

Sputum Induction in Children and Adolescents with Problematic Severe Asthma: Success Rate, Safety and Tolerability

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Abstract: *Background:* In problematic severe asthma (PSA), inflammatory phenotypes can be identified by assessing cellularity in induced sputum (IS) samples. However, there have been few studies employing sputum induction (SI) in pediatric patients.

Objective: To assess the success rate, safety and tolerability of SI, as well as IS sample cellularity, in pediatric PSA patients.

Methods: We conducted a cross-sectional study involving 44 pediatric PSA patients. We collected IS samples using inhalations of nebulized saline solution. On the basis of the post-bronchodilator forced expiratory volume in one second (FEV₁, % of predicted), we administered nebulization with 4.5% hypertonic saline (for patients with an FEV₁ ≥ 60%) or 0.9% isotonic saline (for those with an FEV₁ < 60%). We classified IS samples as satisfactory if there was ≤ 20% squamous cell contamination and cell viability was > 50%.

Results: The observed success rate was 75% (95% CI: 60-86). Most of the patients provided satisfactory samples, although multiple SI sessions were required in some cases (27%). In comparison with the IS samples containing > 20% squamous cells, those containing ≤ 20% showed significantly more neutrophils (P = 0.02) and eosinophils (P = 0.03). The most common adverse events were mild wheezing (in 14%) and salty taste (in 9%). In 8% of the sessions, there was a ≥ 20% decrease in FEV₁.

Conclusion: In our sample of pediatric patients with PSA, sputum induction was safe and generally well tolerated, suggesting that it could be useful in the assessment of inflammatory processes in such patients.

Keywords: Adolescent, asthma, child, eosinophils, inflammation, sputum, safety.

INTRODUCTION

In most children and adolescents with asthma, the symptoms can be controlled with low doses of inhaled corticosteroids. However, patients with problematic severe asthma (PSA) show high morbidity and consume a disproportionate amount of health care resources [1]. The definition of PSA is the persistence of chronic symptoms or severe exacerbations in patients who are using 800 µg of budesonide (or the equivalent) in association with a long-acting bronchodilator or antileukotriene agent [1-4].

To date, sputum induction is the only noninvasive measure of airway inflammation that has a proven role in the

management of moderate-to-severe asthma, and regular monitoring of airway inflammation is required for the optimal treatment of asthma patients [5]. Protocols that have been recommended for PSA patients include assessing the inflammatory process by examining the cellularity of induced sputum samples in order to identify the inflammatory phenotype on the basis of its response to corticosteroid therapy. If the induced sputum sample contains < 2.5% eosinophils, it may be possible to reduce the dose of inhaled corticosteroids [4, 6]. In children with severe refractory asthma, the proportion of eosinophils in sputum samples can be as high as 7.5%, significantly higher than that observed in controls [7]. In a recent study evaluating the effectiveness of thymic stromal lymphopoietin in decreasing allergic responses, the authors used induced sputum sample cellularity as an end point [8]. The authors found a reduction of the sputum eosinophil levels, demonstrating clinical applicability of sputum induction. Other researchers have monitored

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sputum eosinophil levels in order to identify the risk factors for asthma exacerbations [9].

Sputum induction has begun to move from the research context to clinical practice [5]. However, there have been few studies of the use of sputum induction in children with PSA, and its safety and feasibility in this subgroup of asthma patients must be established before it can be routinely used in clinical practice and research [10]. The only such study conducted in a developed country involved children with difficult asthma and had the objective of determining the success rate, safety and tolerability of the technique, using a high-output ultrasonic nebulizer [10]. The authors reported a success rate of 74%. In developing countries, the morbidity associated severe asthma is disproportionately higher than that reported for developed countries [5].

The objective of the present study was to ascertain the success rate, safety and tolerability of sputum induction, as well as to determine the cellularity of induced sputum samples, in children and adolescents with PSA.

MATERIALS AND METHODOLOGY

This was a cross-sectional study involving a non-probability sample of pediatric patients diagnosed with PSA and treated at the University Hospital operated by the Federal University of Minas Gerais, in Belo Horizonte, Brazil, or at the Hospital São Lucas, operated by the Pontifical Catholic University of Rio Grande do Sul, in Porto Alegre, Brazil. The Research Ethics Committees of the Federal University of Minas Gerais and the Pontifical Catholic University of Rio Grande do Sul approved the research project (Protocols no. 0449.0203.000-10 and 10/5084, respectively). All participating patients (or their parents or legal guardians) gave written informed consent.

To be included in the study, the patients had to have been monitored for at least six months, and during that time they had to have exhibited persistent chronic symptoms or frequent exacerbations despite using at least 800 µg of budesonide (or the equivalent) in combination with a long-acting bronchodilator or antileukotriene agent [1]. We excluded patients with cystic fibrosis, bronchiolitis obliterans (post-infectious or post-transplant), immunodeficiency (congenital or acquired), heart disease, α 1-antitrypsin deficiency, neurological diseases, as well as those who had been born prematurely.

SPUTUM INDUCTION PROTOCOL

Spirometry

Using a Spirobank II spirometer (MIR, Rome, Italy), we assessed spirometric parameters before and after the administration of a bronchodilator (400 µg albuterol) [11], obtaining the values for forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), peak expiratory flow (PEF), the FEV₁/FVC ratio and forced expiratory flow between 25% and 75% of FVC (FEF₂₅₋₇₅). We performed all

spirometry tests in accordance with the reproducibility and acceptability criteria of the American Thoracic Society [12]. We conducted functional tests on the same day on which we collected the sputum samples. We considered a bronchodilator test positive when we observed an increase in FEV₁ of at least 12% or 200 mL [12]. We present the spirometric findings as percentages of the predicted values described by Polgar & Promadhat [13] and by Knudson *et al.* [14].

Sputum Induction

All of the patients underwent evaluation by respiratory therapists. In patients who were stable (i.e., those with post-bronchodilator FEV₁ values \geq 60% of the predicted value), we used nebulization with 4.5% hypertonic saline solution, whereas we used 0.9% isotonic saline solution in the patients with post-bronchodilator FEV₁ values $<$ 60% [15]. We prepared all solutions at the time of the procedure. We performed each induction session in four 5-min periods of nebulization, for a total of 20 min [11, 15]. The nebulization procedures follow the standards established by the European Respiratory Society [11, 15]. We used a low-output ultrasonic nebulizer (Pulmosonic Star Premium; Soniclear, São Paulo, Brazil) with a nose clip and a mouthpiece with a breathing valve. The output of the nebulizer was set between 0.75 and 1.25 mL/min, and the particle size (diameter) was set to $<$ 4 µm. Immediately after each induction session [11], we measured FEV₁, peripheral oxygen saturation with a pulse oximeter (Onyx 9500; Nonin, Plymouth, Minnesota, USA), respiratory rate and heart rate, as well as performing pulmonary auscultation. When we observed dyspnea, wheezing or a \geq 20% post-bronchodilator reduction in FEV₁, we suspended the session and administered 400 µg of inhaled albuterol [11]. After each nebulization, we asked the patients to rinse out their mouth and blow their nose in order to minimize the contamination of sputum by saliva. We then asked each patient to cough up sputum into a sterile wide-mouthed flask. We stored the flask containing the sputum sample in a refrigerator at 4°C until the time of processing (2 h after sampling) [16].

We conducted the entire sputum induction procedure in a room that was separate from the clinical examination area. Equipment disinfection procedures followed the standards established by the Committees for Nosocomial Infection Control of the two university hospitals involved [11]. Emergency equipment was available in case of bronchoconstriction. Patients who did not provide a satisfactory sample after the first induction underwent additional rounds of induction over a seven-day period until producing such a sample [17].

Processing of Sputum Samples

We processed induced sputum samples according to the recommendations of Pizzichini *et al.* [18] and Efthimiadis *et al.* [19]. We prepared at least four slides from each sample and stained each slide with May-Grünwald-Giemsa, after which we analyzed the slides using an optical microscope. We considered a sample satisfactory if it showed \leq 20% squamous cells and $>$ 50% cell viability [18, 20]. We

Table 1. Demographic, clinical and functional characteristics of pediatric patients with PSA.

Variable	(N = 44)
Female gender, n (%)	28 (64)
Age (years), mean \pm SD	12 \pm 3
Onset of symptoms (months), mean \pm SD	15 \pm 5
Age at diagnosis (months), mean \pm SD	42 \pm 10
Exacerbations in the last year, mean \pm SD	5 \pm 1
Pre-bronchodilator FVC (% of predicted), mean \pm SD	91 \pm 20
Post-bronchodilator FVC (% of predicted), mean \pm SD	96 \pm 22
Pre-bronchodilator FEV ₁ (% of predicted), mean \pm SD	77 \pm 21
Post-bronchodilator FEV ₁ (% of predicted), mean \pm SD	85 \pm 19
Pre-bronchodilator FEV ₁ /FVC (% of predicted), mean \pm SD	83 \pm 12
Post-bronchodilator FEV ₁ /FVC (% of predicted), mean \pm SD	88 \pm 12
Pre-bronchodilator FEF ₂₅₋₇₅ (% of predicted), mean \pm SD	57 \pm 25
Post-bronchodilator FEF ₂₅₋₇₅ (% of predicted), mean \pm SD	74 \pm 27

Table 2. Success rates according to the number of induction sessions in pediatric patients with PSA.

Induction Session	Successful Sputum Induction	
	n (%)	95% CI
First (N = 44)	24 (55)	39-69
Second (n = 14)	4 (29)	9-58
Third (n = 5)	2 (40)	7-82
Fourth (n = 3)	3 (100)	31-100
Total	33 (75)	60-86

determined cell viability by the trypan blue exclusion method, in which the number of cells that are transparent is multiplied by 100 and divided by the total number of cells (dead cells are stained blue) [20].

STATISTICAL ANALYSIS

We computed the means and standard deviations of all descriptive data obtained from this study sample. We performed comparative analyses using Student's t-tests, chi-square tests or Mann-Whitney tests, depending on the normality of the data distribution. We calculated the success rate by dividing the number of patients from whom satisfactory samples were obtained by the total number of patients [21]. We considered values of $P < 0.05$ statistically significant.

RESULTS

Table 1 shows the descriptive characteristics of the patients. Of the 44 patients with PSA who participated in this study, 28 (64%) were female. The rate of asthma-related morbidity in the last 12 months was high (mean, 5 exacerbations). The FEV₁ values showed clear obstructive disease, reaching 56% of the predicted values. However, FEF_{25-75%} was the index that showed the greatest post-bronchodilator reduction.

Table 2 shows the distribution of success rates according to the number of induction sessions. Of the 33 patients from whom we obtained satisfactory samples, 24 (73%) provided such samples in the first sputum induction session. The overall success rate was 75% (95% CI: 60-86). We observed no statistically significant differences between the patients from

Table 3. Adverse events and FEV₁ after sputum induction in pediatric patients with PSA (N=44).

Variable	n (%)
Wheezing	6 (14)
Sore throat	2 (5)
Salty taste	4 (9)
Nausea	2 (5)
Vertigo	1 (2)
Total number of patients with adverse effects	15 (34)
Number of induction sessions in which there was a $\geq 20\%$ decrease in FEV ₁	5 (8)*

*Based on the total number of induction sessions (N = 66).

Table 4. Cellularity of induced sputum samples in pediatric patients with PSA.

Cell Type	Squamous Cells		P-value
	$\leq 20\%$	$> 20\%$	
	Mean \pm SD	Mean \pm SD	
Neutrophils	47 \pm 31	27 \pm 28	0.02
Eosinophils	7 \pm 11	2 \pm 3	0.03
Macrophages	37 \pm 28	41 \pm 25	0.6
Lymphocytes	5 \pm 8	6 \pm 5	0.6

whom we obtained satisfactory samples and those from whom we did not in terms of gender, age and pre-induction FEV₁ values (P = 0.6, P = 0.7 and P = 0.7, respectively).

Table 3 displays the results related to tolerability, adverse events and safety. Of the 44 patients in the sample, 34% reported some type of discomfort during induction, although all of the reported undesirable effects were considered mild. A total of 66 induction sessions were performed in the 44 study patients. We observed a $\geq 20\%$ decrease in FEV₁ in only 5 (8%) of those 66 sessions.

Table 4 shows the cellularity of the induced sputum samples, in terms of the proportion of squamous cells. We found that the numbers of neutrophils and eosinophils were significantly higher in the samples containing $\leq 20\%$ squamous cells than in those containing $> 20\%$ squamous cells.

Fig. (1) illustrates the importance of a low proportion of squamous cells in visualizing inflammatory cells in induced sputum samples.

Fig. (1). Photomicrographs of induced sputum samples obtained from pediatric patients with PSA (May-Grünwald-Giemsa staining; magnification, $\times 400$). The first sample (left) contained a large number of squamous epithelial cells, indicating a high degree of sputum contamination of in the

upper airways. The second sample (right) contained $\leq 20\%$ squamous cells, allowing the visualization of neutrophils (black arrow), eosinophils (red arrow) and a single lymphocyte (upper right).

DISCUSSION

Although we achieved an overall success rate of 75%, nine patients (27% of the total) required more than one induction session to provide a satisfactory sample. It should be borne in mind that our patients were undergoing sputum induction for the first time, and that the three patients who underwent multiple inductions ultimately did provide satisfactory samples. This is in contrast with the findings of Bossley *et al.* [7], who reported that children with moderate asthma who failed to produce satisfactory samples during the first induction session remained incapable of producing satisfactory samples during a subsequent session [7].

Unlike Lex *et al.* [10], who reported that the sputum induction success rate was significantly higher in 13-year-old patients than in 10-year-old patients, we found no such difference, which could be attributable to the age homogeneity of our sample [22]. Our finding that FEV₁ (i.e., asthma severity) had no significant effect on sputum induction success

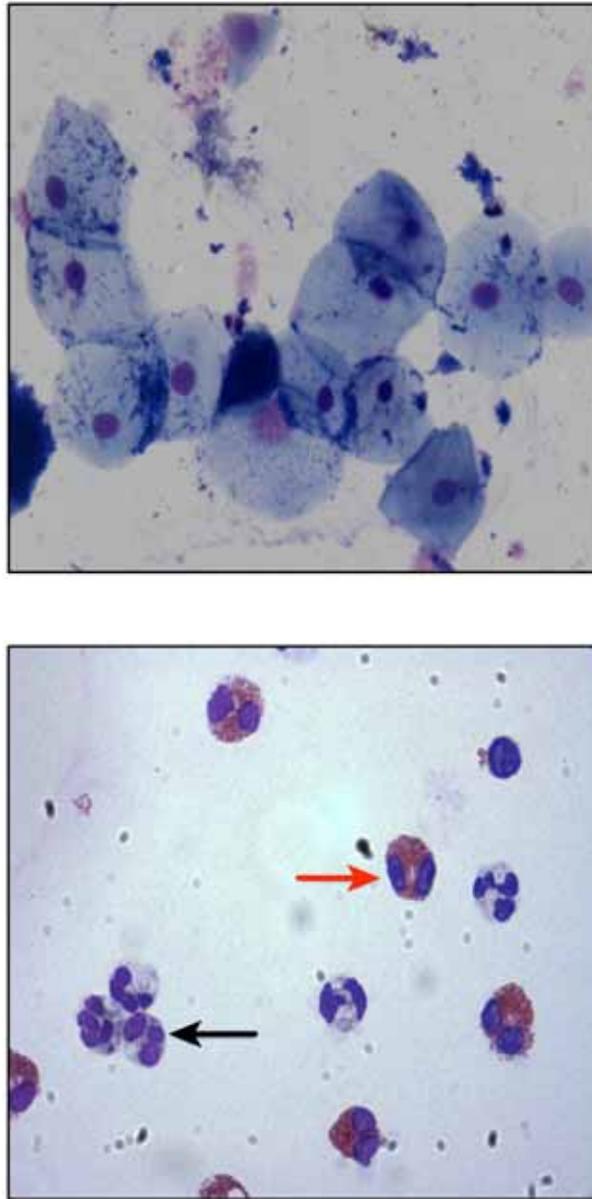


Fig. (1). Slides of induced sputum children with asthma (400x, May Grunwald Giemsa stain). The first sample (above) show a large number of squamous epithelial cells. indicating high upper airway contamination of sputum. The second slide (below) is an adequate sample collected from induced sputum, showing neutrophils (black arrow), eosinophils (red arrow) and a single lymphocyte (upper below).

rates is in agreement with those of various other studies [10, 21, 22]. The success of sputum induction might be more related to the individual characteristics of each patient than to factors associated with the induction technique [11]. In addition, the success rate might depend upon the degree of patient cooperation and understanding of the induction technique [15]. Nevertheless, our findings demonstrate that, for patients in whom the first induction fails to produce a satisfactory sample, satisfactory samples can be obtained via subsequent inductions. This has practical implications, because our results indicate that it is safe to repeat the induction procedure until it is successful. This is especially important when the goal is to study inflammatory patterns and responses to corticosteroid therapy in patients with PSA.

Squamous cell contamination increases the difficulty of visualizing other cells and can therefore impede the proper diagnosis of inflammatory patterns. Despite recommendations to classify a sample as satisfactory only if it contains $\leq 20\%$ squamous cells and has $> 50\%$ cell viability [18, 23], various authors have used squamous cell cut-off values above 20% [10, 24, 25]. In the present study, we observed statistically significant differences, in terms of the proportions of neutrophils and eosinophils, between samples containing $\leq 20\%$ squamous cells (satisfactory samples) with those containing $> 20\%$ (unsatisfactory samples). Therefore, we believe that the 20% cut-off value should be used in research and clinical practice.

It is of note that we used a low-output ultrasonic nebulizer in the present study and achieved a sputum induction success rate similar to that reported by Lex *et al.* [10], who used a high-output nebulizer in children and adolescents with difficult asthma [10]. Other studies have shown no statistically significant differences between high- and low-output nebulizers, in terms of the sputum induction success rate, although some have suggested that the high-output devices are uncomfortable for children [21]. Although there is no consensus in the literature regarding the influence of nebulizer output on sputum induction success rates [21, 24], there is general agreement that ultrasonic nebulizers are preferable [11].

In a study involving patients with asthma of different levels of severity (mild, moderate, severe and currently exacerbated), Palomino *et al.* [26] raised the point that the time required to obtain and process sputum samples (60-90 min) can limit the number of tests that can be performed in a single day [26]. Time constraints and the availability of specialized personnel are both important factors to be considered before this technique can become part of routine clinical practice [26]. In the present study, trained respiratory therapists performed five induction sessions each morning (within the space of 2 h), after which each sample was sent to the laboratory, processed and subsequently examined by a cytopathologist.

Another important variable of induction is the nebulization time. The amount of time between inductions must be kept constant, and the cumulative nebulization time should be between 15 and 20 min [11, 24, 27]. We obtained a mean nebulization time of 17.7 ± 3.4 min, which is within the range recommended in the literature [11]. The requirement of using a constant duration of nebulization has clinical applicability, as different compartments of the respiratory tract are sampled at different nebulization times. Earlier sputum samples, which exhibit a predominance of neutrophils and eosinophils, originate from the central airways, whereas samples obtained later originate from the peripheral airways and alveoli and are characterized predominantly by lymphocytes and macrophages [27].

Regarding adverse effects of sputum induction, 15 patients (34%) reported some mild, self-limiting symptoms. We did not consider cough an adverse event because coughing is necessary to produce sputum. It is difficult to compare adverse events across studies because there are no standardized methods to define such events. Some authors have reported coughing, anxiety and vomiting, whereas others have reported wheezing, sore throat and a salty taste [10, 26]. However, studies examining different clinical types of asthma have reported only mild adverse events after sputum induction [24, 25, 28-30]. In the only study involving sputum induction in children with difficult asthma, the authors reported a 26% incidence of mild events, namely sore throat, a salty taste and general physical discomfort [10]. Our finding that there was a $\geq 20\%$ decrease in FEV₁ in only 8% of the sputum induction sessions is consistent with those of that same study [10]. Despite representing only a small percentage of the total patient population, asthma patients who

exhibit a $\geq 20\%$ decrease in FEV₁ cannot be disregarded. In fact, in the present study, those patients displayed important clinical symptoms that were reversed with short-acting beta-2 agonists, and one required a period of 12 h under observation.

Our results demonstrate that, when properly performed, sputum induction is a safe, well-tolerated, non-invasive technique that facilitates the evaluation of patterns of airway inflammation.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflict of interest.

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