Safety of sputum induction in chronic obstructive pulmonary disease

P.H. Rytilä, A.E. Lindqvist, L.A. Laitinen


ABSTRACT: The aim of the present study was to evaluate the safety of sputum induction in patients with varying severity of chronic obstructive pulmonary disease. The subjects were 28 smokers with baseline forced expiratory volume in one second (FEV1) of (mean and range) 1.8 (0.8–2.9) L that is 53 (28–69)% of the predicted and reversibility of 2.5 (-7.4–9.9)%. Sputum was induced after premedication with 200 μg salbutamol at increasing concentrations (0.9, 3, 4, and 5%) of hypertonic saline nebulized by an ultrasonic nebulizer.

The procedure was well tolerated, and none of the patients reported major side-effects. However, the mean change from prebronchodilator FEV1 during induction was -8.5 (-23–11)%, p=0.001, and from postbronchodilator FEV1 -10.7 (-25–5)%, p=0.0001. Three (11%) of the patients had a fall in FEV1 from the prebronchodilator baseline of >20%, and a further 10 (36%) had a fall of 10–20%. Patients with greater reversibility in airway obstruction seemed to get the best benefit from the bronchodilator pretreatment, since there was an inverse relationship between reversibility in FEV1 and fall in FEV1 during induction (r=-0.4, p=0.03).

It is concluded that sputum induction by hypertonic saline inhalation can cause meaningful bronchoconstriction in patients with chronic obstructive pulmonary disease, despite pretreatment with an inhaled β2-agonist. The results highlight the importance of monitoring spirometry during sputum induction to detect bronchoconstriction.


Sputum induction by inhalation of hypertonic saline has been used as a direct and relatively noninvasive method to investigate airway inflammation. It has been applied in patients with asthma and chronic obstructive pulmonary disease (COPD) [1–4]. Sputum-induction methodology may offer important benefits in diagnosing patients with airflow obstruction and in comparing treatment strategies. Although no standardized method for the induction of sputum has been agreed upon, recently a consensus on the use of sputum induction in asthma has appeared [5]. The challenge procedure should be performed in a standardized manner, because hypertonic saline can cause asthmatics to suffer from airway constriction. This procedure includes spirometry before and during the induction as well as pretreatment with a short-acting β2-agonist.

Some studies assess the safety of sputum induction in patients with asthma [6–8], but information on patients with COPD is scarce [3, 4]. The aim of the present study was to evaluate the safety and success of sputum induction in patients with varying severity of COPD [9].

Materials and methods

Subjects

The authors studied 28 patients with at least a 2-yr clinical diagnosis of symptomatic COPD [10] and a smoking history of 20–103 pack-yrs (table 1). Two (7%) of them were exsmokers, all others were current smokers. Patients had to fulfil the following criteria: 1) prebronchodilator forced expiratory volume in one second (FEV1) of <70% of the predicted [11]; 2) <10% reversibility from prebronchodilator FEV1 to short-acting β2-agonist (200 μg of salbutamol). Patients treated for disease exacerbation or needing antibiotics for respiratory-tract infections during 6 weeks prior to the study were excluded.

FEV1 (mean and range) was 1.8 (0.8–2.9) L, that is 53 (28–69)% pred and FEV1/forced vital capacity was 52 (33–67)% arteries. Reversibility to 200 μg of inhaled salbutamol was 2.5 (-7.4–9.9)% (table 1). Four (14%) patients used inhaled steroids (beclomethasone or budesonide, mean dose 900 μg·day⁻¹, range 800–1200 μg·day⁻¹) and two (7%) oral theophylline. None had regular oral steroid treatment. Five (18%) received short-acting β2-agonists and three (11%) anticholinergics as a rescue medication. Only one (4%) showed clinical evidence of allergy or atopy.

The study was approved by the ethics committee of Helsinki University Central Hospital (Helsinki, Finland), and all subjects gave their informed consent.

Study design

Two weeks before sputum induction, spirometry was performed and reversibility in FEV1 was assessed (Spirotrac III; Vitalograph, Maidstone, UK). For FEV1, the

Copyright ©ERS Journals Ltd 2000
European Respiratory Journal
ISSN 0903-1936

Accepted after revision March 8 2000

The study was supported by a grant from the Ida Montin Foundation, Finland.
The suspension was centrifuged, and the supernatant was aspirated and stored in microfuge tubes at −20 °C for later assay. The cell pellet was resuspended, and the absolute number of cells per milligram of processed sputum was calculated. Coded cytospins were prepared and stained by May-Grünwald Giemsa stain and by toluidine blue in order to obtain a cell differential count. The sputum sample was considered adequate if it had <80% squamous epithelial cell contamination from saliva. The results are expressed as percentage of individual cells of the total nonsquamous cell count.

From sputum supernatant the concentrations (µg·L⁻¹) of eosinophil activation marker eosinophil cationic protein (ECP), and the neutrophil activation marker, myeloperoxidase (MPO), were measured using commercially available immunoassay kits (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

Data analysis
The authors calculated the change in FEV<sub>1</sub> from the prebronchodilator and from the postbronchodilator baseline. Data are expressed as mean and range. Comparisons of different patients groups were analysed by the Mann-Whitney U-test and Chi-squared test, when appropriate. Correlation was analysed by Spearman’s rank correlation test. Wilcoxon’s test for paired data was used to analyse changes in the same patient. Two-tailed p-values <0.05 were considered significant.

## Results
The sputum induction procedure was well tolerated, and no patients reported major side-effects. An adequate sputum sample was obtained from 27 (96%) patients. The mean change from the prebronchodilator FEV<sub>1</sub> during induction was -162 (-541–205) mL that is -8.5 (-23–11)% p<0.001, and from the postbronchodilator FEV<sub>1</sub> -202 (-625–95) mL that is -10.7 (-25–5)%, p<0.0001 (table 2, fig. 1). Three (11%) of the subjects had a fall in FEV<sub>1</sub> from the prebronchodilator baseline value of >20%, and a further 10 (36%) had a fall of 10–20%. All patients with a fall in FEV<sub>1</sub> of ≥20% were followed carefully and treated with inhaled salbutamol.

## Table 1. – Characteristics of subjects

<table>
<thead>
<tr>
<th>n</th>
<th>Age</th>
<th>Male</th>
<th>Smoking pack-yrs</th>
<th>Inhaled steroids</th>
<th>Symptom score*</th>
<th>∆PEF %&lt;sup&gt;#&lt;/sup&gt;</th>
<th>Baseline FEV&lt;sub&gt;1&lt;/sub&gt; L</th>
<th>Baseline FEV&lt;sub&gt;1&lt;/sub&gt; % pred</th>
<th>Baseline FVC L</th>
<th>Baseline FEV/&lt;sub&gt;FVC&lt;/sub&gt; %</th>
<th>Reversibility in FEV&lt;sub&gt;1&lt;/sub&gt; %</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>60 (51–68)</td>
<td>19 (68%)</td>
<td>52 (20–103)</td>
<td>4 (14%)</td>
<td>6.4 (3.3–11)</td>
<td>8.9 (1.9–39)</td>
<td>1.8 (0.8–2.9)</td>
<td>53 (28–69)</td>
<td>3.5 (1.9–5.3)</td>
<td>52 (33–67)</td>
<td>2.5 (-7.4–9.9)</td>
</tr>
</tbody>
</table>

Data expressed as mean and range or number and percentage.

FEV<sub>1</sub>: forced expiratory volume in one second; FVC: forced vital capacity. *: average sum of three symptoms (cough, sputum-production, shortness of breath) per day, see Methods section. #: average diurnal peak expiratory flow (PEF) variation during 1-week follow-up, see Methods section.

The greatest accepted difference between the two highest values among three or more technically acceptable determinations was 4% [12]. During 1–2 weeks time before sputum induction, patients measured their peak expiratory flow (Mini Wright peak flow meter; Clement Clarke Int., London, UK) every morning and evening. They also recorded daily symptoms (cough, sputum-production, shortness of breath) on a scale ranging 0–4 (table 1). Patients were allowed to continue their usual medication for COPD, with the exception of long-acting bronchodilator therapy, which was not allowed for the 2 weeks prior to sputum induction. Short-acting bronchodilators were not allowed 4 h prior to induction.

### Sputum induction
Sputum was induced by the method first described by PIZZICHINI et al. [9]. Before induction, spirometry was performed, after which 200 µg of salbutamol was given by metered dose inhaler. The inhalation technique was checked and medication was given under supervision of a trained nurse. Ten minutes later postbronchodilator spirometry was obtained. If the postbronchodilator FEV<sub>1</sub> was <1 L, only 0.9% hypertonic saline was used, and FEV<sub>1</sub> was checked every 3 min. If the postbronchodilator FEV<sub>1</sub> was >1 L, induction was started with 3% hypertonic saline, and FEV<sub>1</sub> was obtained every 7 min. The inhalation was stopped if FEV<sub>1</sub> dropped by > 20%, if bothersome symptoms occurred, or when the sputum sample was adequate. The inhalation was followed by postinduction spirometry. Duration of inhalation was calculated as time required to reach the end of inhalation or to decrease FEV<sub>1</sub> by >20%. The hypertonic saline solution was nebulized with an ultrasonic nebulizer (Ultra-neb 2000; DeVilbiss Health Care Inc., Somerset, PA, USA); (output 2.5 mL·min⁻¹, particle size 4.5 µm).

### Sputum processing
The authors used the method of sputum examination of PIZZICHINI et al. [13]. Briefly, all sputum macroscopically free of salivary contamination was selected and treated with dithiothreitol (Sputolysin 10%; Calbiochem Corp., San Diego, CA, USA) and phosphate-buffered saline.

### Table 2. – Sputum-induction data

<table>
<thead>
<tr>
<th></th>
<th>Patients n=28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prebronchodilator</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
</tr>
<tr>
<td>Change in FEV&lt;sub&gt;1&lt;/sub&gt; mL</td>
<td>-162 (-541–205)</td>
</tr>
<tr>
<td>% change in FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>-8.5 (-23–11)</td>
</tr>
<tr>
<td><strong>Postbronchodilator</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
</tr>
<tr>
<td>Change in FEV&lt;sub&gt;1&lt;/sub&gt; mL</td>
<td>-202 (-625–95)</td>
</tr>
<tr>
<td>% change in FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>-10.7 (-25–5)</td>
</tr>
<tr>
<td><strong>Final saline concentration</strong></td>
<td></td>
</tr>
<tr>
<td>0.9%</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>3%</td>
<td>21 (75%)</td>
</tr>
<tr>
<td>4%</td>
<td>4 (14%)</td>
</tr>
<tr>
<td>5%</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Induction time</td>
<td>8.3 (2–21)</td>
</tr>
</tbody>
</table>

Data expressed as mean and range or number and percentage. FEV<sub>1</sub>: forced expiratory volume in one second. *: p=0.001; #: p<0.0001.
characteristics and change in FEV1 during induction. No relations among sputum markers and baseline clinical
inhaled steroids and steroid naive patients.

between females and males or between patients on in-
significant differences existed in any of the parameters
from the prebronchodilator baseline FEV1 during induction.

mean fall in FEV1 of -120 (-188–17) mL, that is -13.7 (-20–2)% from the
prebronchodilator baseline, and -120 (-188–17) mL, that is -13.7 (-20–2)% from the
postbronchodilator baseline.

The sputum total and differential cell counts as well as
ECP and MPO results are shown in table 3. A statistically
significant inverse relationship existed between reversi-
bility in FEV1 and fall in FEV1 during induction, (r =-0.4, p =0.03; Spearman rank correlation).

The fall in FEV1 during sputum induction was inversely
correlated with reversibility in FEV1. This indicates that
COPD patients with more reversibility in FEV1 may gain
the best benefit from bronchodilator pretreatment against
bronchoconstriction. Airway hyperreactivity and reversi-

Discussion

It was found that 11% of the patients with COPD had a
fall in FEV1 of >20% during the induction procedure and
another 36% a fall in FEV1 of 10%–20%. The mean fall
from the prebronchodilator baseline FEV1 during induction

Table 3. – Sputum inflammatory markers

| Sputum total cell count $10^9$mg$^{-1}$ | 1.9 (0.09–15) |
| Eosinophils % | 2.0 (0–11) |
| Neutrophils % | 62 (38–89) |
| Lymphocytes % | 0.4 (0–4) |
| Macrophages % | 36 (9–62) |
| Bronchial epithelial cells % | 0.10 (0–4.0) |
| Squamous epithelial cells % | 6.8 (0–35) |
| Metatricameric cells % | 0.10 (0–1.0) |
| ECP g·L$^{-1}$ | 1218 (36–4460) |
| MPO g·L$^{-1}$ | 12320 (531–45540) |

Data are expressed as mean with range in parenthesis. ECP: cosinophil cationic protein; MPO: myeloperoxidase.

was 8.5%, and from the postbronchodilator baseline 10.7%.
However, all patients tolerated the sputum-induction
procedure well and failed to report any major side-effects,
although airway obstruction caused by the induction pro-
cedure did occur.
Pretreatment with a bronchodilator failed to prevent a
fall in FEV1, especially in patients with no or slight re-
versibility in FEV1 caused by pretreatment salbutamol.
The fall in FEV1 during sputum induction was inversely
correlated with reversibility in FEV1. This indicates that
COPD patients with more reversibility in FEV1 may gain
the best benefit from bronchodilator pretreatment against
bronchoconstriction. Airway hyperreactivity and reversi-

It remains unclear whether the magnitude of broncho-
constriction depends on hypertonicity of the saline, the
total amount inhaled, or the rate of delivery, or if it could be
prevented by pretreatment with larger doses of inhaled
salbutamol [17–19]. The current authors used a fairly
high-output ultrasonic nebulizer (output 2.5 mL·min$^{-1}$,
particle-size 4.5 μm). The original paper on asthmatics by
Pis et al. [15] used a relatively low-output ultrasonic nebu-

In conclusion, the authors have shown that sputum
induction by inhalation of hypertonic saline can, in patients
with chronic obstructive pulmonary disease, cause bronchoconstriction. Patients whose airway obstruction is more reversible seem to be better protected by bronchodilator pre-treatment. The results highlight the importance of monitoring spirometry during sputum induction in order to detect bronchoconstriction.

Acknowledgements. The authors wish to thank K. Ahlskog, T. Metso, E. Repo, M. Veneranta, and E-L. Kiiskilä for technical assistance.

References