

# Original Article

## Influence of oscillating positive expiratory pressure and the forced expiratory technique on sputum cell counts and quantity of induced sputum in patients with asthma or chronic obstructive pulmonary disease\*

Influência da técnica de pressão expiratória positiva oscilante e da técnica de expiração forçada na contagem de células e quantidade do escarro induzido em portadores de asma ou doença pulmonar obstrutiva crônica

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### Abstract

**Objective:** To evaluate whether respiratory therapy techniques influence the number of cells within and quantity of induced sputum in patients with asthma or chronic obstructive pulmonary disease (COPD). **Methods:** Randomized clinical trial, in which patients with asthma or COPD under intervention (n = 16 and 10, respectively) were compared with control groups (n = 16 and 10). Patients in the asthma/intervention (A/I) and COPD/intervention (C/I) groups were submitted to oscillating positive expiratory pressure maneuvers for 5 min, followed by 10 forced expiratory technique sequences. These patients were also submitted to an induced sputum protocol with inhaled hypertonic saline (3%, 4% or 5%; A/I group) or inhaled isotonic saline (C/I group). The asthma/control (A/C) and COPD/control (C/C) groups were submitted only to the standard induced sputum protocol. **Results:** The final mean weight of the sputum samples was significantly greater in the A/I group than in the A/C group (2,767.25 ± 998.08 mg vs. 1,689.17 ± 1,189.96 mg; p = 0.03). The mean/median total cell counts (×10<sup>6</sup>/mL) were higher in the A/I and C/I groups than in the A/C and C/C groups (4.06/0.95 and 0.63/0.39, p = 0.05, vs. 5.08/1.77 and 0.64/0.40, p = 0.02). There were no statistically significant differences among the groups in terms of cell viability. **Conclusions:** The use of respiratory therapy techniques can increase sputum sample weight in asthma patients, as well as increasing total cell counts in patients with asthma or COPD.

**Keywords:** Asthma; Pulmonary disease, obstructive chronic; Sputum; Physical therapy modalities.

### Resumo

**Objetivo:** Avaliar se técnicas fisioterápicas interferem no número de células e na quantidade do escarro obtido por coleta induzida, em pacientes com asma e doença pulmonar obstrutiva crônica (DPOC). **Métodos:** Ensaio clínico prospectivo e randomizado, no qual os pacientes com asma ou DPOC sob intervenção (n = 16 e 10, respectivamente) foram comparados com grupos controle (n = 16 e 10). Pacientes dos grupos asma/intervenção (A/I) e DPOC/intervenção (D/I) foram submetidos a manobras de pressão expiratória positiva oscilante por 5 min, seguidas de 10 repetições da técnica de expiração forçada. Além disso, esses pacientes foram submetidos a um protocolo de indução de escarro com a inalação de solução salina hipertônica (3%, 4% e 5%), no caso dos A/I, e de solução salina isotônica, no caso dos D/I. Os grupos asma/controle (A/C) e DPOC/controle (D/C) foram somente submetidos ao protocolo padrão de indução de escarro. **Resultados:** Houve aumento significativo do peso média final de escarro no grupo A/I vs. grupo A/C (2.767,25 ± 998,08 mg e 1.689,17 ± 1.189,96 mg, respectivamente; p = 0,03). O número absoluto de células (×10<sup>6</sup>/mL) foi maior nos grupos A/I e D/I do que nos grupos A/C e D/C (média/mediana, 4,06/0,95 e 0,63/0,39, respectivamente; p = 0,05; e 5,08/1,77 e 0,64/0,40; p = 0,02). A viabilidade celular não apresentou diferença estatisticamente significativa entre os grupos. **Conclusões:** O uso de técnicas respiratórias pode aumentar o peso do escarro em pacientes com asma, assim como aumentar o número absoluto de células em pacientes com asma ou DPOC.

**Descritores:** Asma; Doença pulmonar obstrutiva crônica; Escarro; Modalidades de fisioterapia.

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## Introduction

Chronic inflammatory diseases with airflow limitation, bronchial hyperresponsiveness and inflammation cause dyspnea, cough and wheezing, which are considered nonspecific respiratory symptoms. Airway diseases can be evaluated by spirometry, as well as by measuring peak expiratory flow and bronchial responsiveness.<sup>(1)</sup> In addition, some objective measurements can be used to evaluate the relationship between the alterations observed in ventilatory function and airway inflammation.

The analysis of induced sputum samples can contribute to the evaluation of this inflammatory process by allowing the characterization of cells and cytokines. The induced sputum technique represents a valid, viable, reproducible and minimally invasive tool.<sup>(2)</sup> However, it can be difficult to obtain sputum samples from patients with stable asthma as well as from certain patients with chronic obstructive pulmonary disease (COPD).

It is known that respiratory therapy, through the use of specific techniques and maneuvers, can promote mucus clearance.<sup>(3)</sup> In addition, respiratory therapy maneuvers, such as therapy with oscillating positive expiratory pressure (OPEP) and the forced expiratory technique (FET) or huffing, are used for secretion displacement and expectoration in patients with airway hypersecretion.<sup>(3)</sup> These two respiratory therapy techniques are routinely recommended for the bronchial hygiene of patients with hypersecretion and are typically successful.<sup>(3,4)</sup> However, there is no evidence that respiratory therapy techniques can be adjunct tools in sputum collection for the analysis of the inflammatory process. Therefore, the objective of this study was to evaluate whether these respiratory therapy techniques influence the quantity of and number of cells within induced sputum in patients with asthma or COPD, with or without hypersecretion.

## Methods

Individuals with stable asthma, defined based on the Guidelines for the Diagnosis and Management of Asthma Update on Selected Topics,<sup>(5)</sup> and individuals with COPD (classified as stage III), according to the Global Initiative for Chronic Obstructive Lung Disease criteria,<sup>(6)</sup> all treated at the Pulmonology Outpatient Clinic of the Department of Medicine of the São Paulo Hospital, Federal University of São Paulo School of

Medicine, São Paulo, Brazil, were selected for inclusion in the study. The study was approved by ethics in research committee of the institution.

After giving written informed consent, the patients who met the following inclusion criteria were selected: being 18 years of age or older; having no other lung diseases or severe diseases; and being able to complete the cough score questionnaire, as well as to cooperate with pulmonary function tests and procedures for sputum induction/collection. The exclusion criteria were as follows: being pregnant; currently breastfeeding; being a smoker; using supplemental oxygen; being suspected of having ischemic heart disease; having recently undergone surgery; having a chronic consumptive disease; having participated in pulmonary rehabilitation programs; being suspected of having steroid-induced myopathy; presenting exacerbation of rhinitis; and having active gastroesophageal reflux disease.

In this clinical trial, the patients were assigned, by random drawing, to one of the two procedures: a modified protocol, which consisted in the use of the standard protocol in conjunction with two respiratory therapy techniques during the procedure; or the standard protocol.<sup>(7)</sup>

The patients were categorized into four groups: COPD/intervention; COPD/control; asthma intervention; and asthma/control. Each group was evaluated in terms of the following variables: age; gender; final cough score; forced expiratory volume in one second (FEV<sub>1</sub>); forced vital capacity (FVC); FEV<sub>1</sub>/FVC ratio prior to (for patients with asthma) or after (for patients with COPD) the use of bronchodilators; final weight of the sputum samples after filtering and dilution; total cell counts ( $\times 10^6$ /mL); cell viability (%); time required to obtain sputum samples; peripheral oxygen saturation (SpO<sub>2</sub>); and heart rate (HR).

Before starting sputum induction, the patients completed the cough questionnaire<sup>(8)</sup> on daytime and nighttime symptoms. The questionnaire was administered by the researcher, and the maximum score for each symptom was five points.

All patients underwent evaluation of pulmonary function using spirometry, which was conducted in accordance with the Second Brazilian Consensus on Spirometry (2002).<sup>(9)</sup> Prebronchodilator and post-bronchodilator FVC, FEV<sub>1</sub> and FVC/FEV<sub>1</sub> values were determined using a Koko pneumotachograph and the accompanying PFT software program for

Windows, version 4.1 (PDS Instrumentation, Inc., Louisville, CO, USA). After evaluation of pulmonary function, the patients were instructed to perform nasal and oral hygiene, as well as to drink water, in preparation for the subsequent initiation of sputum induction and collection.

The OPEP can be applied using a pipe-shaped mucus clearance device (Flutter®; Scandipharm, Birmingham, AL, USA). The Flutter is a portable device containing a conical channel, within which there is a metallic sphere. When the patient exhales through the device, the expired airflow raises the metallic sphere, which falls again under its own weight. The rapid succession of these events (risings and fallings of the sphere) makes the air vibrate within the device. This vibration is transmitted to the patient rib cage and tracheobronchial tree, displacing the secretions and facilitating expectoration. Therefore, this device uses the force of gravity to increase or decrease positive pressure. The patients were instructed to use the device while sitting and to hold it firmly, maintaining the mouthpiece horizontal. Lips around the mouthpiece and maintaining the device horizontal, patients performed a deep (unforced) inhalation through the nose and an exhalation through the mouth. During this process, maximal and minimal airway pressure ranges from

20 to 25 cmH<sub>2</sub>O and from 0.8 to 2.5 cmH<sub>2</sub>O, respectively.<sup>(10,11)</sup> At the end of the process, the patients were instructed to stimulate expectoration through efficacious coughing.

The FET or huffing consists of one or two expiratory efforts (huffs), similar to those carried out during peak expiratory flow measurement, performed with an open glottis. A forced expiratory maneuver at medium lung volume until reaching low lung volume was required, followed by a relaxation period, preferably with diaphragmatic breathing.<sup>(4)</sup> The patients, maintained in a sitting position, were advised to expel the sputum through efficacious coughing after the FET maneuver.

#### ***Induced sputum collection: modified protocol***

Sputum collection was performed using an induction method previously described.<sup>(7)</sup> The patients were monitored throughout the protocol period using a portable pulse oximeter (model 9500; Nonin Medical Inc., Minneapolis, MN, USA) to determine SpO<sub>2</sub> and HR.

In order to induce sputum, the patients received 9 mL of inhaled hypertonic saline at 3% (a higher concentration, maximum of 5%, was used if necessary) for 7 min by means of an ultrasonic nebulizer (DeVilbiss, Somerset, PA, USA). Subsequently,

**Table 1** – Characteristics of the patients studied.

Clinical variable	Asthma/intervention	Asthma/control	p
n	16	16	-
Age <sup>a</sup> , years	43.31 ± 13.81	43.69 ± 16.60	0.95*
Gender, F/M	9/6	10/7	1.00**
Pre-BD FEV <sub>1</sub> <sup>a</sup> , L	2.30 ± 0.68	2.10 ± 0.70	0.42*
Pre-BD FVC <sup>a</sup> , L	3.46 ± 0.79	3.24 ± 0.96	0.48*
Pre-BD FVC/FEV <sub>1</sub> <sup>a</sup>	66.50 ± 12.89	64.90 ± 9.95	0.70*
Post-BD FEV <sub>1</sub> <sup>a</sup> , L	2.45 ± 0.68	2.43 ± 0.74	0.94*
Post-BD FVC <sup>a</sup> , L	3.62 ± 0.70	3.48 ± 1.07	0.68*
Post-BD FVC/FEV <sub>1</sub> <sup>a</sup>	67.56 ± 12.47	70.29 ± 9.70	0.50*
Clinical variable	COPD/intervention	COPD/control	p
n	10	10	-
Age <sup>a</sup> , years	66.00 ± 6.46	63.70 ± 7.21	0.46*
Gender, M/F	1/9	1/9	1.00**
Post-BD FEV <sub>1</sub> <sup>a</sup> , L	1.30 ± 0.36	1.54 ± 0.63	0.32*
Post-BD FVC <sup>a</sup> , L	2.71 ± 0.80	3.13 ± 0.85	0.27*
Post-BD FVC/FEV <sub>1</sub> <sup>a</sup>	49.10 ± 7.72	47.90 ± 9.39	0.76*

FEV<sub>1</sub>: forced expiratory volume in one second; BD: bronchodilator; FVC: forced vital capacity; FVC/FEV<sub>1</sub>: FVC/FEV<sub>1</sub> ratio, and COPD: chronic obstructive pulmonary disease.<sup>a</sup>Values expressed as mean and standard deviation. \*Chi-square test; and \*\*Student's t-test.

the patient, sitting straight and maintaining the device horizontal, was instructed to make calm but prolonged exhalations for 5 consecutive min, using OPEP maneuvers. The patient was asked to cough vigorously, subsequently expectorating in a disposable collection container. This expectorated material was placed in a disposable Petri dish, where it was evaluated, and the procedure was interrupted when the weight of the thickest or most viscous portion of the sputum sample was equal to or greater than 100 mg, the event being considered positive. Weight was measured using an analytical precision scale (Gehaka, São Paulo, Brazil). This material was then processed (dilutions and filtering), according to the technique described by other authors,<sup>(7,12,13)</sup> and weighed again, this being considered its final weight. If the expectorated material, after the maneuver using the device, was not considered satisfactory, FET was performed. The FET consisted of 10 expiratory maneuvers at 30-s intervals, followed by vigorous cough and expectoration. If the quantity of material obtained was satisfactory, the procedure was interrupted. Otherwise, 4% saline was administered via inhalation, and the entire procedure described previously was repeated. This continued, with successive concentrations of inhaled saline (maximum, 5%). The test was considered a negative event if, in the final phase, the patient could not expectorate the necessary quantity.

After each inhalation, FEV<sub>1</sub> was measured. If there was a greater than 20% decrease in FEV<sub>1</sub>, sputum induction was interrupted. If the decrease in FEV<sub>1</sub> was between 10% and 20%, the saline concentration used in the last inhalation was maintained. It is important to emphasize that sequential variation in

saline concentration was used only in the group of patients with asthma, since, in the group of patients with COPD, the saline concentration, in the successive inhalations, was fixed at 0.9%.

The interval between the performance of each respiratory therapy technique and the production of the sputum sample was timed: after the first inhalation of saline solution and OPEP, 15 min; after the first FET, 20 min; after the second inhalation, 30 min; after the second OPEP, 35 min; after the second FET, 40 min; after the third inhalation, 50 min; after the third OPEP, 55 min; and after the third FET, 60 min. The maximum time was 60 min.

The material was processed by an experienced laboratory technician, who was blinded to the protocol performed to obtain sputum samples. This procedure was started within the first 2 h, as recommended by some authors.<sup>(7,12,13)</sup>

After the expectorated material had been collected, the patients were observed clinically for a minimum of 30 min.

### ***Induced sputum collection and processing: standard protocol***

The procedures for collecting and processing the material were performed using a method previously described.<sup>(7,12,13)</sup> Although the induced sputum protocol was identical to that previously presented, the respiratory therapy techniques described above were not employed.

### ***Statistical analysis***

The variables were presented as mean and standard deviation values for parametric data, as

**Table 2** - Sputum sample weight, cell viability and total cell counts in the groups studied.

Variable studied	Asthma/intervention	Asthma/control	p
n	12	12	
Sample weight <sup>a</sup> , mg	2.767.25 ± 998.08	1.689.17 ± 1.189.96	0.03*
Total cell counts <sup>b</sup> , ×10 <sup>6</sup> /mL	4.06 (0.95)	0.63 (0.39)	0.05**
Cell viability <sup>b</sup> , %	56.00 (59.00)	43.17 (48.50)	0.10*
Variable studied	COPD/intervention	COPD/control	p
n	10	10	
Sample weight <sup>a</sup> , mg	3.457.10 ± 1.721.29	2.805.20 ± 1.752.28	0.41*
Total cell counts <sup>b</sup> , ×10 <sup>6</sup> /mL	5.08 (1.77)	0.64 (0.40)	0.02**
Cell viability <sup>b</sup> , %	67.30 (69.50)	58.80 (55.50)	0.23**

COPD: chronic obstructive pulmonary disease. <sup>a</sup>Values expressed as mean ± SD; and <sup>b</sup>values expressed as mean (median). \*Student's t-test; and \*\*Mann-Whitney test.

median for nonparametric data and as percentages for categorical data. Numerical independent variables were compared using the unpaired Student's t-test. Preintervention and postintervention data were analyzed using the paired Student's t test.

Spearman's correlation coefficient was used to analyze how well the time required to obtain sputum samples correlated with cell viability and with total cell counts.

In order to compare the time required to obtain sputum samples from patients under intervention (asthma/intervention and COPD/intervention groups), Kaplan-Meier curves were generated. In those curves, the event was defined as obtaining an adequate quantity of sputum. The log-rank test was used in the analysis of the significance of the curves obtained.

The level of statistical significance required to reject the null hypothesis was set at  $p < 0.05$ .

## Results

In this prospective and randomized clinical trial, 52 patients were studied. Of those, 32 had asthma (16 in the intervention group and 16 in the control group) and 20 had COPD (10 in the intervention group and 10 in the control group). All COPD patients were successful in producing an adequate quantity of sputum for analysis, whereas only 75% of the patients with asthma produced a quantity of sputum sufficient for analysis. None of the patients included in the study presented a greater than 20% decrease in  $FEV_1$  during any of the study phases.

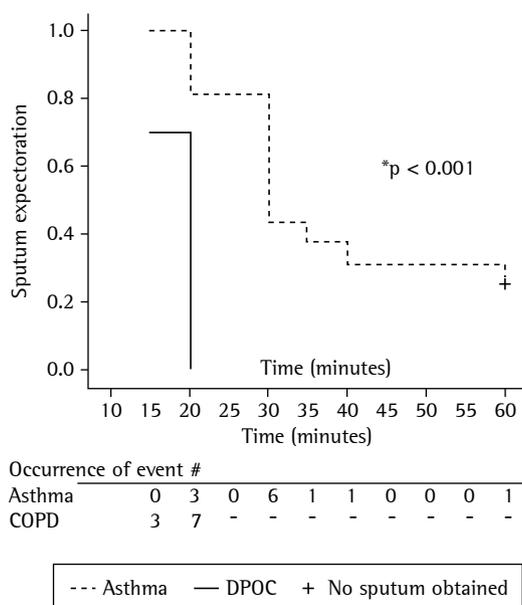
Table 1 presents the characteristics of the patients studied.

Daytime and nighttime cough scores were lower in the patients with asthma than in the patients with COPD (median values, 1 and 2, respectively;  $p < 0.01$ ).

Table 2 presents the results regarding final sputum sample weight, total cell counts and cell viability in the patients studied.

There was no correlation between cell viability and total cell counts over time with the use of the techniques in the groups.

Using the Kaplan-Meier curves, we observed that all patients with COPD managed to expectorate adequately within the first 20 min of study. However, among the patients with asthma, 4 (25%) reached the end of the intervention (60 min) without



**Figure 1** – Analysis of the Kaplan-Meier curves generated by comparing the time required to obtain sputum samples from patients with asthma and patients with chronic obstructive pulmonary disease (COPD) using the \*log-rank test (event # = adequate quantity of sputum).

producing an adequate quantity of sputum. The time required to obtain sputum samples was significantly shorter in the COPD group ( $p < 0.0001$ ; Figure 1).

## Discussion

In the present study, we demonstrated that OPEP and FET were efficient in inducing sputum for collection, increasing the final weight of the material expectorated by the patients with asthma.

In the literature, there are no studies describing associations between respiratory therapy techniques and increased total cell counts in induced sputum protocols. In our study, we observed a statistically significant postintervention increase in total sputum cell counts in the asthma group and in the COPD group (Table 2).

We also observed that the respiratory therapy maneuvers did not influence sputum cell viability.

The patients included in our study were fully comparable to the controls in terms of gender, age and pulmonary function, demonstrating the validity of the results observed (Table 1).

The pulmonary function profile was more severe in the patients with COPD, who presented lower

postbronchodilator FEV<sub>1</sub>, FVC and FEV<sub>1</sub>/FVC values, this being consistent with the character of the disease (stage III).

The respiratory therapy techniques used in the present study are routinely recommended and typically successful for bronchial hygiene in patients with hypersecretion.<sup>(3)</sup> In the literature, there are various studies evaluating the use and effectiveness of respiratory therapy techniques in patients with cystic fibrosis,<sup>(3,14)</sup> asthma<sup>(15,16)</sup> and COPD.<sup>(17-19)</sup> However, there have been no studies analyzing the use of these interventions to obtain induced sputum.

One group of authors<sup>(16)</sup> studied 20 patients with asthma, over a two-week period, using OPEP, and observed a significant increase in the quantity of sputum obtained after six days of therapy. In our study, we observed that, among the patients with asthma, the expectoration volume and baseline cough scores were lower than those found in patients with COPD. However, only 75% of the patients in the asthma/intervention group were successful in producing an adequate quantity of induced sputum, with a significant increase in the final sample weight.

It has been reported that FET can induce bronchospasm in individuals with asthma if it is not accompanied by relaxation periods.<sup>(20)</sup> In our study, the recommended periods of relaxation were respected, and none of the patients with asthma studied presented bronchospasm after FET, as confirmed by monitoring FEV<sub>1</sub>. These patients were also monitored by means of pulse oximetry throughout the intervention and presented no significant alterations in this parameter.

In studies on the effectiveness of respiratory therapy techniques in promoting expectoration, the patients most often studied are those with cystic fibrosis. One group of authors<sup>(21)</sup> found that, in patients with cystic fibrosis, there was no difference in terms of sputum volume or FEV<sub>1</sub> between the use of OPEP and the use autogenic drainage, although mucus viscosity was lower after the use of the former. Other authors<sup>(22)</sup> observed an increase in sputum volume after the use of OPEP in patients with cystic fibrosis, when compared with the use of postural drainage and the use of pure cough.

In one study,<sup>(17)</sup> patients with airway obstruction and abundant secretion, without cystic fibrosis, in whom respiratory therapy techniques (postural

drainage and thoracic percussion) were used, were compared with patients in whom only OPEP was used. The quantity of sputum was found to be significantly increased in the two groups studied. In our study, the COPD group was expected to present a greater quantity of sputum and a better response to the stimuli provided by the respiratory therapy techniques, since this group presents bronchial hypersecretion, as well as higher daytime and nighttime cough scores. However, there was no statistically significant difference in terms of the weight or viability of the material.

Other authors,<sup>(19)</sup> in a study involving in patients with chronic bronchitis, compared the effects of postural drainage, OPEP (using the Flutter device), and slow exhalation with an open glottis in the lateral decubitus position. The authors observed an increase in sputum production, obtained 30 min after the intervention, for all of the respiratory therapy techniques evaluated. The increase in sputum production was more significant when the OPEP technique was used. In our study, the mean interval between the performance of an intervention and the production of a sputum sample was shorter in the patients with COPD (20 min) than in the patients with asthma (Figure 1).

The inclusion of the two respiratory therapy techniques in an induced sputum protocol was efficient in terms of increasing final sputum sample weight in patients with asthma, also increasing total inflammatory cell counts for the analysis of the samples from patients with asthma or COPD. This represented an improvement in the induced sputum method conventionally used, since some induced sputum tests are excluded due to the small volume of material obtained. During the study period, there were no episodes of bronchospasm, nor were there any alterations in SpO<sub>2</sub> or HR.

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