REPORT OF WORKING GROUP 1

Sputum induction

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The aim of sputum induction is to collect an adequate sample of secretions from lower airways in subjects who do not produce sputum spontaneously in order to study the features of airway inflammation in asthma and other respiratory disorders. Inhalation of isotonic or hypertonic solutions administered by nebulisation has been demonstrated to induce a small amount of airway secretion that can be expectorated and analysed. The mechanisms by which this occurs are not known, but both direct and indirect mechanisms are likely to be involved. It is believed that the increased osmolarity of the airway lining fluid increases vascular permeability in the bronchial mucosa and induces production of mucus by submucosal glands. Animal studies have shown that hypertonic saline increases vascular permeability in the airways, and this effect can be modulated by capsaicin [1]. However, *in vivo* instillation of hypertonic solution into the airways of animals and humans induces an increase in the levels of several mediators but no rise in levels of albumin or other markers of increased vascular permeability [2, 3]. Therefore, this hypothesis has not been confirmed, and only preliminary measurements of sputum osmolarity after induction have been reported with conflicting results [4]. Conversely, previous reports have demonstrated increased clearance of secretions from the airways in humans after administration of hypertonic saline aerosol [5], perhaps by facilitating the collection of small amounts of pre-existing airway secretions.

Although sputum induction has been used extensively since the 1990s, few methodological studies have examined the influence of various technical factors on the feasibility and repeatability of sputum induction and collection. As with other techniques, the lack of a "gold standard" makes it hard to evaluate the influence of these technical factors on the adequacy and accuracy of sputum induction.

The technique of sputum induction consists of inhaling an aerosol of saline (either normal or hypertonic) over different time-periods. Volunteers/

patients are instructed to expectorate into a container. Various issues must be considered with regard to this procedure: 1) facilities, equipment and personnel; 2) bronchodilator pretreatment; 3) pulmonary function monitoring; 4) concentration of saline solution and nebuliser output; 5) duration of inhalation; 6) expectoration technique; 7) spontaneous *versus* induced sputum; and 8) frequency of sputum induction. The influence of these factors on the feasibility of the technique must be evaluated in terms of the success rate, repeatability and accuracy of the results obtained.

Facilities, equipment and personnel

Ultrasonic nebulisers are recommended since other nebulisers do not usually exhibit sufficient output of saline aerosol. The output of nebulisers should be accurately determined. Spirometry is necessary to assess baseline airway calibre and avoid excessive bronchoconstriction during saline inhalation. Spirometers are preferable to peak flow meters because of the greater sensitivity of the forced expiratory volume in one second (FEV₁) in detecting induced bronchoconstriction. Oxygen saturation should be monitored if there is any question of resting hypoxaemia. Supplemental oxygen should be immediately available for chronic obstructive pulmonary disease patients with hypoxaemia. Full resuscitation equipment and trained personnel should be available, as recommended for other specific and nonspecific bronchial challenge procedures.

Sputum induction requires a high degree of cooperation from the patient. It is recommended that the procedure be performed in a quiet environment, separate from other routine activities. The procedure should be conducted by an experienced technician under the supervision of an experienced physician. Patients may prefer to collect sputum in a separate room (*e.g.* a bathroom); if this is available, it

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Received: April 4 2002; Accepted: April 16 2002

must be immediately adjacent to the laboratory in which the sputum induction is performed.

Sterile saline solution should be prepared freshly. Rescue bronchodilator medication (inhaled or nebulised salbutamol or other β_2 agonist) together with any other emergency drugs must be immediately available. Infection control procedures for the protection of personnel and patients must be carried out according to local anti-infection policy.

Bronchodilator pretreatment

Hypertonic saline causes bronchoconstriction in asthmatic subjects [6]. The mechanism of this effect is unknown, but may involve activation of airway mast cells [3] or sensory nerve endings [7]. Although some authors do not pretreat the patient before studying the functional response of the airways to this bronchoconstrictor stimulus [8, 9], pretreatment with a short-acting β_2 -agonist is to be recommended as the standard procedure in order to prevent excessive bronchoconstriction [10–12]. The potential for sputum induction to cause bronchoconstriction should not be underestimated. A severe asthma exacerbation leading to death in an asthmatic subject undergoing a distilled water challenge test has been reported [13]. Apart from the safety issue, excessive bronchoconstriction occurring after only a few minutes of inhalation may result in premature discontinuation of induction and collection of an inadequate sputum sample. This may influence both the success rate and repeatability of the results (see *Duration of inhalation* section). Furthermore, the usefulness of measuring bronchial hyperresponsiveness to hypertonic saline at the same time as sputum collection has not been clearly demonstrated, although it is well known that bronchial hyperresponsiveness to indirect stimuli is not related to clinical asthma in the same way as bronchial hyperresponsiveness to direct stimuli, such as methacholine or histamine [14]. Therefore, pretreatment with bronchodilators should be routine, except for special research purposes.

Salbutamol, usually 200–400 μg , *i.e.* 2–4 puffs from a standard metered-dose inhaler, has generally been used for pretreatment. Pretreatment with high doses of salbutamol has not been universally effective in preventing hypertonic saline-induced bronchoconstriction [12, 15–17]. In addition, subsequent bronchospasm may be more severe or more difficult to reverse. Therefore, a single dose of salbutamol 200 μg is recommended, with measurement of FEV₁ before and after 10 min.

The effect of β_2 -agonist pretreatment on the cellular constituents of induced sputum has been investigated in two studies [15, 18], both reporting no effect of salbutamol on sputum inflammatory cell percentages. With regard to the effects on soluble mediators in sputum supernatant, salbutamol pretreatment has no effect on eosinophil cationic protein (ECP) levels, but tends to reduce histamine concentrations [15]. This finding is in agreement with the observation that hypertonic solution activates mast cells when directly instilled into the airways by endobronchial challenge

[3, 19] and that salbutamol may prevent this. No data have been published on the effect of different doses (200–400 μg) of salbutamol on levels of other soluble mediators in the supernatant (cytokines, albumin, neutrophil elastase, *etc.*) or on the expression of cell activation markers detected by immunocytochemistry. Furthermore, no comparison between different bronchodilators (*e.g.* β_2 -agonists *versus* anticholinergic agents) has been reported.

Thus, outstanding questions regarding this issue include: 1) comparison of different inhaled bronchodilators; and 2) assessment of possible significant effects of bronchodilator pretreatment, particularly at high dose, on fluid-phase measurements.

Pulmonary function monitoring

Monitoring pulmonary function during sputum induction is necessary for safety reasons, in order to assess excessive bronchoconstriction (see article entitled "Safety of sputum induction" [20]), and to standardise the forced expiratory manoeuvre during the procedure as its influence on sputum production and/or composition is not known.

No standardised protocol for pulmonary function monitoring during sputum induction has been suggested to date. Many authors measure pulmonary function every 5–10 min, with further measurements performed in case any symptoms develop [8–11, 21]. Different methods have been employed. Most investigators use spirometry, although some have used peak flow meters [12]. Considering the low sensitivity of peak expiratory flow measured using portable peak flow meters [22], spirometry is preferable. The relative advantages of frequent *versus* occasional monitoring of lung function have not been formally studied. However, since "poor perceivers" of dyspnoea exist and bronchospasm may occur early during inhalation, it is probably safer to measure pulmonary function within the first minute of nebulisation in order to detect subjects who are very sensitive to hypertonic saline. FEV₁ should be monitored at intervals of ≤ 5 min during aerosol inhalation. A single measurement is appropriate if the change in FEV₁ is $<10\%$ of the postbronchodilator value. Monitoring of arterial oxygen saturation should be performed in patients who are hypoxaemic before sputum induction. One study reported a significant reduction in saturation during sputum induction [23], but this has not been the experience of others [24].

Concentration of saline solution and nebuliser output

The concentration of saline used for sputum induction has ranged 0.9–7% in different studies [5, 8, 12, 25]. Some investigators change concentration during the procedure, starting with 3% and subsequently increasing to 4 and 5% [9, 10]. Saline concentration and nebuliser output might be expected to influence the safety, tolerability and success rate of the procedure as well as the cellular and biochemical characteristics of the induced sputum collected.

Hypertonic saline solutions are reportedly more effective than normal saline in inducing sputum [18]. However, the latter should be considered in patients at greater risk of bronchospasm (see article entitled "Safety of sputum induction" [20], including table 1 therein).

There is no difference in the cellular composition of sputum induced with either isotonic or hypertonic saline [25], and different saline concentrations do not affect total and differential cell counts in selected portions of induced sputum [18]. To date, only one study has investigated whether increasing saline concentration during induction has any advantage over using a single concentration [18]. This study showed that hypertonic saline 3% is as successful as 3–5% given sequentially.

Furthermore, the effect of different saline concentrations on levels of soluble mediators in induced sputum is not known. Preliminary results have found no difference in ECP and histamine level in the supernatant of sputum induced using either isotonic or hypertonic solution [26]. Sputum supernatant osmolality varies over a wide range, 70–360 mOsm, and sputum concentrations of sodium, chloride and magnesium vary considerably from subject to subject, although they do not exceed the physiological concentrations in blood [4]. The importance of these variations on any *ex vivo* release has not been studied in great detail, but one study has shown no effect of 4.5% physiological saline on peripheral histamine release by blood basophils [4]. There is a consensus to recommend induction of sputum with 4.5% sodium chloride solution, which is commercially available, effective and generally well tolerated, as standard.

The type and output of the nebuliser are important. One study compared jet nebulisers with ultrasonic nebulisers, and reported higher success rates with the latter [18]. Other studies have used ultrasonic nebulisers with lower output, in general showing little influence of these variables on outcome [27, 28]. However, there is no clear indication as to the volume of inhaled solution that might be required to induce an adequate sputum sample, nor whether this is best achieved by changing the duration of inhalation, the nebuliser output or both. It is important, however, to determine the total volume of inhaled solution required. Finally, the size of the inhaled particles affects their airway deposition and distribution (proximal *versus* peripheral airways) [29], which, in turn, may have effects on sputum composition and success rate. Furthermore, the influence of different nebuliser set-ups (length of tubing, valve, *etc.*) has not been systematically evaluated. There is a consensus that an ultrasonic nebuliser should be used and that an output of $\sim 1 \text{ mL} \cdot \text{min}^{-1}$ is sufficient to achieve a high success rate.

Duration of inhalation

The duration of inhalation is another important variable in sputum induction. At least two studies have reported that the cellular and biochemical constituents of induced sputum change during the

course of sputum induction [30, 31]. These studies found that neutrophils and eosinophils are prominent in samples collected early during sputum induction, whereas lymphocyte and macrophage counts are increased in samples collected later. In addition, mucin concentrations are higher in samples collected early (0–4 min) than in samples collected later (16–20 min), whereas surfactant concentrations are higher in samples collected later compared to those collected early [30, 31]. This suggests that different compartments of the respiratory tract are sampled at different time-points during induction, *i.e.* central airways are sampled early, whereas peripheral airways and alveoli are sampled later. However, in another study, when the duration of induction in the same patients changed according to pretreatment with either salbutamol or placebo, no difference in sputum cell composition was shown [15]. Some authors have suggested that the first sputum samples be discarded and subsequent samples be collected and analysed [11].

The data from these studies highlight the need to standardise the duration of sputum induction. Furthermore, the duration of induction should be reported in each paper to enable comparison with other studies. Moreover, stopping induction when an adequate sample has been obtained may no longer be an acceptable research procedure. The maximum acceptable duration of induction has not been formally studied, although it depends on a reasonable compromise between success rate and tolerability/safety. Shorter inhalation times (*e.g.* 15–20 min) appear to have similar success rates and feasibility to longer inhalation times (30 min). It is important to keep the duration of inhalation constant between inductions, especially in the same subject, in order to obtain comparable results. For most purposes, the consensus is to use a cumulative duration of nebulisation of 15–20 min.

Other additional points may be relevant. Ideally, the respiratory frequency during nebulisation should be standardised between different inductions in the same individual. It is unclear whether slow deep inhalations, as opposed to tidal breathing, during challenge has any influence on the success rate and outcome of induction. The influence of different patterns of distribution of inhaled saline on the success rate has not been evaluated.

Expectoration technique

The methods of subject preparation for sputum induction and expectoration of induced sputum during sputum induction vary in different protocols. Some protocols have recommended that subjects fast for several hours before sputum induction, because of the risk of nausea and vomiting; others recommend that subjects brush their teeth immediately beforehand to minimise oropharyngeal contamination of induced sputum. These procedures do not appear necessary. Some authors have recommended that subjects rinse their mouth with water and dry it with tissue paper,

then proceed with spitting saliva and finally cough up sputum. Others have suggested that mouth rinsing or drying may increase oropharyngeal inflammation. Finally, some researchers advocate the use of nose clips. The utility of these measures in sputum induction protocols is unclear. The incidence of nausea during sputum induction in subjects who are not acutely ill has not been reported in large series, but most authors believe that it is low, and the value of brushing teeth or rinsing the mouth has not been proven. Concern has been raised that these protocol details are unnecessarily complex.

Sputum induction protocols differ with respect to schedule of sputum collection. Subjects may be asked to stop inhalation at regular intervals in order to cough up sputum (*e.g.* every 5 min), or to stop only when they feel the urge to cough. Methods of sputum expectoration also differ; some protocols require subjects to spit saliva into one container before coughing sputum into another, whereas others do not. Spitting saliva into one cup before coughing sputum into another has been shown to decrease the percentage of squamous cells in sputum (whole expectorate) by 30% and increase the concentration of ECP in the supernatant by 80% [32].

In general, the influence of induced sputum techniques on the feasibility and validity of the procedure has not been assessed. Preliminary results have not shown any correlation between ease of expectoration and quality of slides obtained [33]. Furthermore, no relationship has been observed between the clinical characteristics of asthma and success rate or sputum quality [33]. Therefore, it appears that production of a good sputum sample is related more to the characteristics of the individual subject than to technical factors. It is considered desirable that patients be given a written description of the procedure in order to familiarise them with the different steps (inhalation, FEV₁ measurement, sputum collection, *etc.*).

Spontaneous versus induced sputum

Some asthmatics, in particular those with acute exacerbations or severe current symptoms, and patients with chronic obstructive pulmonary disease [34, 35] produce sputum spontaneously. Spontaneously produced sputum has been demonstrated to contain similar percentages of inflammatory cells and mediators to induced sputum [24, 36]. However, cell viability in spontaneous sputum is considerably lower than that in induced sputum [24, 36], and the quality of samples (and preparations) is poorer, particularly in patients with more severe asthma [33]. It is possible that a longer residence time of mucus secreted into the airways leads to reduced cell viability and a poorer distinction between different types of inflammatory cell. In order to obtain better samples, some authors have suggested collecting spontaneous sputum first and then inducing sputum by inhalation of saline. It may be important to employ the induced sputum technique even in subjects with spontaneous expectoration in order to make appropriate comparisons

with subjects who do not produce sputum spontaneously.

The exact airway level(s) from which samples obtained spontaneously are derived are not known. Induced sputum may be more representative of central airways cell populations, but may also include more contamination with nasal or pharyngeal secretions. More studies are needed to assess comparisons of induced and spontaneous sputum in different clinical situations.

Frequency of sputum induction

It has been suggested that frequent repetition of sputum induction can itself lead to (late) airway inflammation resulting in a change in cell populations obtained by subsequent inductions. Some authors have reported that repeating sputum induction 8–24 h after an initial induction can cause an increase in neutrophil levels in the second sputum sample [37, 38]. Therefore, sputum induction should not be repeated frequently. An interval of 48 h between two inductions gave reproducible cell counts in normal subjects [39]. More research is needed to determine the minimum interval required between repeated inductions in order to ensure that "carry-over" effects are avoided. An interval of 2 days between subsequent inductions is currently recommended.

Key points

It is important to: 1) standardise the sequence of inhalation, FEV₁ measurement and sputum collection; 2) pretreat with bronchodilators (except for specific research questions); 3) monitor airway function during induction; 4) use an ultrasonic nebuliser with a sufficient and measured output; and 5) use adequate instruments and facilities.

Outstanding questions

The research questions that still need to be addressed include: 1) the influence of different types and doses of bronchodilator on sputum markers; 2) the effect of hypertonic solution on soluble mediator concentrations; 3) the effect of expiratory manoeuvres on sputum outcomes; and 4) the effect of nebuliser characteristics (set-up, particle size, *etc.*) on sputum outcomes.

Task Force recommendations regarding methods of sputum induction

The following recommendations are based primarily on evidence obtained from the literature; in addition, for some recommendations, a consensus was reached amongst members of the Task Force based on observations that have not yet been published.

Standard (use alternative procedure for high-risk patients)

1) Give detailed information and clear instructions to the patient prior to the procedure. 2) Check safety equipment and set up ultrasonic nebuliser (output $\sim 1 \text{ mL} \cdot \text{min}^{-1}$). 3) Measure prebronchodilator FEV₁. 4) Administer 200 μg inhaled salbutamol before commencing. 5) After 10 min, measure postbronchodilator FEV₁. 6) Use either a fixed concentration of sterile saline solution (e.g. 3 or 4.5%) or incremental concentrations (3, 4 and 5%). 7) Perform induction at 5-min intervals for ≤ 20 min. Alternatively, induction can be conducted at 1, 4 and 5 min followed by three further 5-min periods. 8) Measure FEV₁ at the end of each induction interval. Stop induction if there is a fall in FEV₁ of $\geq 20\%$ compared with the postbronchodilator value or if symptoms occur. 9) Ask the patient to cough and spit after 5, 10, 15 and 20 min of induction or whenever they get the urge to do so.

Alternative (for high-risk patients)

1) Give detailed information and clear instructions to the patient prior to the procedure. 2) Check safety equipment and set up ultrasonic nebuliser (output $\sim 1 \text{ mL} \cdot \text{min}^{-1}$). 3) Measure prebronchodilator FEV₁. 4) Administer 200 μg inhaled salbutamol. 5) After 10 min, measure postbronchodilator FEV₁. 6) Start with 0.9% sterile saline solution and perform induction for 30 s and 1 and 5 min, measuring FEV₁ after each period as a safety precaution. If this fails to induce sputum, increase the saline concentration to 3%, induce for 30 s and 1 and 2 min. If this also fails to induce sputum, increase saline concentration further to 4.5% and induce for 30 s and 1, 2, 4 and 8 min. 7) If normal saline is successful at inducing sputum, there is no need to progress to higher concentrations. The same applies for 3% saline. 8) Measure FEV₁ at the end of each induction interval. Stop induction if there is a fall in FEV₁ of $\geq 20\%$ compared with the post-bronchodilator value or if symptoms occur. 9) If the patient does not cough spontaneously, ask them to attempt to cough and spit after the 4- and 8-min periods.

Additional recommendations

1) In a given study, the protocol should be kept as constant as possible, especially the timing of inhalation when repeating the procedure in the same individual (e.g. pre- and post-treatment); 2) do not repeat induction within 48 h of the first induction; and 3) follow Task Force conclusions regarding the safety of sputum induction in article entitled "Safety of sputum induction" [20].

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