Noninvasive Monitoring of Airway Inflammation

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Airway inflammation is central to the pathogenesis, exacerbation and persistence of asthma and other airway conditions. Asthma is a disease characterized by airway inflammation, variable airflow limitations, airway hyper-responsiveness, lung function impairment and the presence of symptoms such as dyspnoea, wheezing, chest tightness and cough. Airway inflammation is considered to be the cause of the symptoms and physiological abnormalities of asthma and guidelines suggest that it should be the primary target for treatment. Assessment of inflammation and oxidative stress in lung is important in clinical management of not only asthma, but other lung diseases like COPD, cystic fibrosis and interstitial lung disease. Ongoing inflammation can cause irreversible structural changes such as epithelial damage, sub-epithelial basement membrane thickening, increased vascularization, myofibroblast proliferation and hypertrophy and hyperplasia of smooth muscle. Prevention and treatment of airway inflammation is therefore the mainstay of management. It may also allow the clinician to assess the efficacy of anti-inflammatory or anti-oxidant treatment.

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 measurement of symptoms, spirometry, peak expiratory flow, tests of hyperresponsiveness or peripheral blood inflammatory markers. Bronchoscopy with tissue diagnosis remains the gold standard for assessment of airway inflammation, but is limited by discomfort to the patient, risk of procedure and expense. The indirect measures relate variably with each other and reflect more than airway inflammation, for which they are non-specific.

Recently, a variety of non-invasive approaches, such as exhaled breath analysis (exhaled gases and condensate) and induced sputum have been developed, which are changing the understanding about the speed of action of drugs (especially corticosteroids) and their effects in asthma.

SPUTUM INDUCTION

Sputum has been used for more than a hundred years to characterize airway diseases.

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 is known to be altered in asthma, showing an increase in eosinophils. Initially, sputum cells were examined on stained smears and it was for a brief period in clinical practice in the 1950s, 60s and 70s. 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 the 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 as proposed first by Pin et al in 1992. Today, the method is considered to be reliable, valid, responsive and reproducible. The inflammatory findings correlate best with bronchial washings and more variably with bronchial biopsies and BAL. Both induced sputum and bronchial washings reflect secretions from the more central airway lumen, BAL from the peripheral lumen and bronchial biopsies from the central airway walls. Sputum eosinophils are more sensitive and specific for the presence of asthma than blood eosinophils or serum eosinophilic cationic protein (ECP) or the more recently introduced analysis of non-respiratory gases in exhaled air such as nitric oxides.

METHOD OF SPUTUM INDUCTION

Hypertonic saline (3-5%) has been used for sputum induction, as isotonic saline has been shown to result in a lower success rate. However, in two studies on severe asthma, induction with normal saline has been better tolerated. Thus, in patients with severe airway disease, it is advisable to start with isotonic saline and to continue with hypertonic saline only if this is not successful. The methods of sputum induction have been reviewed in detail elsewhere. After each inhalation, the patient is asked to cough and try to expectorate into a universal container. Safety is of prime concern. A β2 agonist is inhaled first to inhibit any bronchoconstriction caused by inhalation of the saline aerosol and the forced expiratory volume in first second (FEV1) is monitored before and after each inhalation. In addition, if the baseline value is <70%, the inhalations are started with normal saline and given for shorter durations, if the FEV1 falls during the induction by 10-19%, any further inhalations are given cautiously, and if it falls by 20% the procedure is discontinued and a bronchodilator may be prescribed.

The procedure can be performed safely, even in patients with exacerbation of asthma or moderate chronic airflow limitation provided appropriate precautions are taken. Measures are also taken during the procedure to prevent contamination with post-nasal drip and to limit salivary contamination by blowing the nose, rinsing the mouth with water and swallowing the water before expectoration. The expectorated sputum is a mixture of sputum and saliva. The procedure can be successful in >80% of adults and older children who cannot produce spontaneous sputum.

Accurate sputum analysis requires that the specimen is examined fresh within ~2 hrs. Two methods were recently suggested to overcome the problems, that storage of sputum could cause a lower quality of cytospins and a change in sputum composition. One method consists of simultaneous homogenization and fixation of freshly expectorated sputum with Saccomanno’s fixative plus 0.2% dithiothreitol (DTT) in order to enable longer storage. This method is currently used for automated sputum cytometry. The second and more promising approach was reported by Papov et al who demonstrated that the freezing of cells using dimethylsulfoxide to avoid water crystallization does not impair cellular morphology to an extent that effects the differential cell count. Preliminary data shows a good correlation between frozen samples and corresponding freshly prepared aliquots.

Processing of samples can be done by two methods. In one, salivary squamous contamination can be reduced further by pouring the expectorate into a petridish and selecting all of the more opaque or dense portions, as little as 50g is sufficient. In the other method, the whole expectorant of sputum and saliva is processed together. In both the methods the sputum is treated with 0.1% DTT 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 non-squamous cell count and viability (with Trypan blue) are determined in a haemocytometer. Cytospins are made and stained and differential count is performed.

OBSERVATIONS OF SPUTUM CELL COUNTS RELEVANT TO CLINICAL PRACTICE

Types and Causes of Inflammation

In normal healthy subjects, there is predominance of macrophages and neutrophils with few eosinophils
and lymphocytes. Different types of abnormalities of differential cell count indicate different causes of various airway inflammations.

**SPUTUM EOSINOPHILIA**

An increase in sputum eosinophils is known to be characteristic of asthma. It can be induced by inhaled allergen or chemical sensitizers in sensitized subjects, and can also be increased by reducing the steroid dose in corticosteroid-dependent asthma. In occupational asthma too, it can help in investigations by serial measurement during periods at work and away from work. There is an increase in eosinophils at work followed by a fall away from work.

Eosinophilic bronchitis without asthma was a condition recognized by sputum cell counts. The condition was seen in patients presenting with chronic cough who had normal spirometry and normal airway response to challenge. The sputum eosinophils and chronic cough were reversed by corticosteroid treatment. The condition is encountered in 10-15% of patients presenting with chronic cough. It can be transient or persistent unless suppressed by steroid treatments. If untreated, it can progress to asthma or progressive airflow limitation without airway hyper-responsiveness. Thus it is important to identify the condition early and treat it.

**SPUTUM NEUTROPHILIA**

Cigarette smoking, pollutants such as ozone, endotoxins and infections are known causes of sputum neutrophilia. The intensity of neutrophilia is most pronounced in bacterial infections. Occupational neutrophilic asthma has also been described.

**INFLAMMATION AND TREATMENT**

The differentiation of inflammation into eosinophilic and neutrophilic is important with respect to the treatment. In patients presenting with either chronic cough or asthma or chronic airflow limitation, the presence of sputum eosinophilia predicts a positive response to steroid treatment. Neutrophilic treatment usually indicated resistance to added steroid treatment. To monitor the steroid treatment too, sputum eosinophils can be measured serially till they are in normal range. Persistent sputum eosinophilia in patients with “difficult asthma” should raise the suspicion of under-treatment or patient non-compliance.

Smoking related chronic airflow limitation is usually associated with sputum neutrophilia which is not responsive to corticosteroid treatments. Some smokers however have a concomitant sputum eosinophilia that is responsive to corticosteroid treatment. In a single blind, sequential cross-over trial of placebo and prednisone (30mg/d) each given for two weeks, in which sputum measurement was double blind, improvement in clinical and inflammatory variables with prednisone were observed only in those patients with sputum eosinophilia of at least 3%.

Long acting β2-agonists significantly improve symptoms and pulmonary function, however, they do not reduce sputum eosinophils and indeed can mask them. McIvor et al demonstrated that salmeterol controlled symptoms and lung function in asthmatics undergoing steroid withdrawal allowing development of sputum eosinophilia without exacerbation of clinical symptoms.

Leukotriene antagonists’ anti-inflammatory properties have been studied using bronchoscopy and confirmed by induced sputum. In a double blind, randomized, controlled, parallel-group study over 4 weeks, montelukast, improved clinical outcome and reduced sputum eosinophil count.

**OTHER CELLULAR ABNORMALITIES**

The demonstration of lipid-laden macrophages in induced sputum can be an indicator of gastric content aspiration. BAL examination for lipid-laden macrophages has earlier been suggested as a useful diagnostic test for aspiration. Recently, induced sputum examination for lipid-laden macrophages was shown to have high sensitivity (89%) for oropharyngeal reflux measured by 24-hours dual channel esophageal pH monitoring, for an alveolar macrophage lipid index of 7.0. Lipid index is a composite score derived from counting 100 consecutive macrophages, grading the intracellular lipid from 0 (no lipid) to 4 (many intracellular oil red O stained lipid droplets). After adding up the score of...
each cell the lipid index can range from 0-400.

Left ventricular dysfunction if suspected in a breathless patient, can be confirmed by presence of hemosiderin-laden macrophages (HLM) in induced sputum. HLM more than 2% had a sensitivity of 80% and specificity of 94% and positive predictive value of 96% for presence of left ventricular dysfunction confirmed by 2D echocardiographic criteria\textsuperscript{58}. More studies are required to investigate the role of these counts in patients with chronic airflow limitation with dyspnoea to rule out concomitant cardiac disease with left ventricular dysfunction.

Involvement of lungs in storage disorders like Fabry’s disease have been successfully confirmed using induced sputum examination. Typical lamelliar inclusion bodies within bronchial epithelial cells were demonstrated in sputum using electron microscopy\textsuperscript{59}.

D’ppolito et al studied induced sputum in patients with newly diagnosed untreated sarcoidosis and compared it with bronchial wash and BAL. They found induced sputum comparable to bronchial washings or BAL to demonstrate lymphocytosis\textsuperscript{60}. Initially, induced sputum was developed for cyto-diagnosis of lung cancer. But this role has been superseded by bronchoscopy.

**SPUTUM EOSINOPHIL CATIONIC PROTEIN IN ASTHMA**

With the activation and/or lysis of eosinophis, there is release of eosinophil granule proteins such as ECP\textsuperscript{61}. Sputum supernatant ECP level is used as an index of eosinophil degranulation. Reported level of sputum ECP vary greatly depending on procedures and subject selection. There is a 2-3 fold increase in sputum ECP levels in stable asthma. Extremely high levels (>1000µg/L) have been reported during acute severe asthma\textsuperscript{62}, suggesting intense eosinophil degranulation. The levels have been found reduced with steroid treatment and reduction of asthma symptoms\textsuperscript{63}.

Gibson et al chose a different, approach to simplify sputum analysis. They used the cellular component by lysing the cell pellet after homogenization and measured the amount of ECP as a marker of the number of eosinophils in sputum\textsuperscript{64}. The method has the advantage of being automated and hence cheaper. They found a good correlation between the number of eosinophils and the concentration of ECP in the cell lysate. This concept, however, requires further validation.

**PROBLEMS WITH SPUTUM INDUCTION**

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 time and cost of the procedure. At present, reliable measurements are only available to clinical practice in a few centers, where they are established in research. The process of induction is usually performed by pulmonary function technologists and precautions taken are similar to challenge tests.

**EXHALED NITRIC OXIDE**

Recent studies have revealed a good correlation between exhaled nitric oxide (NO) and the level of bronchial hyper-responsiveness as well of the number of eosinophils in the sputum of asthmatics\textsuperscript{65-66}, thus suggesting the potential use of these investigations in the monitoring of asthma severity. Piacentini et al found good concordance between exhaled nitric oxide and sputum eosinophil count in evaluating the degree of airway inflammation in patients with mild to moderate asthma\textsuperscript{67}. However, sputum induction can not be used for day to day monitoring, as it provokes transient neutrophilia\textsuperscript{68}.

The presence of endogenous nitric oxide in exhaled breath of animals and humans was first described in 1991\textsuperscript{69}. Soon after, several publications reported high levels of orally exhaled NO in subjects with asthma as compared with unaffected subjects\textsuperscript{70-72} and a fall in these high levels after treatment with steroids\textsuperscript{73}. Exhaled NO has been validated against invasive measurement of inflammation by bronchoscopy and induced sputum in asthma. The inflammatory origin of exhaled nitric oxide and accumulating evidence of its association with asthma severity make NO an effective and practical marker to monitor the effect of corticosteroids and other anti inflammatory treatments in asthma or any other lung disease.
**TECHNICAL FACTORS AFFECTING EXHALED NITRIC OXIDE MEASUREMENT**

Exhaled NO is usually measured by chemiluminiscence analysers based on the photochemical reaction between NO and ozone generated in the analyser. The method has been validated against gas chromatography-mass spectrophotometry.

In summary, exhaled NO may be measured on-line using a manoeuvre to close the soft palate and avoid nasal gas contamination of the sample. If ambient NO concentration is high, then breathing NO-free air is advised. An alternative method is to use a gas impermeable bag and measuring later by the same technique74,75.

A few technical factors should be considered while monitoring asthma using exhaled NO Low exhalation or sampling flow rate and breath holding can give erroneous high exhaled NO levels. Decreased levels may be seen with high sampling flow rate and spirometric manoeuvres.

The physiological and pathophysiological conditions affecting exhaled NO levels are enumerated in Table I.

The European Respiratory Society guidelines on exhaled and nasal NO measurement were established in 1997, and recently the recommendations for standardized procedures for on-line and off-line measurement in both adults and children have been published76.

**Table I. Physiological and Pathophysiological Factors Affecting Levels of Exhaled Nitric Oxide**

<table>
<thead>
<tr>
<th>Increased Levels</th>
<th>Decreased Levels</th>
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<tbody>
<tr>
<td>Allergen exposure</td>
<td>Menstruation</td>
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<tr>
<td>Air pollution</td>
<td>Smoking</td>
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<tr>
<td>Occupational exposure</td>
<td>Alcohol ingestion</td>
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<tr>
<td>Asthma</td>
<td>Excess mouth washing</td>
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<tr>
<td>Unstable/ severe COPD</td>
<td>Pulmonary Hypertension</td>
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<tr>
<td>Allergic rhinitis</td>
<td>Kartagener’s syndrome</td>
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<tr>
<td>URTI</td>
<td>Primary ciliary dyskinesia</td>
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<tr>
<td>Bronchiectasis</td>
<td>Cystic fibrosis</td>
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<tr>
<td>Tuberculosis</td>
<td>Sarcoidosis</td>
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**USE OF EXHALED NITRIC OXIDE TO MONITOR ASTHMA**

Exhaled nitric oxide has been shown to significantly fall or even get normalised whilst other markers of airway inflammation (e.g. sputum eosinophils) are still high. It is also the first marker to rise during asthma deterioration suggesting it to be used as a loss of control indicator77.

In patients with severe persistent asthma (despite treatment with high dose steroids)78 or in acute asthma the levels of exhaled nitric oxide are found to be increased. The levels also rise when maintenance dose of corticosteroids is reduced77. During acute exacerbation the levels are better indication than serum levels of ECP or interleukins79.

In children too, these observations have been extended Exhaled nitric oxide appears to be related to bronchial responsiveness and shows little correlation with isolated spirometric measurements or acute changes in FEV₁80.

A good correlation has been found between symptom frequency, β₂ agonist use and the levels of exhaled nitric oxide. The levels are significantly higher in patients with difficult/severe asthma having highest symptom score where changes in lung function may have limited sensitivity79.

The patients of asthma in whom the level of exhaled nitric oxide remains persistently elevated despite corticosteroid treatment, the use of an alternative type of therapy for example, iNOS inhibitors, anti-oxidants or leukotriene antagonists may be considered76,81.

**ROLE OF NITRIC OXIDE IN SCREENING**

Exhaled nitric oxide levels are elevated not only in asthma71,72 but some other airway diseases too. Inspite of that, nitric oxide measurement may be useful in screening for asthma and atopy.

Exhaled NO is increased in both asthma and atopy82,83, and the levels are known to rise with conditions known to increase inflammation in these patients, such as allergen Provocation challenge84, pollen season85, animal allergy82, etc. In allergic rhinitis, Henriksen et al found exhaled NO to be elevated even in non-pollen season with a further rise.
in pollen season\textsuperscript{86}. In such cases it reflects airway inflammation and an increased risk for developing asthma. The levels of exhaled NO are higher in atopic asthmatics than non-atopic asthmatics\textsuperscript{77,87}. LP Ho \textit{et al} found a correlation between exhaled nitric oxide and atopy in asthmatics using skin test scores and total IgE levels\textsuperscript{88}.

**EFFECTS OF TREATMENT ON EXHALED NITRIC OXIDE ASTHMA**

**Oral Steroids**

Yates \textit{et al} found decrease in levels of exhaled Nitric oxide in asthmatic patients on oral prednisolone (30mg/dX3d) with no effect on exhaled NO in normal subject\textsuperscript{76}. This could be explained on the basis of inhibition of inducible NOS in asthmatics. The constitutive NOS in normal subjects does not get suppressed by steroids. In asthmatics NOS is the major source of exhaled NO. Yates \textit{et al} found a significant (22\%) reduction in exhaled NO with prednisolone within 72 hrs in mild asthma. A high dose of methylprednisolone (180-500mg) caused a 36\% fall within 50 hrs during exacerbation of asthma\textsuperscript{76}.

**INHALED CORTICOSTEROIDS**

Exhaled nitric oxide has been found to be a very sensitive and rapid marker of the effect of corticosteroid treatment especially inhaled ones. Kharitinov \textit{et al} observed a significant reduction in exhaled NO 6 hrs after a single dose (8mg) of nebulized budesonide in symptomatic moderate asthma\textsuperscript{88}. Jatakanon \textit{et al} reported dose-dependent changes in NO during 3-week treatment with Budesonide in mild asthma\textsuperscript{89}. Kharitinov \textit{et al} showed that the onset of action of inhaled budesonide on exhaled NO and the time to reach maximum reduction are also dose-dependent\textsuperscript{90}.

The effect of inhaled corticosteroids may be direct via inhibition of transcription factors activity (NF-kB)\textsuperscript{91} or inflammatory cytokmes or indirectly by inhibiting the recruitment of inflammatory cells\textsuperscript{92}.

**INHALED P2 AGONISTS**

No effect of short-acting β2 agonists (salbutamol) or long-acting ones (salmeterol) has been found on exhaled NO\textsuperscript{93}. This is consistent with the fact that inhaled (32 agonists do not have any role to play in chronic inflammation in asthma. Thus exhaled NO can be used to measure inflammation independent of airway calibre in patients using β2 agonists.

**LEUKOTRIENE ANTAGONISTS**

Leukotriene antagonists like Pranalukast and Montelukast have been studied to test the effect of inflammatory cytokines that induce iNOS in eosinphils\textsuperscript{94,95}. Pranalukast inhibits the rise in exhaled NO expected during corticosteroid dose reduction\textsuperscript{94}. Montekulast has been tried in childhood asthma where Bratton \textit{et al} reported a rapid reduction of exhaled NO\textsuperscript{96}.

**INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITORS, PROSTAGLANDINS AND OTHER DRUGS**

Inducible NOS inhibitors\textsuperscript{84,85} and Prostaglandins\textsuperscript{97} are still under clinical research but have the potential of being used in management of severe asthma where combination of airway inflammation and oxidative stress together with resistance to steroids make their treatment difficult.

**EXHALED NITRIC OXIDE AND OTHER LUNG DISEASES**

**Cystic Fibrosis**

Measurement of exhaled NO levels in cystic fibrosis (CF) can be of diagnostic importance. Significantly low levels of NO in both exhaled and nasal air has been reported in patients of CF\textsuperscript{98,98}. Kelley \textit{et al} observed loss of expression of iNOS leading to decreased NO production in CF murine model as well as in human epithelial cells\textsuperscript{100}. Meng suggested the possibility of increased susceptibility to infection in CF due to lack of iNOS in bronchial epithelium\textsuperscript{101}. It has been found that the increased oxidative stress in the airways of patients with CF is secondary to pulmonary inflammation\textsuperscript{102,103}. The airway influx of neutrophils releases large amount of superoxide dismutase which converts NO to nitrate, leading to reduced levels of NO\textsuperscript{104}. The total sputum nitrite and nitrate levels have been found to be raised during acute pulmonary infections in CF and they do not respond to steroids or antibiotics\textsuperscript{105}.
Thus the dramatic reduction of iNOS expression in airway and nasal epithelium in patients with CF hinders an important first-line defense mechanism and increases the susceptibility of airway to infection.

**CHRONIC OBSTRUCTIVE PULMONARY DISEASE**

The usefulness of exhaled NO measurement in assessing the inflammation in COPD is not established. In patients of stable disease, the levels are found to be normal\textsuperscript{106,107}. The lack of elevation may be due to several reasons including cigarette smoking and oxidative stress. The levels are found to be lower in smokers than in non-smokers\textsuperscript{106,107}. Su Y\textit{ et al} suggested a role of cigarette smoke extract in inhibiting NOS\textsuperscript{110}. Another possibility is the conversion of NO produced to peroxynitrite by neutrophils\textsuperscript{104}.

However, exhaled NO does play a role in identifying patients with severe, unstable COPD, having elevated NO levels compared to stable patients\textsuperscript{107,111}. This may be explained by an exaggerated neutrophilic inflammation, oxidant/antioxidant imbalance and microbial infection in more severe patients with COPD.

**BRONCHIECTASIS**

Exhaled NO has been found increased in bronchiectasis and the level being proportional to extent of disease\textsuperscript{112}. Exhaled NO measurement plays an important role in identifying patients of bronchiectasis with acute infective exacerbations where levels rise further. In clinically stable patients the levels are not elevated. This may be due to NO getting trapped in viscous airway secretions or getting removed by reacting with reactive oxygen species.

Another role of NO measurement in bronchiectasis is to identify patients with cystic fibrosis and primary ciliary dyskinesia whose NO levels are abnormally low\textsuperscript{113}.

**PRIMARY CILIARY DYSKINESIA**

It is a genetic disorder characterized by defective motility of cilia and male infertility. Its another example of abnormally low nasal NO which is helpful in diagnosis\textsuperscript{113}. Lack of endogenous NO production might also account for recurrent chest infections.

Treatment with NO donor (L-arginine)\textsuperscript{114} has been found to improve mucociliary transport in these patients\textsuperscript{115}. Thus measurement of exhaled NO in PCD is not only diagnostic but also a therapeutic target.

**FUTURE CONSIDERATIONS**

In recent years, the method of sputum induction has been adopted by a large number of research groups. The basic protocols have been modified in multiple respects and the procedures used by different research groups vary considerably in details. The European respiratory Society (ERS) Task Force was formulated with these problems in mind and will soon provide a review as well as recommendations concerning the induction protocol, safety aspects, processing and analysis of sputum sample. This will be the first step in standardization of the process, allowing comparability between the results of different groups.

At present, the number and percentage of eosinophils remain the most important information gathered from sputum analysis, particularly in asthma and COPD. During infection, the total cell count and proportion of neutrophils can be helpful in assessments.\textsuperscript{9} Lymphocytes have seldom been used as a marker except in patients of sarcoidosis.\textsuperscript{60} However, in future, analysis of lymphocytes by flow cytometry yielding their subtypes and cytokine production may be useful. The components of fluid phase of sputum (sputum supernatant) have been shown to be reproducible and responsive markers of the state of airway disease. Despite this, their value appears limited in clinical practice. Regarding the usefulness of sputum induction in clinical practice, three different time scales have to be considered. The first refers to a single, one-time application of sputum induction in diagnosis of airway disease and decision on initial therapy or repeated use in monitoring of inflammation and control of treatment or use of sputum induction to predict the long-term outcome of disease. Controlled studies are required to actually establish the extent to which knowledge of type and degree of airway inflammation from induced sputum is useful for diagnosis and treatment of patients.

Measurement of markers in exhaled air is totally non-invasive and can be performed even in infants.
Also, the on-line analysis of NO can provide immediate results. However, the NO analysers are still expensive. It has been demonstrated in several studies that level of exhaled NO and percentage of sputum eosinophils correlate quite well. Thus it seem to indicate the presence of an eosinophilic airway inflammation. However, the pathophysiologic pathways involved are different and there may be circumstances in which both parameters dissociate.

Currently, the measurement of markers in breath condensate is a rapidly expanding field. One of the most commonly studied marker is hydrogen peroxide, which has been shown associated with airway inflammation in COPD and asthma, however its measurement seems to be hampered by yet unknown intrinsic factor resulting in poor reproducibility. Other markers being studied are protein and lipid mediators. There are no published data on the reproducibility of these markers.

In summary, the methods of sputum induction and the measurement of exhaled NO represent two techniques, that have been extensively applied in clinical research and have the potential to be incorporated into future clinical practice. Their future use will largely depend on a favorable cost: benefit ratio. Thus in near future, these methods may continue to be restricted to specialized research centers which already have the equipment and expertise.

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