 905-521-6158, e-mail hargreav@fhs.mcmaster.ca

Can Respir J Vol 6 No 2 March/April 1999 169
amination has made this possible (1). Because this technique is relatively noninvasive, it can be applied at random, in severe disease and repeatedly to examine the effects of treatment.

MEASUREMENT OF AIRWAY INFLAMMATION

Sputum is defined as lower respiratory tract secretions. It can be induced with an aerosol of hypertonic saline (2,3). A short-acting beta2-agonist is inhaled to inhibit possible bronchoconstriction from the saline aerosol. The aerosol is generated by an ultrasonic nebulizer; we initially used the Fisoneb, which is no longer produced, and now use the Universal (Methapharm Inc), which is similar but improved. We use 3%, 4% and 5% concentrations of saline, each inhaled for 7 mins, although for a 3% or 4% concentration a shorter period may be sufficient. Forced expiratory volume in 1 s (FEV1) is measured before and after each inhalation, or at any time if symptoms develop, for safety. The subject is asked to blow their nose, rinse their mouth and swallow water to limit contamination with postnasal drip and saliva, and to try to cough sputum into a container. The method is safe in patients with mild asthma (4,5). It can be made safe in subjects with more severe asthma or chronic airflow limitation by beginning with normal saline and using shorter periods of inhalation (6,7). In clinical practice, the procedure can be discontinued when enough sputum is obtained. It is successful in 80% or more of adults and older children who are healthy or have airway disease.

The specimen of sputum needs to be processed as soon as possible, within 2 h. It is an advantage if the cells can be preserved for longer, in case a specimen is collected at night or during the weekend; how to accomplish this is being investigated. When sputum is expectorated, it is often mixed with saliva. Some investigators examine the whole expectorate (8), but we select out the sputum from saliva with blunt forceps. This is then treated with 0.1% dithiothreitol, followed by saline, and filtered (3,9). Dithiothreitol breaks disulphide bonds in the mucus and allows a uniform dispersal of the cells. A total cell count and cell viability are obtained by using a hemocytometer and trypan blue stain, respectively. Cytospins are made and stained with Wright’s stain, and a differential cell count is performed on 400 cells. The remaining suspension is centrifuged, and the fluid phase stored at −70°C for later measurements, if these are required. However, for clinical practice, only cell counts are needed. The results can be obtained within 1 to 2 h and involve 30 to 40 mins of technologist time, with the remainder of the process being automated.

This method of sputum induction and examination has been evaluated. The cell counts in induced sputum are similar to those in spontaneous sputum (10). The selection of sputum from the expectorator is usually very successful at minimizing salivary contamination as indicated by less than 5% of squamous epithelial cells and improves the recognition of the types of nonsquamous cells (11). This is relevant because squamous contamination of greater than 20% is associated with poorer repeatability of nonsquamous counts (12). Clearly, the total cell count is reduced by selection and filtering, but measurements can be expressed per milligram of selected sputum and the differential cell count is not altered. The neutrophil, eosinophil and macrophage differential cell counts, and several of the fluid-phase measurements are reproducible (reliable) (9), valid and responsive (6,13), but the total cell and lymphocyte counts are not as reliable as those. The cell counts obtained from sputum induction are different from the cell counts obtained from bronchial washings, bronchoalveolar lavage (BAL) and bronchial biopsies (14-18). This is to be expected given the different compartments being sampled. Eosinophils and neutrophils are highest in number in sputum, whereas macrophages and lymphocytes are higher in number in BAL. The predominant cell in biopsies is the lymphocyte. In a comparison of sputum versus blood eosinophils and serum eosinophilic cationic protein in patients with asthma, and a control group of healthy subjects and others with smokers’ nonobstructive chronic bronchitis, sputum eosinophils and eosinophilic cationic protein were more sensitive and specific to the blood measurements (19).
Sputum examination has drawn attention to the different types of airway inflammation and their causes; eosinophilic existence of bronchitis without asthma; asthma without an eosinophilic bronchitis; neutrophilic exacerbations of asthma; the frequent failure of clinical assessment to recognize the presence or type of inflammation in sputum and the influence of the type of inflammation on the effects of different treatments.

Sputum examination has emphasized the occurrence of different types of inflammation based on increases in specific cells and the different causes of these inflammations (Figure 1, Table 1). Eosinophilic inflammation is recognized to be a feature of asthma (9,20), defined as a condition in which there is variable narrowing of the airways of the lungs (modified from Scadding [21]), sputum eosinophilia occur from airway reactions to allergens (13,22) and occupational chemical sensitizers (23,24) in sensitized subjects, and after withdrawal of corticosteroid treatment in steroid-dependent asthma (25). Neutrophilic inflammation occurs in cigarette smokers (9,26), during bacterial and viral infections (27), and after the inhalation of endotoxin (28) and some pollutants such as ozone (29). A sputum lymphocytosis has been observed early in a subject with cough associated with chlamydia infection (30). Many of these causes are common, and can occur at the same or different times in the same individual. The colour of the sputum can identify intense neutrophilia when it is mucopurulent or purulent, but not eosinophilia, which can occur in mucoid, mucopurulent or purulent sputum (31). The clinical significance of the different types of inflammation relates to differences in clinical and physiological presentation, and the effects of treatment.

Sputum examination was responsible for the recognition of eosinophilic bronchitis without asthma (32), which may be asymptomatic but can present with a chronic cough which can be dry or productive. Spirometry, diurnal variation of peak expiratory flow (PEF) and responsiveness to methacholine or adenosine monophosphate are normal (33). The sputum contains an increase in eosinophils and metachromatic cells, and mRNA for interleukin-5 and granulocyte-macrophage colony-stimulating factor as in eosinophilic bronchitis with asthma (34). Eosinophilic bronchitis without asthma occurs in atopic or nonatopic subjects, and may be caused by inhaled allergens or occupational chemical sensitizers (35). It may occur in smokers or nonsmokers, and be transient (36) or persistent and require regular treatment. If left untreated, airway hyperresponsiveness and other features of asthma may occur (33,36), or there may be a progressive fall in FEV1 without airway hyperresponsiveness (37) (Table 2). The recognition of this cause of chronic cough is important with respect to treatment.

The examination of sputum has identified what has also been observed with BAL (38), that the severity of eosinophilic bronchitis does not necessarily correlate with the severity of clinical and physiological parameters in asthma (32,39). There may be no eosinophilic bronchitis in a patient with many symptoms due to variable airflow limitation and severe airway hyperresponsiveness. In contrast, a pronounced eosinophilic bronchitis can occur in someone with only mild airway hyperresponsiveness. This may make it difficult to recognize the presence or severity of an eosinophilic bronchitis from clinical parameters.

An increase in the proportion of neutrophils in sputum is a feature of asthma (9). However, some exacerbations of asthma can be characterized by a more intense neutrophilia without an eosinophilia. The exacerbation can be mild (40), severe (6,41) or fatal (42). Causes of neutrophilic exacerbations include respiratory virus infection (27) and certain oc-

### Table 1: Induced sputum: markers of inflammation

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Stable asthma</th>
<th>Smokers' bronchitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells x10⁶/mg</td>
<td>3.1 (4.0)</td>
<td>3.3 (6.0)</td>
<td>3.9 (3.5)</td>
</tr>
<tr>
<td>Percentage eosinophils</td>
<td>0.5 (1.1)</td>
<td>5.2 (19.4)</td>
<td>0.3 (0.7)</td>
</tr>
<tr>
<td>Percentage neutrophils</td>
<td>24.1 (26.8)</td>
<td>46.9 (42.0)</td>
<td>33.0 (27.2)</td>
</tr>
</tbody>
</table>

Data are median (interquartile range) (9). More comprehensive data on all counts in health subjects have been submitted for publication (unpublished data)

### Table 2: Changes in clinical features and sputum cell counts in a patient with prednisone-dependent eosinophilic bronchitis

<table>
<thead>
<tr>
<th>RV 65 Y</th>
<th>April 11</th>
<th>April 18</th>
<th>April 25</th>
<th>June 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Forced expiratory volume in 1 s (FEV1) (L)</td>
<td>1.9</td>
<td>1.9</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Vital capacity (L)</td>
<td>2.7</td>
<td>2.5</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Provocation concentration of methacholine to cause a fall in FEV1 of 20% (mg/mL)</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td></td>
</tr>
<tr>
<td>Percentage sputum eosinophils</td>
<td>1.3</td>
<td>27.3</td>
<td>71.3</td>
<td>61.5</td>
</tr>
<tr>
<td>Prednisone (mg/day) (number of days on that dose)</td>
<td>30 (8)</td>
<td>20 (6)</td>
<td>17.5 (7)</td>
<td>10 (35)</td>
</tr>
<tr>
<td>Budesonide (mg/day)</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Prednisone was reduced progressively. + Cough and sputum
occupational exposures (43,44). How often neutrophilic exacerbations occur is unknown, but, if viral exacerbations of asthma are common, they also may be common. Neutrophilic exacerbations respond differently than eosinophilic exacerbations to treatment. Sputum eosinophilia responds to treatment with corticosteroids whether the clinical presentation is of asthma (6,37,45,46) (Figure 2), chronic cough (32-34) or chronic airflow limitation (7). Sputum examination has illustrated the kinetics of change in sputum eosinophilia with changes in prednisone treatment. During severe exacerbations of asthma, the improvement in sputum eosinophils lags behind improvement in FEV1 (6). Persistence of sputum eosinophilia has been observed in subjects whose symptoms and FEV 1 seem to be controlled (46). In these individuals, the sputum eosinophilia can be reversed by higher doses of steroid, but the clinical relevance of this still needs investigation (47). When prednisone is reduced in patients who are prednisone-dependent (37), and when inhaled steroid is reduced in inhaled steroid-dependent patients (48), sputum eosinophilia recurs before there is worsening of symptoms, FEV1 or PEF (Table 2). The serial observations raise the possibility that the control of sputum eosinophilia will produce better outcomes than control of symptoms and FEV1. This hypothesis deserves investigation.

In contrast, it appears that when there is no eosinophilia in asthma, chronic cough or chronic airflow limitation, patients fail to respond clinically to corticosteroid treatment (6,7,49,50). Such noneosinophilic (usually neutrophilic) exacerbations of asthma seem to be steroid resistant. This observation needs to be confirmed and alternative treatment for the exacerbations investigated.

Sputum examination has also helped increase the knowledge of the actions of drugs used in the treatment of asthma. Salmeterol was shown to have no effect on sputum eosinophilia (51) and to mask slightly the clinical effects of developing sputum eosinophilia (52), thus delaying the recognition of a clinical exacerbation. Montelukast (Singulair, Merck Frosst Canada) has been shown to reduce sputum eosinophilia, although the magnitude of its effect and the clinical relevance still need investigation (53).

THE PLACE OF SPUTUM EXAMINATION IN PRACTICE

Research observations indicate that it can be difficult for physicians to recognize the presence or type of airway inflammation (54). The significance of this in relation to the effects of treatment needs further investigation, but observations do identify places in practice where sputum examination can be useful.

- When patients present with a chronic cough, sputum examination can be helpful to diagnose eosinophilic bronchitis, which responds to corticosteroid treatment. The presence of sputum eosinophilia indicates a need to search for a causal allergen or occupational chemical sensitizer, and for initial treatment with avoidance strategies and corticosteroid. If sputum eosinophilia is not present, other causes of the chronic cough and other treatment should be considered first.

- Sputum examination provides an additional objective measurement to diagnose occupational asthma. Occupational asthma is usually caused by allergens or occupational chemical sensitizers to which the person has become sensitized, and is associated with sputum eosinophilia (55) (Figure 3). Hence, when the person is at work, sputum eosinophilia develops, and, when the patient is away from work for a period, the
The recent description of work-related neutrophilic exacerbations of asthma raises the question of how often this occurs and in what situations (43,44).

- When there is difficulty controlling asthma, sputum cell counts can indicate whether the patient is prednisone-dependent (37), or whether failure to respond to prednisone might be due to a noneosinophilic exacerbation of asthma or other disease (50). The persistence of sputum eosinophilia despite high doses of corticosteroid treatment should raise the possibility of patient noncompliance with treatment.
- In patients with moderate to severe chronic airflow limitation, sputum eosinophilia predicts benefit from corticosteroid treatment (7).
- The value of sputum examination to monitor anti-inflammatory treatment requires investigation (56).

PROVISION OF SPUTUM CELL COUNTS IN PRACTICE

The provision of reliable sputum cell counts in practice is problematic. There is no billing system (public or private) to cover the cost of sputum induction or appropriate sputum examination.

REFERENCES


The procedure for sputum induction is not dissimilar to the procedure for a methacholine inhalation test. However, it would be inappropriate to perform sputum induction if it is not backed up by reliable sputum examination. The latter needs to be performed by hematologically trained, registered technologists and to involve ongoing quality control checks. The procedure can be performed by a hematologist laboratory, but this will likely be difficult to arrange when health care funds to hospitals have been reduced. An alternative is to employ a technologist to perform sputum examination in the pulmonary function laboratory.

For the moment, in Hamilton, Ontario, we have introduced sputum induction by registered pulmonary function technologists in the pulmonary function laboratory and reliable sputum examination by research staff. The service is available at one centre in the city. Other physicians in the city and surrounding areas can refer patients for the procedures as for any other pulmonary function test.

Discussions need to be undertaken with medical insurance providers to introduce an appropriate billing system to allow the procedures to be made available in other areas of Canada.

ACKNOWLEDGEMENTS: I thank Lori Burch for typing the manuscript, Ann Efthimiadis for providing Figure 1, and Marcia and Emilia Pizzichini for Figures 2 and 3.
Hargrave


