Update on clinical inflammometry for the management of airway diseases


Airway inflammation is a central feature of many airway diseases such as asthma, chronic bronchitis, bronchiectasis and chronic cough; therefore, it is only logical that it is measured to optimize its treatment. However, most treatment recommendations, including the use of anti-inflammatory therapies such as corticosteroids, are based on assessments of only airflow and symptoms. Over the past 10 years, methods have been developed to assess airway inflammation relatively noninvasively. Quantitative cell counts in sputum and the fraction of exhaled nitric oxide are the most validated tests. Judicious use of currently available drugs, such as corticosteroids, bronchodilators and antibiotics, and other anti-inflammatory therapies guided by sputum eosinophil and neutrophil counts, have been demonstrated to decrease exacerbations of asthma and chronic obstructive pulmonary disease, ameliorate cough, improve quality of life in patients with these diseases and is cost effective compared with treatment strategies based on guidelines that do not incorporate these measurements. Thus, it is unfortunate that this is not used more widely in the management of airway diseases, particularly in patients with severe asthma and chronic obstructive pulmonary disease who experience frequent exacerbations.

Key Words: Asthma; COPD; Cough; Exhaled nitric oxide; Inflammometry; Sputum

Methods to assess airway inflammation

Sputum

Sputum cell counts are a relatively noninvasive and reliable method of identifying airway inflammation. The method of sputum collection is well described and standardized (5). Hypertonic saline inhalation is safe in patients with forced expiratory volume in 1 s (FEV1) as low as 0.9 L (6). Spontaneously expectorated sputum, when available, provides information equally as useful as induced sputum (7). The method is successful in almost all patients with smoker's bronchitis and COPD, in 80% of patients with asthma and in 60% of patients with a dry, chronic cough (8). Sputum processing and the quantification of cell counts are also standardized (9), and normal values have been established (10). It has recently been simplified by the introduction of a commercially available sputum filtration device (Accufilter, Cellometrics, Canada) and a kit (11). The cell counts can accurately discriminate eosinophilic airway inflammation from non-eosinophilic airway inflammation. Eosinophilic airway inflammation is steroid responsive (12) while non-eosinophilic (usually neutrophilic) inflammation generally is not (13). Monitoring of airway inflammation using sputum cell counts helps to identify impending loss of asthma control and adjust anti-inflammatory medications in patients with a variety of airway diseases such as asthma, smoker's bronchitis and chronic cough. It helps to increase corticosteroid dosing in exacerbations associated with an eosinophilic bronchitis, limit the use of corticosteroids in exacerbations associated with a non-eosinophilic bronchitis and use antibiotics in exacerbations associated with a neutrophilic bronchitis. This also helps to identify patients with an ‘eosinophil phenotype’ for targeted therapy with anti-eosinophil strategies such as anti-interleukin 5 molecules (14), and to develop protocols to investigate patients with persistent eosinophilic (15) or neutrophilic bronchitis (16). Such treatment strategies help to significantly reduce asthma exacerbations (17,18) and hospitalizations due to exacerbations of COPD (19). Sputum examination provides additional useful information in patients with airway diseases. For example, macrophages containing hemosiderin can be useful in detecting left ventricular dysfunction (20), and macrophages containing lipid are suggestive of oesophagogastric reflux with microaspiration (21), both of which can complicate...
TABLE 1  
Adjustment of treatment based on sputum cell count

<table>
<thead>
<tr>
<th>Bronchitis Type</th>
<th>Sputum Cell Count</th>
<th>Medication Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophilic bronchitis</td>
<td>N&gt;80%</td>
<td>2%–3% or EFGs increase if symptomatic</td>
</tr>
<tr>
<td></td>
<td>1%–2% dose unchanged</td>
<td>Evaluate prednisone-sparing therapy</td>
</tr>
<tr>
<td></td>
<td>0%–1% consider reducing dose</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neutrophilic bronchitis</th>
<th>TCC &lt;25×10^6/g, N&gt;80%</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCC 25×10^6/g–25×10^6/g, N&gt;80%</td>
<td>Consider antibiotics</td>
</tr>
<tr>
<td></td>
<td>TCC &lt;10×10^6/g OR isolated N&gt;80%</td>
<td>No antibiotics</td>
</tr>
<tr>
<td></td>
<td>TCC &gt;60×10^6/g, N&gt;80% bronchiectasis</td>
<td></td>
</tr>
</tbody>
</table>

Recheck sputum 4–6 weeks after treatment change
Add LABA after controlling bronchitis
Same strategy for COPD, hemosiderin in Mito to assess LVEDP
Same strategy for chronic cough, lipid in Mito to assess reflux

COPD: Chronic obstructive pulmonary disease; EFG: Eosinophil-free granules; LABA: Long-acting beta-agonist; LVEDP: Left ventricular end-diastolic pressure; Mito: Macrophages; N: Neutrophils; TCC: Total cell count.

Exhaled nitric oxide
This measurement, which is a reflection of the activity of the nitric oxide synthase enzyme, is perhaps the most widely used assessment of airway inflammation in clinical practice today. A United States Food and Drug Administration-approved device (Niox, Aerocrine, Sweden) is available commercially, and the measurement is very well standardized and normal values have been established (22). However, it does not help to identify the cellular nature of airway inflammation associated with exacerbations of airway diseases, particularly in patients who are already being treated with corticosteroids. Thus, it may be a predictor of steroid responsiveness, but does not help to reduce asthma exacerbations (23) when used in clinical practice, except in patients with mild airway disease (24). It may be an alternative measurement in patients who are unable to produce sputum; however, this requires more investigation.

Exhaled breath measurements (mediators, pH, temperature)
Two commercially available devices (EcoScreen [Jaeger GmbH, Germany], RT tubes [Respiratory Research, USA]) are widely used to collect breath condensate. An array of cytokines, mediators and growth factors have been measured that appear to discriminate, with a reasonable degree of accuracy, between healthy and diseased states (25). Although breath condensate pH, which considers the relationship among airway acidification, buffering capacity and inflammation, is the most rigorously validated measurement and may provide some information regarding airway inflammation (i.e., low pH representing an inflamed airway), it is not yet ready to be useful for monitoring therapy and anticipating inflammatory lung disease exacerbations in individual patients. However, this measurement holds promise, with the primary advantage being that it is totally noninvasive and can be applied to children. Temperature measurement in exhaled air, which is a reflection of mucosal blood flow, is also now possible with a commercially available instrument (X-halo, Delmedica, Singapore). While it has the advantage of being easy to use and may discriminate among healthy volunteers and patients with airway diseases (26), it is too early to apply to clinical practice to monitor disease, adjust medications or discriminate among severities of bronchitis.

Metabolomics in breath and in urine
The analysis of volatile organic compounds in exhaled breath using gas chromatography and mass spectrometry (electronic-Nose) is a novel, attractive and completely noninvasive technology that is currently being evaluated to assess airway inflammation. Volatile compounds that are identified are analyzed using statistical methods, such as principal component analysis, using a systems biology approach to recognize patterns consistent with physiological or pathological abnormalities. It remains a research tool that has shown that it can discriminate between eosinophilic and neutrophilic bronchitis in patients with asthma and with COPD (27). The technology is currently undergoing evaluation for possible application in routine clinical practice.

A similar approach is being developed to analyze metabolites in urine. Urine metabolites, analyzed using nuclear magnetic resonance spectroscopy, discriminate between children with asthma and those with other obstructive airway diseases, including infective bronchitis and pneumonia (28). This technology is particularly attractive because it can be applied in subjects as young as one day of age because urine can be obtained from diapers or catheters. It remains to be determined whether a metabolomics approach to measurements in breath condensate or in urine would be useful tests that can be applied routinely in clinical practice.

Clinical practice in Hamilton
Spontaneous sputum is collected from patients with airway diseases (eg, chronic cough, COPD, asthma, bronchiectasis) at the time of initial assessment and at the time of every exacerbation. In practical terms, these are patients who are usually already being treated with moderate to high doses of inhaled corticosteroids and a long-acting beta-agonist. They fall into one of three categories: patients who require daily or frequent courses of prednisone; patients who experience recurrent ‘bronchitis’; and patients who experience frequent ‘exacerbations’. If the patient is not able to produce sputum spontaneously, it is induced by the inhalation of hypertonic saline in the pulmonary function laboratory as a routine, scheduled test. Pre- and post-salbutamol spirometry are recorded, and the patient subsequently inhales 3%, 4% and 5% saline solutions, each for 7 min, delivered through a low-output ultrasonic nebulizer with a particle size of approximately 3 to 4 mass median aerodynamic diameters. FEV1 is measured after each concentration is inhaled and the flow-volume curves are carefully inspected for evidence of vocal cord dysfunction. If the FEV1 drops by 15%, salbutamol is administered and the time taken for the FEV1 to return to preinduction baseline is recorded. Failure of salbutamol to protect against saline-induced bronchoconstriction and the refractoriness to bronchodilation are interpreted as indicators of possible tolerance to the bronchoprotective effects of beta-agonists (or moderate airway hyper-responsiveness) and increased mast cell activity, respectively. Sputum is transported to a combined clinical/research laboratory that operates under the Hamilton Regional Laboratory Medicine program, where it is processed within 15 min to 20 min and a result is made available within 2 h. The spirometry and sputum cell counts are captured in a database (approximately 12,000 to date) and the results (with a clinical interpretation) are made available to the referring physician at the end of the day. Patients are then telephoned the same evening or the next day with recommendations based on the sputum cell counts (Table 1). The same approach is now being evaluated to treat rhinitis based on processing of cell counts in blown nasal secretions (29).

CONCLUSIONS
Clinical guidelines and regulatory agencies have begun to recognize the importance of measuring bronchitis to optimize the management of airway diseases and to guide anti-inflammatory therapies. Of the currently available methods, quantitative cell counts in sputum and exhaled nitric oxide are well validated and recommended by professional organizations for clinical use. Sputum cell counts have the advantage of providing information about the cellular nature of...
TABLE 2
Information obtained from the process of sputum collection and examination

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum induction</td>
<td>Helps design protocols to investigate types of bronchitis</td>
</tr>
<tr>
<td>Airflow obstruction</td>
<td></td>
</tr>
<tr>
<td>Vocal cord dysfunction</td>
<td></td>
</tr>
<tr>
<td>Hyper-responsiveness</td>
<td></td>
</tr>
<tr>
<td>Tolerance to bronchodilators</td>
<td></td>
</tr>
<tr>
<td>Sputum examination</td>
<td></td>
</tr>
<tr>
<td>Infection: Bacterial versus nonbacterial</td>
<td></td>
</tr>
<tr>
<td>Eosinophilic bronchitis</td>
<td></td>
</tr>
<tr>
<td>Microaspiration</td>
<td></td>
</tr>
<tr>
<td>Left ventricular dysfunction</td>
<td></td>
</tr>
<tr>
<td>Possible postnasal drip</td>
<td></td>
</tr>
<tr>
<td>Environmental exposures, cigarette smoking</td>
<td></td>
</tr>
</tbody>
</table>

Clinical inflammometry for the management of airway diseases

**TABLE 3**
Some common misconceptions about sputum cell counts

1. **Sputum should always be induced**
   - No, spontaneous sputum can be used for reliable cell counts
   - Only a single examination is needed
   - Only in severe patients not responding to usual therapy or exacerbating frequently

2. **Sputum eosinophilia diagnoses asthma**
   - It does not. It simply identifies the bronchitic component of an airway disease
   - No, because the nature of inflammation changes over time, sputum must be examined at each exacerbation

3. **It is all about eosinophils**
   - Sputum neutrophil count provides valuable information about the presence of infective bronchitis

4. **Selection method**
   - Helps design protocols to investigate types of bronchitis

5. **Sputum needs to be examined in all patients with airway diseases**
   - No, only in severe patients not responding to usual therapy or exacerbating frequently

medications for the treatment of asthma or COPD in decreasing exacerbations and hospitalizations (32), and because the majority of physicians and patients consider them useful and necessary (33), we are doing our patients with severe asthma and COPD a disservice by delaying the introduction of sputum cell counts in clinical practice.

DISCLOSURES: Dr Nair is supported by a Canada Research Chair in Airway Inflammometry. This review is a reflection of more than 20 years of research program in Hamilton pioneered by Dr Jerry Dolovich and Dr Freddy Hargreave, and the many clinical fellows who trained under their supervision. The author is listed on a patent for a ‘biological fluid filtration device’ and has provided scientific consultation to Cellometrics Inc.

REFERENCES


