INDUCED SPUTUM – A METHOD FOR CYTOLOGIC ANALYSIS OF BRONCHIAL SPECIMENS

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SUMMARY – Sputum induction by inhalation of hypertonic saline is a noninvasive method to obtain secretions from the lower respiratory tract for cell counting and bacteriologic analyses. The aim of this study was to introduce the method of induced sputum in our clinical practice and to get an insight into airway inflammation by examining cell count differences in 15 asthmatic patients, 30 chronic obstructive pulmonary disease (COPD) patients and 15 healthy controls. Gradually increasing concentrations (3%, 4%, 5%) of hypertonic saline were inhaled. Subjects were encouraged to cough in order to expectorate. The quality of the sample was scored on the volume of the plugs (considered to represent lower respiratory tract secretion) and salivary contamination (proportion of squamous cells in the slides). A sample score ≥4 was considered adequate, 3 intermediate, and ≤2 inadequate. Cytologic sputum analysis was done after microscopic selection of the plugs. The results showed that inhalation of hypertonic saline is a safe method to get adequate sample for investigating various inflammatory mechanisms in lower airways. There were differences in sputum from three groups of subjects, i.e. a higher proportion of neutrophils in COPD (32%) in comparison to asthmatics (15%) and healthy controls (10%). The percentage of alveolar macrophages also differed (COPD 56.5%, asthmatics 46%, healthy 40%). Asthmatic patients had a higher proportion of eosinophils (asthmatics 18.5%, COPD 1.9%, healthy 0.06%) and metachromatic cells (asthmatics 0.3%, COPD 0.039%, healthy 0.014%).

Key words: Sputum, cytology; Pulmonary disease – chronic obstructive, diagnosis; Asthma, diagnosis

Introduction

The analysis of cells and mediators in the airway lining fluid from patients with pulmonary diseases has traditionally been performed in samples of spontaneously expectorated sputum or on the fluid obtained by bronchoscopy and lavage. Both sampling methods are limited in their applicability. Many subjects are unable to produce sputum spontaneously. Bronchoscopy can only be employed to collect airway samples from study subjects who are well enough to tolerate it. In addition, bronchoscopy cannot easily be applied repeatedly to follow the evolution of changes in airway lining fluid over short periods of time.

Inhalation of hypertonic saline to produce sputum was introduced almost four decades ago by Bickerman et al.¹. However, it was only for the last 8 years that the method has been used to assess the indices of airway inflammation. The results have shown the method to be suitable, valid, reliable²,³, and responsive⁴. The noninvasive nature of the procedure is important because repeated examinations are needed to learn more about the causes and treatment of airway diseases. The mechanism by which hypertonic saline induces sputum is not known. It may involve
an osmotic effect\textsuperscript{5,6}, increase in the mucociliary clearance\textsuperscript{7,8}, or stimulation of glandular secretions.

There is evidence for inflammation in chronic obstructive pulmonary disease (COPD) but aspects of the inflammation have not been studied as much as those in asthma\textsuperscript{9}. The study of Pin \textit{et al.} resembles most of other published analyses of induced sputum in asthmatic subjects\textsuperscript{10}. Evidence for bronchial inflammation have been shown\textsuperscript{11}. The aim of this study was to introduce the method of induced sputum in our clinical practice and to compare cellular characteristics of the specimens of COPD and asthma patients, and healthy controls.

\section*{Subjects and Methods}

\subsection*{Subjects}

Subject characteristics are shown in Table 1. The study population comprised 30 smokers with COPD, 15 stable asthmatics, and 15 healthy subjects. Subjects with COPD were current smokers (smoking is the main feature of COPD) with a history of productive cough on most mornings for more than 3 months/year for two consecutive years, and forced expiratory volume in first second (FEV\textsubscript{1}) <70\% of the predicted value. Asthma was identified by a history of asthma symptoms and previous physician diagnosis, and objectively confirmed by metacholine airway hyperresponsiveness (PC <2 mg/ml). Healthy subjects had no history of respiratory disease, and had FEV\textsubscript{1} >81\% of the predicted value.

\subsection*{Study design}

Study subjects attended the laboratory for 3 days within a 2-week period, at the same time of the day. On the first visit, subject characteristics were documented by a questionnaire, spirometry (Jaeger body plethysmography), and allergy skin prick tests. Then they were instructed to record peak expiratory flow (PEF) in the morning and in the evening, for 2 weeks, and to enter values in a diary. On the second and third visit, the diary was checked, spirometry was performed, and sputum was induced. If sputum could not be obtained on one of two consecutive visits, another visit was scheduled and, if unsuccessful, the subject was excluded.

\subsection*{Sputum processing}

The reservoir of a Sonix 2000 ultrasonic nebulizer was filled with 30 ml of sterile 3\% saline, and all subjects inhaled the nebulized solution for 10 min. The nebulizer generates particles with a mean mass diameter of 3.9 mm and output of 3.5 ml/min. The concentration of saline was increased at 10-min intervals from 3\% through 4\% to 5\%. If the PEF, which was measured after each concentration, fell by >20\% from the starting value, or if troublesome symptoms occurred, nebulization was discontinued. Subjects were encouraged to cough throughout the procedure, and they regularly interrupted inhalation of hypertonic saline in order to expectorate sputum into a Petri dish, where its macroscopic characteristics were recorded. Sputum was processed as soon as possible within 2 h. The quality of samples was assessed by estimating the volume of lower respiratory tract secretions and the degree of salivary contamination, as described by Pin \textit{et al.}\textsuperscript{12}. The quality of sputum sample was scored by: (a) visual inspection and inverted microscope examination (no plugs=0, ≤4.5x9 mm= 1, >4.5x9 mm = 2); (b) salivary contamination in total cell count defined as percentage of squamous cells among nucleated cells (>10\%=0, ≤10\%=1, 0\%=2); and (c)
salivary contamination in differential cell count by assessing the proportion of squamous cells (too many squamous cells to permit counting=0, enough squamous cells to permit counting=1, no squamous cells=2). A sample score ≥4 was considered adequate, 3 intermediate, and ≤2 inadequate. Freshly prepared trypsin at a dilution of 1:10 in distilled water was added to the sample for homogenization. The samples were gently mixed and placed in the incubator at 37 °C for 2 hours. Ten microliters of the homogenized sputum were used to determine total cell count using a standard hemacytometer. The remainder of the homogenized sputum and saliva was centrifuged at 2000 rpm for 10 min. Cell pellets were cytocentrifuged. Differential cell counts were performed by counting 400 nucleated cells on each of two slides fixed with methanol and stained with May-Grünwald-Giemsa. Two further slides were stained with 0.5% toluidine blue in 0.7 N hydrochloric acid at pH 0.1, and 1500 nucleated cells were counted on each to obtain a differential count of metachromatic cells. The last two slides were fixed with phosphate buffer and stained with diluted Giemsa solution for eosinophil count analysis.

**Statistical analysis**

Differential and total cell counts were calculated as arithmetic means with 95% confidence intervals. Two-tailed unpaired t-tests were used to compare cell counts between groups. Significance was accepted at the 95% level.

**Results**

The procedure was well tolerated in 57 patients. Significant fall in PEF was observed in three patients, and the induction was stopped. Adequate samples (one sample of each concentration) were produced in 51 patients, whereas six patients produced inadequate sputum samples.

Differences in total and differential cell counts in induced sputum between study groups are shown in Table 2 and Fig. 1. There was no difference between the subjects with COPD, asthmatics and normal subjects in total cell counts, lymphocytes and bronchial epithelial cells. In comparison with asthmatics and healthy subjects, the sputum from COPD patients contained higher proportions of neutrophils (COPD 32%, asthmatics 15%, healthy 10%) and alveolar macrophages (COPD 56.5%, asthmatics 46%, healthy 40%). Asthmatic patients had significantly higher...
of airway inflammation. The results of sputum examination in asthmatic and COPD patients show differences in inflammatory cells, which suggests different pathophysiological mechanisms and helps in differential diagnosis.

References

INDUCIRANI SPUTUM – SUVREMENA METODA ZA CITOLOŠKU ANALIZU BRONHALNIH UZORAKA

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Inducirani sputum je metoda potpomognutog iskašljavanja putem inhaliranja rastućih koncentracija (3%, 4%, 5%) hipertonične otopine NaCl. Tim neinvazivnim načinom mogu se izbjeći invazivnije dijagnostičke metode poput bronhoskopije, a dobiti prikladni uzorci sekreta iz donjih dišnih putova. Cilj ovoga istraživanja bio je: a) uvesti metodu induciranog sputuma u našu kliničku praksu, b) ispitati njenu sigurnost i uspješnost indukcije i c) usporediti ukupni i diferencijalni broj stanica u 15 bolesnika s astmom, 30 s KOPB i 15 zdravih ispitanika. Kvaliteta sputuma procjenjivana je na temelju količine bronhalnih odljevaka (čepići koji dokazuju da se radi o sekretu iz donjeg dišnog sustava) te udjela stanica pljačastog epitela (dokaz da uzorak čini slina). Bodovi ≥4 pokazuju da je uzorak prikladan, 3 da je srednje kvalitete, a bodovi ≤2 da nije prikladan za analizu. Rezultati su pokazali da je inhalacija hipertonične otopine sigurna metoda za dobivanje prikladnih uzoraka kojima se može dobiti uvid u različite upalne mehanizme kod bolesnika s opstruktnim plućnim bolestenima. U bolesnika s KOPB utvrđen je značajno veći udio neutrofila (KOPB 32%, astma 15%, zdravi 10%) i alveolarnih makrofaga (KOPB 56,5%, astma 46%, zdravi 40%) u odnosu na druge dvije ispitivane skupine. U bolesnika s astmom utvrđen je značajno veći udio eozinofila (astma 18,5%, KOPB 1,9%, zdravi 0,06%) i metakromatskih stanica (astma 0,3%, KOPB 0,039%, zdravi 0,014%).

Ključne riječi: Sputum, citologija; Plućne bolesti – kronične opstrukтивne, dijagnostika; Astma, dijagnostika