Eur Respir J 2000; 16: 1175–1179
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European Respiratory Journal
ISSN 0903-1936

SERIES "NONINVASIVE MONITORING OF AIRWAY INFLAMMATION"
Edited by H. Magnussen and F.E. Hargreave
Number 6 in this Series

Noninvasive methods to measure airway inflammation: future considerations

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Noninvasive methods to measure airway inflammation: future considerations. H. Magnussen, O. Holz, P.J. Sterk, F.E. Hargreave. ©ERS Journals Ltd 2000.

ABSTRACT: This last contribution to the series focuses on open questions regarding: 1) methodological issues; and 2) the potential clinical application of the noninvasive methods such as induced sputum and the analysis of exhaled air for the assessment of airway inflammation. In addition their potential future role in occupational health and the early diagnosis of neoplastic lesions are briefly discussed.

The future clinical application of noninvasive methods will depend on the progress made to improve their practicability, particularly in rendering them less time consuming and cheaper. To assess their clinical value, prospective studies are needed to establish whether patients actually benefit from the results obtained. This is also important to implement the methods into the healthcare system and to obtain adequate financial compensation.

Therefore, it is necessary to know: 1) whether the assessment of airway inflammation can aid in coming to an earlier and better defined diagnosis; 2) whether by repeated monitoring it is possible to avoid exacerbations through earlier interventions; and 3) whether the long-term outcome of patients is improved through knowledge of the type and degree of airway inflammation that is taken into account in selecting the appropriate treatment.

In the meantime a wealth of data has become available, both for induced sputum and the analysis of exhaled air, which give these methods the potential to be incorporated into future clinical practice. This, however, will, amongst the other issues, depend on favourable cost-benefit ratios which should also be the subject of future prospective studies.

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Keywords: Breath condensate
clinical application
cost-benefit-ratio
induced sputum
nitric oxide

Received: October 16 2000
Accepted: October 26 2000

This is the final contribution to the series on methods for the noninvasive assessment of airway inflammation, which appeared in the last five issues of the *European Respiratory Journal* (ERJ). The series has mainly focussed on the best current validated methods of the techniques of sputum induction and the determination of exhaled NO. Both methodological issues and potential clinical applications were covered in the consecutive articles. Here the authors will discuss methodological issues that remain open and consider the prospects of these methods regarding their future use in clinical practice.

Induced sputum

Induction and processing

In recent years, the method of sputum induction has been adopted by a large number of research groups. Basically two protocols describing the procedure of induction and subsequent processing of samples have been proposed [1, 2]. These protocols have been modified in multiple respects subsequently, and the procedures used by different research groups vary considerably in detail. As a consequence, a European Respiratory Society (ERS) Task Force was inaugurated, which will soon provide a review as well as recommendations concerning the induction protocol, safety aspects, processing and analysis of sputum samples to be published in the ERJ early 2001. This will be the first step towards a standardization of the methods, which seems particularly important for maximal comparability between the results of different research groups.

To further improve the practicability of the method, however, additional efforts are necessary, in particular regarding a shortening of the procedure. This improvement appears to be a prerequisite for a broader use either in

Previous articles in this series: No 1: L. Jayaram, K. Parameswaran, M.R. Sears, F.E. Hargreave. Induced sputum cells counts: their usefulness in clinical practice. *Eur Respir J* 2000; 16: 150–159. **No. 2:** O. Holz, J. Kips, H. Magnussen. Update on sputum methodology. *Eur Respir J* 2000; 16: 335–359. **No. 3:** R.A. Jörres. Modelling the production of nitric oxide within the human airways. *Eur Respir J* 2000; 16: 555–560. **No. 4:** S.A. Kharitonov, P.J. Barnes. Clinical aspects of exhaled nitric oxide. *Eur Respir J* 2000; 16: 781–792. **No. 5:** P.G. Gibson, R.L. Henry, P. Thomas. Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. *Eur Respir J* 2000; 16: 1008–1015.

standard clinical practice or in epidemiologal settings, when markers of inflammation are to be included into the analysis. At present the time needed for induction, processing, and assessment of total and differential cell counts amounts to approximately 1.5 h, rendering the method relatively time consuming and expensive.

In most instances, the costs for the clinical assessment of airway inflammation by sputum examination are not covered by health insurance, although in some countries it is possible to get at least part of them reimbursed by declaring the induction as an inhalation challenge. In Ontario, Canada, there is a technical fee, which can be charged for the induction, while the fee for the professional covers the cost of processing and analysis.

A second important problem is the need to process sputum samples shortly after induction, currently a maximum of 2 h for storage is recommended. This renders it difficult to batch-process samples or to send them to a central laboratory for analysis. It has been suggested that freezing of cells in dimethylsulphoxide (DMSO)-containing medium does not result in a significant deterioration of cell morphology or composition. Therefore, this approach might be suited to separate induction from processing [3, 4]. Alternative methods are also being tested for their feasibility for storing samples without effects on cellular composition and there is evidence to be presented on the American Thoracic Society (ATS) meeting 2001, that a storage time of up to 9 h between induction and processing at 4°C might be achieved.

Analysis of samples

From current knowledge, the number and percentage of eosinophils remain the most important and informative outcome parameters of sputum analysis, particularly in asthma and chronic obstructive pulmonary disease (COPD). During infections and in patients with COPD, also the total cell count per mL sputum and the proportion of neutrophils can provide relevant information [5]. Owing to their usually low numbers, lymphocytes have seldom been used as a marker, except in patients with sarcoidosis [6]. However, since these cells can be analysed by flow cytometry, yielding their subtypes and profile of intracellular cytokine production, their analysis might play a more important role in the future.

A number of components in the fluid phase of sputum, *i.e.* sputum supernatants, have been shown to be reproducible and responsive markers of the state of airway diseases, in particular within intervention studies. Despite these results, however, the value of these markers in clinical practice appears to be limited. This is partially due to difficulties in standardization and assessment of normal values, which are based on variable dilution by saline or saliva. It may also be due to the potential interference between immunoassays or other detection procedures and the mucolytic agent dithiotreitol (DDT), proteases [7, 8], and yet unknown formations of complexes between components of interest and, *e.g.* other proteins [9].

Clinical issues

Similar to the situation encountered at the introduction of flexible bronchoscopy and BAL in the 1970s, sputum induction is generally currently considered only as a research tool. In the meantime, however, the situation has changed for bronchoscopy and it may well be that in a few years sputum induction finds its way into clinical practice.

Regarding the usefulness of sputum induction, at least three issues playing on three different time scales have to be considered. The first issue refers to the single, one-time application of sputum induction in the diagnosis of airway diseases and the decision on the initial therapy. The second one refers to its repeated use in the monitoring of airway inflammation and the steady control of treatment, whereas the third comprises its potential to predict the long-term outcome of the disease. Unfortunately, however, there are no controlled studies available so far, which have actually established the extent to which knowing the type and degree of airway inflammation from induced sputum is useful for the diagnosis and treatment of patients with airway diseases.

Therefore, a worthwhile aim of future studies could be to determine: 1) whether the noninvasive assessment of airway inflammation adds significant information to that provided by history, lung function data and airway hyperresponsiveness; and 2) whether this results in a relevant improvement regarding the patient's diagnosis or the optimization of treatment. Unfortunately, there are no data available from large-scale controlled studies which demonstrate that patients benefit from a therapy that targets airway inflammation. Naturally, this would require repeated monitoring of airway inflammation which can be achieved only by noninvasive methods.

A closely related question is whether such a strategy improves the outcome to an extent which leads to a significant reduction of treatment costs. There is one well-known study that targeted corticosteroid therapy on the basis of airway hyperresponsiveness as a surrogate marker of structural and inflammatory alterations within the airways, in addition to symptoms and lung function [10]. This strategy resulted in a greater reduction of the number of mast cells in biopsies and in a more pronounced decline of reticular layer thickness. At the same time, larger improvements of peak expiratory flow (PEF) and forced expiratory volume in one second (FEV_1), as well as a lower exacerbation rate and lower airflow variability were observed, when compared to a conventional dosing strategy. Irrespective of the unresolved issue of the relationship between airway hyperresponsiveness and inflammation, these data suggest that it will be worth the effort to follow an analogous approach using more direct measures of airway inflammation as the target parameter. Such a study would have to be performed prospectively over a considerable period of time.

Therefore, to assess the clinical value of the method of induced sputum, more data are needed. Regardless of the difficulties to get the costs of measurements reimbursed, a number of centres have already implemented sputum induction into clinical practice. Some of the advantages of this approach have been described in Part I of this series by JAYARAM *et al.* [5]. This paper provides two figures which show in detail how the method of induced sputum can be well incorporated into the diagnostic decision tree for difficult asthma and chronic cough. Although the group of patients with these characteristics, in whom conventional methods do not lead to a firm diagnosis, is likely to be small, it is probable that patients benefit from the

analysis of induced sputum prior to the start of treatment, simply because the treatment can be chosen with a knowledge of the type and severity of any underlying inflammatory processes.

In addition, it seems to be reasonable to consider the currently available alternatives, when there is no access to facilities experienced in sputum induction, or when the induction is considered to be too time consuming and expensive. Patients, with for example persistent cough, and no obvious diagnosis resulting from the data on lung function and airway responsiveness, can be treated with inhaled corticosteroids over a short period of time, solely for diagnostic purposes. If there is no response, the treatment needs to be changed or adjusted, and the nonresponse is the only additional information that was not available at the beginning of the treatment. On the other hand, if symptoms improve, it is not known whether this occurred due to the treatment or to natural remission, although the latter case, admittedly, is less likely. But most importantly, the initial treatment and, even more relevant, its continuation is based on an educated guess and not on solid information describing the individual patient′s type and state of airway disease. Although the additional benefit of knowing type and degree of airway inflammation is not known yet, the assumption that it confers important information, is one of the leading "working hypotheses" that currently drive the cellular and biochemical research in asthma.

This line of reasoning suggests that there are two questions that have to be addressed in the evaluation of sputum induction. Firstly, on the basis of current knowledge, should we continue to diagnostically treat those patients with steroids, in whom no obvious diagnosis could be achieved by conventional means, or should we use the opportunity to assess inflammation noninvasively to learn about the underlying inflammatory process? Secondly, from a practical point of view, is a steroid trial in those difficult to diagnose patients actually adequate, more convenient and cheaper than sputum induction? These two points obviously also need prospective studies.

The answer to the first question involves the initiation of a debate on how basically to deal with airway diseases in future clinical practice. Unless studies actually demonstrate an additional benefit regarding the patient′s quality of life and prognosis as well as overall costs, health insurances are not likely to cover the costs for sputum induction. Currently a treatment trial with steroids for a 1-month period costs about Canadian $80. Based on this figure, the assessment of airway inflammation is equally expensive, when performed according to common protocols. On the other hand, it needs to be taken into account that valuable time has been lost in the case when the treatment did not improve the patient′s status.

In addition, there are patients who require only intermittent, rather than chronic, treatment. The open question is then, which criterium should be used for stopping the anti-inflammatory treatment in these patients. If it is the improvement in symptoms and lung function, it may well be that an underlying inflammatory process, which elicits subsequent exacerbations, is not recognized and properly treated [11]. It has already been shown that the increase in the number of sputum eosinophils can be considered as an early marker for asthma exacerbations [12]. Possibly regular monitoring would result in early interventions with increased doses of steroids, avoiding expensive hospital admissions and impaired quality of life due to full-fledged asthma exacerbations. This might result in an improved cost/benefit ratio in these patients.

Concerning clinically relevant applications of sputum induction in diseases other than asthma, so far there has not been much work. Regarding its potential usage in COPD, a particularly interesting question which needs to be examined is, whether patients with eosinophilia in sputum are the ones that respond to inhaled steroid treatment, as has been demonstrated for systemic steroids [13, 14]. Furthermore, a recent study has used the method to assess the bacterial colonization in the airways of patients with COPD [15]. Further issues of interest that need to be explored, include the question, whether sputum induction is helpful to distinguish between central (bronchitis) and peripheral (pneumonia) infections, or how bacterial colonization affects sputum composition in general.

When BICKERMAN *et al.* [16] introduced the procedure of sputum induction in 1958, it was first considered as a method that was potentially useful for the early detection of lung cancer. A number of studies have used spontaneous sputum to screen patients with a high risk for lung cancer [17, 18]. In the past tumour cells were primarily detected through their morphology. Nowadays specific polymerase chain reaction (PCR) or immunological methods are available to detect relevant mutations or tumour-specific proteins in sputum specimens [19–21]. Chances of detecting tumour cells or tumour markers in sputum seem to be highest in centrally located tumours [22]. As some of the newly developed methods could possibly be automated, they have the potential to play a significant role in future for the early detection of neoplastic lesions in high risk groups.

Sputum induction has been used successfully in the area of occupational health [23, 24] including the diagnosis of occupational asthma as well as the detection of workplace-related fibers or dust particles. Until now, biomonitoring studies which have looked for protein or DNA adducts produced after exposure to suspected genotoxic agents, were mainly limited to the analysis of the peripheral blood [25]. Using induced sputum it is now possible to assess alterations in cells that are derived directly from the airways or lung, *i.e.* exposed organ [26]. Indeed, there are first reports on the use of sputum in this kind of quest. Owing to the higher sensitivity which can be expected by analysis of airway cells, occupational and environmental health studies are likely to include induced sputum more frequently in the future.

Markers in exhaled air and breath condensate

The measurement of markers in exhaled air is totally noninvasive and can be performed even in infants [27]. In addition, the analysis of exhaled nitric oxide (NO) can be performed on-line, with an immediate read out of the results, making this method fast and versatile. Unfortunately, however, NO analysers are still expensive. Despite this, it has been a great accomplishment that meanwhile the method has been fairly standardized in guidelines published by the ERS [28] as well as the ATS [29].

Several studies have demonstrated that the level of exhaled NO and the percentage of sputum eosinophils

correlate quite well. Therefore, in many instances this method seems to indicate the presence of an eosinophilic airway inflammation. However, it has to be kept in mind that the pathophysiological pathways that are involved differ and that there may be circumstances in which both parameters dissociate. Accordingly, it has to be clarified in future studies, whether, for practical purposes, the more time-consuming and demanding analysis of induced sputum might be performed only in patients with unexpected changes in the level of exhaled NO or a discrepancy between their history, clinical state, and NO level. The exhaled NO, as an indirect marker of airway inflammation, has been shown to be extremely responsive to treatment with corticosteroids. Some researchers have even argued that it might be oversensitive making it less useful to identify eosinophilic inflammation in those already on corticosteroid treatment. For those having access to an analyser, the measurement might also be useful for monitoring the patient′s compliance to inhaled corticosteroids. Alternatively, it may be particularly useful in young children where sputum induction is not possible. Currently clinical algorithms are being developed to implement exhaled NO in clinical decision making, particularly in paediatrics, and most progress is probably being made in this field. It should be repeated that until now there are no prospective studies demonstrating the benefits of NO measurement in asthma management and that these studies are urgently needed.

Recently novel approaches of interpreting or measuring exhaled NO have been proposed, such as the use of a model for the assessment of airway diffusing capacity for NO [30] or the so-called step responses for the assessment of the bronchial volume within which NO is produced [31]. Future studies will have to reveal whether these sophisticated methods are helpful tools in clinical research, particularly for the assessment of the effects of anti-inflammatory treatment in clinical studies.

Currently, the measurement of markers in the breath condensate is a rapidly expanding field, as reflected by an explosively increasing number of presentations on national and international meetings. One of these markers is hydrogen peroxide (H_2O_2). Although H_2O_2 in exhaled air has been shown to be associated with airway inflammation in COPD and asthma, its measurement seems to be severely hampered by unknown intrinsic factors that result in poor reproducibility, in contrast to exhaled NO [32, 33]. The same seems to be true for pH measurements in samples of breath condensate that have been collected repeatedly in the same subjects [34]. The fact that proteins and lipid mediators can be detected in the breath condensate, has been discussed for a long time [35]. Although differences between different groups of patients have been reported, there are currently no published data on the reproducibility of these measurements. Owing to the enormous potential offered by this completely noninvasive approach, the clinical value of the analysis of breath condensate will hopefully be assessed by carefully designed, controlled studies in near future. To facilitate such studies, the ERS has recently set up a Task Force on exhaled condensate.

In summary, the methods of sputum induction and the measurement of exhaled nitric oxide represent two techniques, that have been extensively applied in clinical research and have the potential to be incorporated into future clinical practice. While there is little doubt on the benefit, at least in certain groups of patients, the costs of performing these methods or acquiring the required equipment are still high. Their future use will largely depend on a favourable cost/benefit ratio that has to be achieved under stronger restrictions by health care insurances than before. Therefore, it may be that in the near future the application of these methods continues to be restricted to specialized research centres which already have the equipment and expertise. Within that scope, the efforts, that have been undertaken on standardization, should facilitate the comparison of data between different centres and enable prospective collaborative studies that aim to assess the clinical value of these methods. Hopefully, the results of these efforts will result in the spread of noninvasive methods into clinical practice. Through the set-up of appropriate Task Forces, the European Respiratory Society is devoting much effort to each of the fields of sputum induction, nitric oxide, and condensate measurement in order to provide clear recommendations to researchers and clinicians on using these techniques.

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