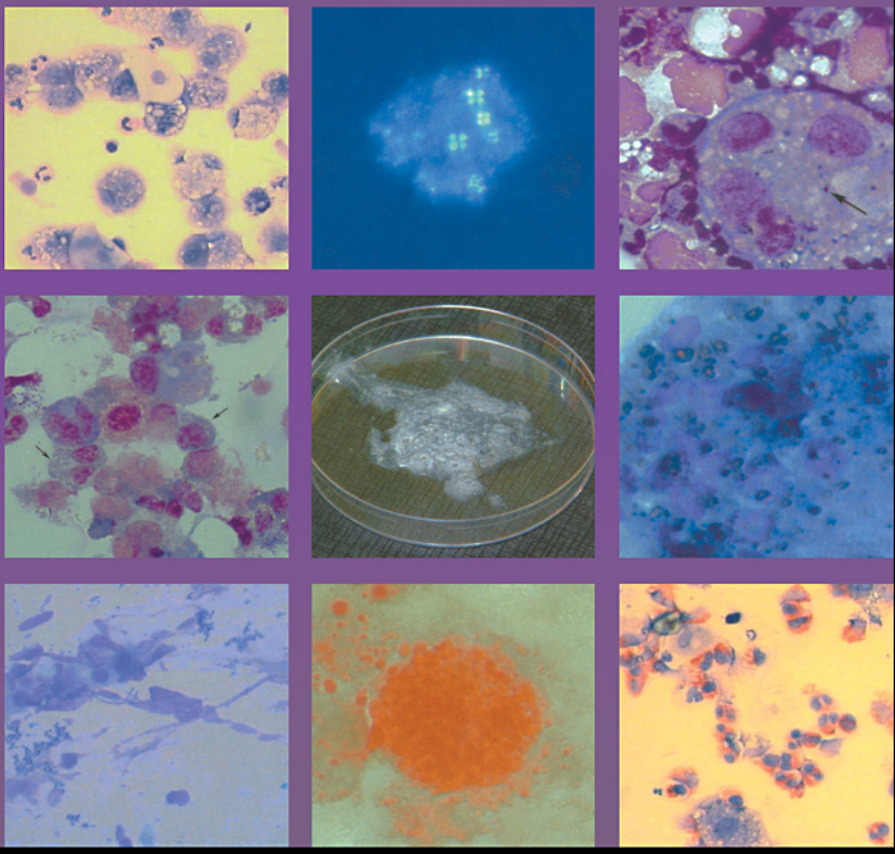
THE ENCYCLOPEDIA OF VISUAL MEDICINE SERIES

An Atlas of INDUCED SPUTUM

An Aid for Research and Diagnosis



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Introduction

Ratko Djukanovic and Peter J. Sterk

The ability to understand disease mechanisms and make a correct diagnosis is greatly enhanced by analyzing tissue or body fluid samples; this information complements that obtained by functional tests. This principle has long been applied in nephrology, hepatology and dermatology but was not fully accepted until recently in respiratory medicine. Thus, asthma and chronic obstructive pulmonary disease (COPD) were diagnosed on the basis of history and lung function tests, and the study of pathogenesis was limited by the relative lack of insight into the pathological changes in the airways and parenchyma. In the mid 1980s, first attempts to study airway pathology were made using bronchoscopy. This tool has provided extremely valuable insight into the pathology of asthma, and more recently COPD, but the invasive nature of the technique has meant that its use was limited mainly to academic centres with appropriate facilities and significant expertise to undertake a procedure that is not without risk.

It has long been appreciated that sputum provides insight into mechanisms of diseases of the lungs. Hippocrates identified sputum as one of the four essential humors in the body. Ever since the development of cytology and microbiology, sputum has been used to diagnose cancer and respiratory infections, including tuberculosis. In the last century Gollasch observed an increase in eosinophils in the sputum of asthmatics. In 1958 Dr Morrow Brown recognized the value of assessing sputum for the presence of eosinophils, which predicted which patients would respond to corticosteroids. After a period during which the use of induced sputum to assess airway diseases was almost forgotten, refinements in methodology have progressed significantly in the last 10–15 years to the point that today it is one of the more referenced methods used to assess airway inflammation in research and clinical practice. Recently, a task force of the European Respiratory Society (ERS) investigated the value of sputum induction and guidelines were provided for how sputum should be induced, processed and interpreted. These can be found in a supplement of the European Respiratory Journal, 2002; volume 37.

The aim of this Atlas is to be a useful reference book, with many illustrations and easy-to-read text. We wish to help researchers, clinicians, laboratory technicians, and all others involved in clinical trials and drug development to acquire insight into some of the major advances in the use of sputum to study lung disease, excluding lung cancer since this is usually and better covered by lung pathologists. The Atlas has been put together by experts in the field of induced sputum, who have selected the most relevant and exciting data and have presented them in a way that is easy to read and allows easy recognition of the potential of this method.

Sputum is defined as secretions that are expectorated from the lower respiratory tract. As such, it is composed of a fluid phase, which contains abundant mucins, and a cellular phase; which contains inflammatory cells and epithelial cells that are shed into the airway

lumen. The Atlas covers the important topics of methods and safety of induction, the principles of processing and analysis of the fluid and cellular phases of sputum; and the exciting application of the technique to study a variety of lung diseases.

Ratko Djukanovic
Peter J.Sterk

CHAPTER I
Sputum induction: methods and safety

Pierluigi Paggiaro, Antonio Spanevello and Elena Bacci

The aim of sputum induction is to collect an adequate sample of lower airway secretions in patients who are not able to produce sputum spontaneously. Sputum induction requires a high degree of patient cooperation and is best conducted in a quiet; secluded area to minimize embarrassment for the patient (Figure 1.1). Adequate facilities and equipment are needed (Table 1.1, Figure 1.2). Infection control procedures for the protection of personnel and patients must be carried out according to the local antiinfection policy, which sometimes includes purpose-built chambers (Figure 1.1).

For successful induction it is essential that a sufficiently high output of saline aerosol with an adequate particle size is achieved. This is best done with ultrasonic nebulizers, the output of which needs to be accurately determined and set at the same level for a given study. Fresh sterile saline solution should be used. There is no evidence to suggest that larger volumes of saline are more successful than smaller ones, and the consensus at present is that an output of approximately 1 ml/min is sufficient to achieve a high success rate.

Inhalation of isotonic or hypertonic solutions administered by nebulization induces small amounts of airway secretions that can be expectorated more or less easily (Figure 1.3). Some patients, such as those with acute asthma exacerbations or severe current symptoms, and patients with chronic obstructive pulmonary disease (COPD) or cystic fibrosis, can produce significant amounts of sputum spontaneously, i.e. without the need for inhalation of saline. This can be sufficient for some studies in that spontaneous sputum contains similar percentages of inflammatory cells and amounts of some mediators as induced sputum (Figure 1.4), albeit at the expense of cell viability and the quality of samples. However, the majority of patients need to inhale saline before they can produce an adequate sample.

The technique of sputum induction has been standardized to enable comparison between researchers and to ensure adequate reproducibility of the technique. Frequent repetition of sputum induction can itself lead to airway inflammation, resulting in a change in cell populations obtained by subsequent inductions. Thus, repeating induction 8–24 h after an initial induction can cause an increase in neutrophils (Figure 1.5), and it is possible that mediator levels also change. For this reason, an interval of 2 days between inductions is recommended.

SAFETY

As with all procedures, safety of sputum induction is paramount. The risk of excessive

bronchoconstriction caused by sputum induction in subjects with susceptible airways cannot be underestimated. Predictors of excessive bronchoconstriction reported

Figure 1.1 Sputum induction carried out in a volunteer participating in a research project. The subject is wearing a nose-clip to avoid breathing through the nose and thus reduce contamination of sputum by nasal secretions. A technician or nurse must be present throughout the procedure, measuring peak exploratory flow using a hand-held device (Wright's mini peak flow meter shown)

Table 1.1 List of facilities and instruments required for sputum induction

Facilities:
– quiet environment
– sterile saline solutions
Instruments:
– ultrasonic nebulizer
– spirometer
– safety equipment (resuscitation equipment, oxygen supply, rescue medications)

Figure 1.2 Trolley with an ultrasonic nebulizer. The sputum sample is expectorated into a Petri dish and placed on ice in the white polystyrene box

so far are the degree of baseline airflowlimitation and the degree of airway hyperresponsiveness to methacholine or histamine. However,this has not been universally confirmed andmeasurement of hyperresponsiveness does notremove the need for vigilance. Studies havereported a strong correlation between recentoveruse of a short-acting β2-agonist and the magnitude of fall in forced expiratory volume (FEV_1after sputum induction, and continuous ß2-agonist use can result in a reduction of the bronchoprotective effect against a variety of both specific andnon-specific bronchoconstrictor stimuli. It has alsobeen demonstrated that 200 or 400 µg of salbutamol does not protect against excessive bronchoconstriction if there is exposure to a relativelystrong bronchoconstrictive stimulus.

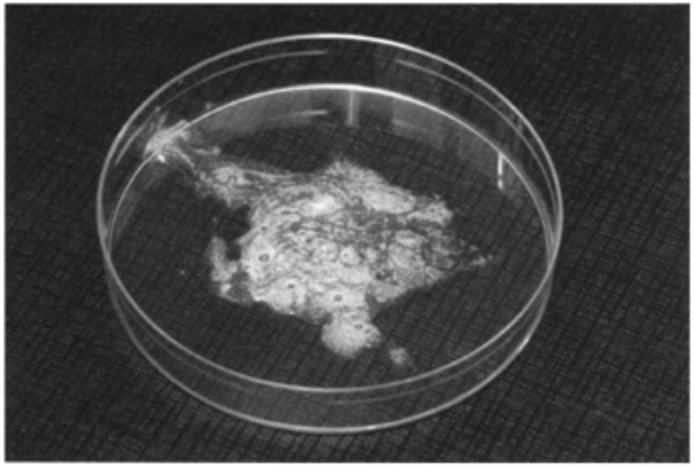

Figure 1.3 Sputum sample expectorated and collected in a Petri dish over a period of 15 min. A good sample contains small amounts of saliva only, with the majority of the sample being viscous due to the presence of highmolecular-weight mucins that form the gel phase of sputum

In view of these concerns; sputum induction should be conducted by an experienced technician or nurse and supervised by an experienced physician. This is needed for early and dependable recognition of bronchoconstriction in patients with hyperreactive airways. Full resuscitation equipment and additional rescue bronchodilator medication together with any other emergency drugs must be immediately available. Oxygen saturation should be monitored if there is any concern about resting hypoxemia. Supplemental oxygen must be immediately available.

Based on considerable experience accrued over the past 10 years, recommendations have been made for how sputum induction should be conducted. The method varies depending on whether the investigator sees the patient as being at low risk of bronchoconstriction—in which case a standard protocol is applied—or at high risk, when an alternative protocol is used (Table 1.1).

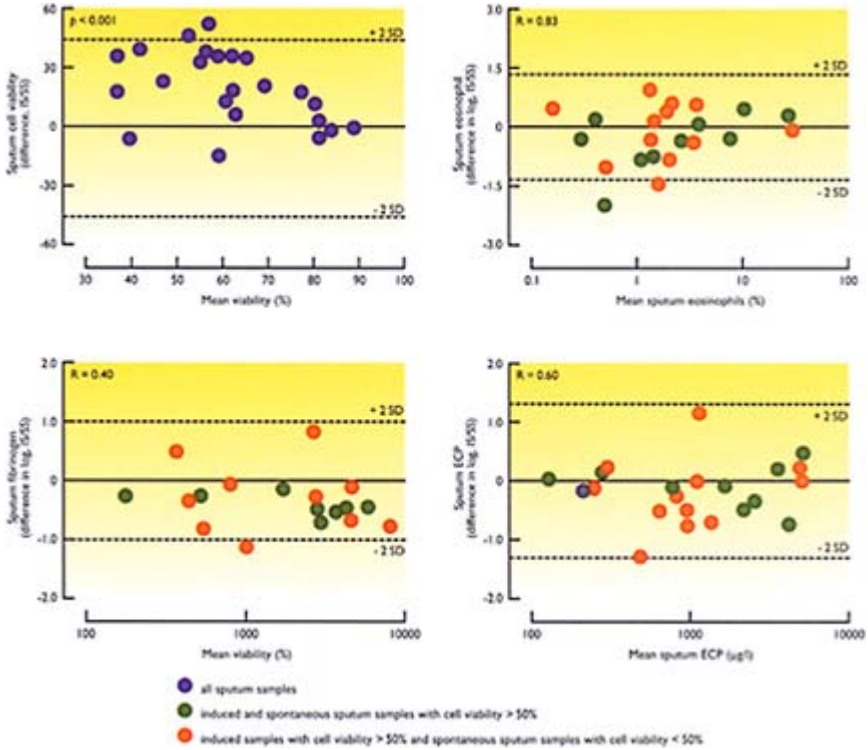

Figure 1.4 Comparison of sputum obtained spontaneously with sputum obtained by induction using hypertonic saline in respect of cell viability, eosinophil counts, concentration of fibrinogen, and eosinophil cationic protein (ECP). The difference (in logs, except for cell viability) between induced (IS) and spontaneous (SS) sputum is plotted against the mean of the two values. R is the intraclass correlation coefficient and the marked area is ± 2 standard deviations (SD) of the mean of the two differences. Adapted with permission from Pizzichini MM, Popov TA, Efthimiadis A, et al. Spontaneous and induced sputum to measure indices of airway inflammation in asthma. *Am J Respir Crit Care Med* 1996; 154:866–9

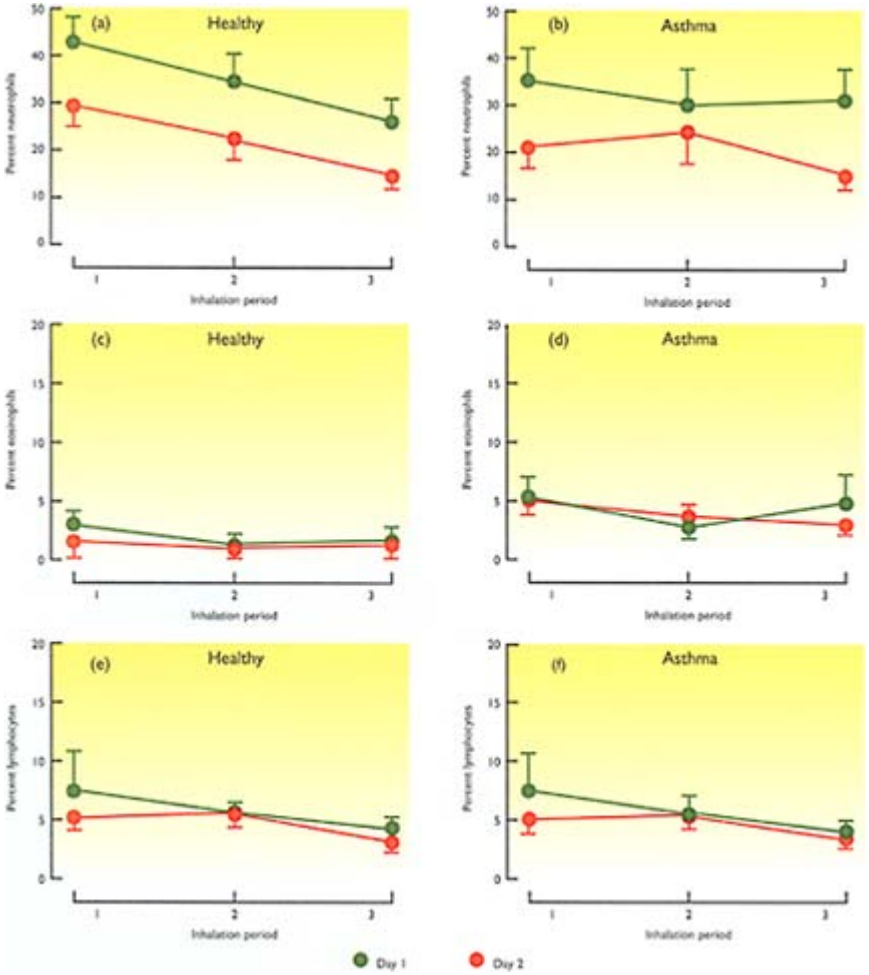

Figure 1.5 The effect of repeat sputum induction on sputum cell counts. Mean (SE) percentage of neutrophils (a, b) eosinophils (c, d) and lymphocytes (e, f) measured on day I and 24 h later on day 2 are shown. Inhalation periods 1–3 indicate consecutive 10-min sampling periods within each of the two sputum inductions. The increase in levels of neutrophils in the second sputum induction, compared with the first, was statistically significant in both healthy and asthmatic subjects ($p < 0.01$). Adapted with permission from the BMJ Publishing Group from Holz O, Richter K, Jorres RA, et *al.* Changes in sputum composition between two inductions performed on consecutive days. *Thorax* 1998;53:83–6

Pretreatment with a short-acting ß$_2$-agonist, salbutamol (200 µg), delivered via a standard metered dose inhaler is generally used in all cases. Higher doses are not