ORIGINAL ARTICLE

Induced Sputum in Children: Success Determinants, Safety, and Cell Profiles

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Abstract

Background: Sputum induction is a noninvasive method for the assessment of airway inflammation.
Objectives: To evaluate the safety of the procedure and the clinical predictors of successful induction, and to analyze the relationship between sputum cell counts and clinical features in asthmatic and nonasthmatic children.
Methods: We reviewed sputum inductions performed in our department between 2006 and 2008 in individuals under 18 years; 34 asthmatic and 24 nonasthmatic children were included. Sputum induction was performed with 4.5% saline for 5-minute periods with salbutamol pretreatment. The most viscid portions were selected for processing. Inductions which were tolerated for less than 4 minutes or which produced a sample volume of less than 1 mL or a sample with a squamous cell percentage of over 80% were considered unsuccessful.
Results: Sputum induction was successful in 43 (74%) of the 58 children studied. The total median induction time was 15 minutes (interquartile range, 10-15 minutes). Only 7 individuals (12%) experienced mild symptoms, which were easily reversed with salbutamol inhalation in all cases. The mean (SD) overall PEF variation with induction was -2.5% (7%), with no significant differences between asthmatics and nonasthmatics. Asthmatics had significantly higher total cell counts (*P*=.007), macrophages (*P*=.033), and relatively fewer neutrophils (*P*=.003) than nonasthmatics; metachromatic cells were rare and seen only in asthmatics (*P*=.026). We found a positive correlation between exhaled nitric oxide and sputum eosinophil count (r=0.363, *P*=.017).
Conclusions: Sputum induction is a safe, noninvasive, and feasible procedure that allows the direct assessment of airway inflammation in most children.

Key words: Asthma. Safety. Induced Sputum. Children. Airway inflammation. Exhaled nitric oxide.

Resumen

Antecedentes: La inducción de esputo es un método no invasivo para la evaluación de la inflamación de las vías respiratorias.
Objetivos: Evaluar la seguridad del procedimiento y los factores clínicos predictivos de una inducción eficaz y analizar la relación entre los recuentos celulares en el esputo y las características clínicas en niños asmáticos y no asmáticos.
Métodos: Se revisaron inducciones de esputo realizadas en nuestro departamento entre 2006 y 2008 en personas menores de 18 años; se incluyó a 34 niños asmáticos y 24 niños no asmáticos. La inducción de esputo se realizó con solución salina al 4,5% durante períodos de 5 minutos con pretratamiento con salbutamol. Se seleccionaron las porciones más viscosas para el procesamiento. Las inducciones que se toleraron durante menos de 4 minutos o que produjeron un volumen de muestra inferior a 1 ml o una muestra con un porcentaje de células escamosas de más del 80% se consideraron insatisfactorias.
Resultados: La inducción de esputo fue eficaz en 43 (74%) de los 58 niños estudiados. La mediana de tiempo de inducción total fue de 15 minutos (amplitud intercuartil, 10-15 minutos). Solo 7 pacientes (12%) experimentaron síntomas leves, que remitieron fácilmente con la inhalación de salbutamol en todos los casos. La variación global media (DE) del FEM con inducción fue del -2,5% (7%), sin diferencias significativas entre los pacientes asmáticos y los no asmáticos. Los asmáticos mostraron un recuento celular total (*p*=0,007) y de macrófagos (*p*=0,033) significativamente mayores y una cifra relativamente menor de neutrófilos (*p*=0,003) que los no asmáticos; las células metacromáticas se observaron en raras ocasiones, y solo en los pacientes asmáticos (*p*=0,026). Se halló una correlación positiva entre el óxido nítrico exhalado y el recuento de eosinófilos en el esputo (r = 0,363, *p*=0,017).
Conclusiones: La inducción de esputo es un procedimiento seguro, no invasivo y factible que permite la evaluación directa de la inflamación de las vías respiratorias en la mayoría de niños.

Palabras clave: Asma. Seguridad. Esputo inducido. Niños. Inflamación de las vías respiratorias. Óxido nítrico exhalado.

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Introduction

Asthma is a common chronic inflammatory airway disease characterized by recurring symptoms, variable airflow obstruction, and bronchial hyperresponsiveness [1]. It is one of the most common chronic diseases of childhood.

Inflammation plays a critical role in asthma pathogenesis and increasing evidence suggests considerable variability in the pattern of inflammation that may influence treatment responses [1].

The induction of sputum is a safe, easily performed, well-tolerated and noninvasive method for the investigation of inflammatory airway diseases. It allows clinicians to characterize the inflammatory profiles of a variety of airway diseases including asthma, chronic obstructive pulmonary disease, and chronic cough. Its use for diagnostic or scientific purposes has increased dramatically in recent years, and it has replaced the invasive technique of bronchoalveolar lavage in the monitoring of changes in airway inflammation [2,3].

The identification of sputum eosinophilia currently has the greatest clinical value as it predicts a favorable response to corticosteroids and can therefore guide treatment. In asthma management, protocols aimed at normalizing sputum eosinophil count have markedly reduced exacerbations without an overall increase in therapy [4].

However, only a few studies have evaluated the value of induced sputum analysis in children with asthma. Previous studies have shown a significant increase in sputum eosinophils in children with asthma compared to healthy children [5-13]. Airway inflammation is also a feature of asthma exacerbations in children, where an eosinophilic or a mixed eosinophilic/neutrophilic pattern might be found, depending on the severity and cause of the exacerbation [14,15]. Eosinophilic inflammation in induced sputum can also predict exacerbations induced by corticosteroid reduction [16]. These studies establish that airway inflammation is associated with current asthma both in the stable state and during an exacerbation, and that sputum induction is a valuable, noninvasive method for its assessment.

One study comparing airway inflammation between childhood and adult-onset asthma showed that sputum inflammatory cell counts, T-lymphocyte subpopulations, and inflammatory cytokines did not differ between adults with adult-onset asthma and children with asthma, suggesting that the underlying airway inflammatory mechanisms are similar and that the immunopathogenic pathways might begin and continue from childhood into adulthood [17].

In this study, we aimed to evaluate the safety of sputum induction in children, to assess the clinical predictors of successful induction, and to analyze the relationship between sputum cell counts and the clinical features of asthmatic and nonasthmatic children.

Materials and Methods

Patients

From 2006 to 2008 more than 100 individuals underwent sputum induction for the investigation of their pathology or as a part of the protocol of 2 clinical studies [18,19] at our outpatient allergy clinic. They were required to have a baseline forced expiratory volume in the first second (FEV_1) of above 70% and to be clinically stable. For this study, we selected all sputum inductions performed in pediatric patients (aged <18 years). If more than 1 induction was performed, only the first attempt was considered to prevent possible bias from learning effects.

All procedures and the 2 clinical studies [18,19] were approved by the local ethics committee, with informed written consent from patients/parents.

The participants were considered to be asthmatic if they had a previous medical diagnosis of asthma, if they were under current treatment with inhaled corticosteroids (ICSs), or if they had a positive bronchodilation test (FEV_1 of >20% and 200 mL) in the previous year.

Study Measurements

Atopy was defined by a positive skin-prick test result (wheal of 3 mm in diameter when the control solutions produced the expected results) to at least 1 aeroallergen (house dust mites, pollen, animal dander and molds supplied by Alk-Abelló, Madrid, Spain) or specific immunoglobulin E levels of over 0.35 kU/L measured by fluorometric enzyme immunoassay (Phadia, Uppsala, Sweden) to 1 or more aeroallergens.

Spirometry using a calibrated computerized pneumotachograph spirometer (SensorMedics Vmax 22; SensorMedics, Yorba Linda, California, USA) and exhaled nitric oxide (eNO) measurements using a chemiluminescence analyzer with an exhalatory flow rate of 50 mL/s (NIOX; Aerocrine, Stockholm, Sweden) were performed in accordance with the recommendations of the American Thoracic Society [20,21].

Sputum Induction and Processing

Sputum induction was performed using an inhalation of hypertonic saline (4.5%) through a mouthpiece connected to an ultrasonic nebulizer (OMRON NE-U17; Omron Healthcare Europe, Netherlands) with maximum output settings. Sputum induction and processing were performed according to the European Respiratory Society task force recommendations [22,23] using 4.5% saline for periods of 5 minutes. Baseline and post 200 μg of salbutamol peak expiratory flow (PEF) were registered using a Mini-Wright Peak-Flow Meter (Clement-Clarke International, Harlow, Essex, UK). Induction was stopped when the child had produced an adequate sample of sputum, when 20 minutes of inhalation were completed, or when PEF dropped below 80% of the baseline value. After induction, all sputum macroscopically free of salivary contamination was selected for processing.

The procedure was classified as unsuccessful in the following cases: when sputum induction was tolerated for less than 4 minutes, when the volume of the sample was less than 1 mL, or when the percentage of squamous cells exceeded 80% as this would have prevented the performance of differential cell counts of intact bronchial epithelial cells and leukocytes up to a total of 500 nonsquamous cells [24].

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Statistical Analysis

Categorical variables were summarized as percentages and continous variables as means and SDs for normal distributions and medians (interquartile range [IQR]) for nonnormal distributions. The one-sample Kolmogorov test was used to test for normal distribution. Proportions were compared using the χ^2 test with Yates correction. The *t* test and analysis of variance or the Wilcoxon and Kruskal-Wallis tests were used for comparing variables between groups, according to whether or not their distribution was normal. The correlation between variables was assessed using Spearman rank correlation coefficients. Statistical significance was set as $P<.05$, and statistical analyses were performed using the statistical package SPSS version 16.0 (Chicago, Illinois, USA).

Results

A total of 58 individuals were analyzed (mean [SD] age, 13 [3] years, 64% boys). Thirty-four (59%) were asthmatics; 25 of these were under treatment with low-dose ICSs (<400 mcg budesonide or equivalent) and 5 were under treatment with medium-dose ICSs (400-800 mcg budesonide). Compared to nonasthmatics, asthmatics had significantly more medical diagnoses of rhinitis, a higher frequency of atopy, and higher eNO values (Table 1). All the nonasthmatic individuals and 3 of the asthmatic children were competitive swimmers.

Safety and Feasibility of Sputum Induction

Sputum induction was successful in 43 (74%) of the 58 individuals. Of the 15 in whom induction was unsuccessful, 9 (60%) produced a sputum sample with a percentage of squamous cells of over 80%, 4 (27%) were unable to produce any sputum or produced a sample volume of less than 1 mL after 20 minutes of induction, and 2 (13%) had to stop induction before an adequate sample was obtained due to adverse effects (1 experienced chest tightness and a decrease in PEF of over 20% and 1 had chest tightness only). The total median induction time was 15 (IQR, 10-15) minutes (Table 2).

PEF variation with induction was –2.5% (7.0%) and significantly higher in asthmatic children (Table 2). Only 7 individuals (12%), all asthmatics, experienced adverse effects: 4 experienced chest tightness, 1, bronchospasm with a decrease in PEF of over 20%, 1, distressing cough, and 1, chest tightness and bronchospasm. The adverse effects were responsible for the interruption of the procedure before a sufficiently large sputum sample was obtained in just 2 individuals. There were significantly more asthmatics than nonasthmatics among those with unsuccessful induction (87% vs 49%, $P=.014$) but no significant differences were found for age, sex, atopy, eNO levels, induction time, variation in PEF with salbutamol and after induction, or adverse effects between those with successful and unsuccessful (n=15) induction.

Sputum Analysis and Asthma Diagnosis

Of the 43 children with successful sputum induction, those with asthma (n=21) had a significantly higher frequency of atopy (100% vs 18%; $P<.01$), higher eNO levels ($P=.029$), greater PEF variation with salbutamol inhalation ($P=.03$), and a more pronounced decrease in PEF with hypertonic sputum induction ($P<.01$) than nonasthmatics (Table 3).

Overall, sputum cell viability was 57.7% (14.7%). Table 3 shows the induced sputum results for asthmatics and nonasthmatics. The former had significantly higher total cell counts ($P=.007$). With respect to differential counts (% of nonsquamous cells) asthmatic children also had more macrophages ($P=.033$) and relatively fewer neutrophils ($P=.003$) than nonasthmatic children; metachromatic cells were rare and only seen in asthmatics ($P=.026$). Regarding the absolute number of inflammatory cells in the sputum samples, asthmatic children had a significantly higher number of eosinophils (15.6 [IQR, 1.8-134.1] $\times 10^3$ mL^{-1}) than nonasthmatics (3.2 [IQR, 0.0-84.0] x10^3 mL^{-1}) ($P=.008$), but no significant differences were noted in the absolute number of neutrophils (108.8 [IQR, 59.2-298.6] $\times 10^3$ mL^{-1} vs 142.9 [IQR, 50.5-296.2] ×103 mL^{-1}) ($P=.706$).

We found a positive correlation between eNO and both relative sputum eosinophil count (r=0.363, $P=.017$) and absolute sputum eosinophil (r=0.341, $P=.025$). A negative correlation was found between eNO and absolute sputum neutrophil count (r=–0.418, $P=.005$).

Table 1. Characteristics of Asthmatic and Nonasthmatic Children Included in the Study

	Asthmatics (n=34)	Nonasthmatics (n=24)	Total (n=58)	*P*
Age, mean (SD), y	12.8 (3.3)	12.7 (2.6)	12.8 (3.0)	.891
Male/female, No.	21/13	16/8	37/21	.786
Rhinitis, No. (%)	34 (100)	11 (46)	34 (59)	<.01
Atopy, No. (%)	34 (100)	4 (17)	38 (66)	<.01
eNO, median (IQR), ppb	37.5 (21.1-59.3)	21.1 (16.3-33.8)	27.0 (17.0-50.7)	.038

Abbreviations: eNO, exhaled nitric oxide; IQR, interquartile range; ppb, parts per billion.

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Table 2. Characteristics of Population and Safety Parameters According to Induced Sputum Success

	Successful Induction (n=43)	Unsuccessful Induction (n=15)	Total (n=58)	*P*
Age, mean (SD), y	13.0 (2.9)	12.2 (3.3)	13.0 (3.0)	.385
Male/female, No.	28/15	9/6	37/21	.762
Asthma, No. (%)	21 (49)	13 (87)	34 (59)	.014
Atopy, No. (%)	25 (58)	13 (87)	38 (66)	.061
eNO, median (IQR), ppb	27.0 (17.0-48.1)	41.3 (18.8-77.6)	27.0 (17.0-50.7)	.38
% Δ PEF BD,[a] mean (SD)	6.1 (3.9)	5.8 (6.4)	6.0 (4.6)	.16
% Δ PEF induction,[b] mean (SD)	–1.5 (6.5)	–5.2 (7.6)	–2.5 (7.0)	.843
Induction time, min	15 (10-15)	15 (10-20)	15 (10-15)	.24
Adverse effects,[c] No. (%)	4 (10)	3(25)	7 (12)	.175
PEF decrease >20%, No. (%)	0 (0)	2 (14)	2 (4)	NA
Chest tightness, No. (%)	3 (7)	2 (14)	5 (9)	NA
Distressing cough, No. (%)	1 (2)	1 (0)	1 (2)	NA

Abbreviations: eNO, exhaled nitric oxide; NA, not applicable; PEF, peak expiratory flow; ppb, parts per billion.
[a]% variation in PEF after salbutamol inhalation.
[b]% variation in PEF after sputum induction with 4.5% saline.
[c]1 patient had a PEF decrease >20% and chest tightness.

Table 3. Characterization of Children With Successful Sputum Induction According to Asthma Diagnosis[a]

	Asthmatics (n=21)	Nonasthmatics (n=22)	Total (n=43)	*P*
Age, mean (SD), y	13.3 (3.2)	12.7 (2.7)	13.0 (2.9)	.518
Male/female, No.	13/8	15/7	28/15	.755
Atopy, No. (%)	21 (100)	4 (18)	25 (58)	<.01
eNO, ppb	37.5 (20.0-57.7)	21.0 (15.0-33.3)	27.0 (17.0-48.1)	.029
% Δ PEF BD,[b] mean (SD)	7.41 (3.60)	4.82 (3.89)	6.06 (3.93)	.030
% Δ PEF induction,[c] mean (SD)	-6.11 (4.97)	2.64 (4.76)	-1.53 (6.53)	<.01
Total cell count, No. x10^6/mL	1.00 (0.40-1.85)	0.34 (0.18-0.62)	0.50 (0.28-1.50)	.007
% cell viability, mean (SD)	60.5 (16)	54.9 (13)	57.68 (14.7)	.210
% neutrophils	15.5 (7.1-28.9)	41.9 (25.0-62.0)	26.2 (13.6-54.0)	.003
% eosinophils	2.0 (0.4-11.8)	0.7 (0-1.4)	0.8 (0.2-5.4)	.073
% lymphocytes	3.9 (1.7-6.0)	3.0 (2.3-5.3)	3.6 (2.2-5.4)	.584
% macrophages	50.8 (29.5-62.4)	29.0 (19.8-42.6)	36.0 (24.0-52.7)	.033
% metachromatic cells	0 (0-0.75)	0 (0-0)	0 (0 -0)	.016
% bronchial epithelial cells	17.8 (11.5-21.9)	11.8 (3.6-21.9)	15.8 (7.6-21.8)	.215

[a]Data are expressed as medians (interquartile range) unless otherwise indicated.
[b]% variation in PEF after salbutamol inhalation.
[c]% variation in PEF after sputum induction with 4.5% saline.

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Discussion

Sputum induction with 4.5% saline was successfully performed, with differential cell counts, in 43 (76%) of the 58 children studied. Our results are in accordance with previous studies, which have reported success rates ranging from 56% to 100% in children [9,25]. Asthma was the only clinical condition associated with less successful sputum induction (62% success rate in asthmatics vs 92% in nonasthmatics). Wilson et al [9] reported no difference in the proportion of asthmatic and nonasthmatic children able to produce a valid induced sputum sample. The asthmatics included in our study were clinically stable and under ICS treatment in most cases; it has been shown that sputum induction success might decrease after ICS treatment, with the proportion of children successively completing induction dropping from 82% at baseline to 68% after 6 months of treatment [12].

Sputum induction was safe, with only 7 children (12%) presenting mild symptoms during induction; these symptoms were easily reversed with salbutamol inhalation. Moreover, the overall PEF variation with induction was –2.5% (7%), with no significant differences between asthmatic and nonasthmatic patients. Our safety data are in agreement with previous studies that have shown that sputum induction is safe, even in children with difficult asthma [8,13, 26,27], and also associated with mild adverse effects in a minority of individuals. We used salbutamol pretreatment before induction. It has been shown that bronchodilator inhalation before sputum induction results in higher success rates and fewer adverse effects in children [26]. Moreover, Cianchett et al [28] have shown that pretreatment with salbutamol does not significantly affect inflammatory cells counts or levels of soluble mediators in induced sputum.

The patients with asthma in our study had significantly higher total cell counts and relative numbers of macrophages and metachromatic cells and fewer neutrophils (%) than healthy individuals. Our differential cell count results are in agreement with previous published reports [6,8,12]. Our sample had a high percentage of bronchial epithelial cells (15.8% [IQR, 7.6%-21.8%]), but similar results have been reported in asthmatic children [6].

The asthmatic children in our study had a significantly higher absolute number of eosinophils (15.6×10^3 mL^{-1}) than the nonasthmatic children (3.2×10^3 mL^{-1}) but no significant differences were found for absolute neutrophil count. However, it should be noted that all the nonasthmatic children were competitive swimmers. We have previously reported that competitive asthmatic swimmers had a higher proportion of neutrophils than nonswimmer asthmatics and a higher percentage of lymphocytes than healthy swimmers in induced sputum [19].

We found a significant correlation between eNO and both relative and absolute sputum eosinophil counts. Some authors have reported similar results [29,30] while others [27] have not detected this correlation. These conflicting results might be due to population differences. Further studies and data synthesis are needed in this area.

Despite the relative safety and tolerability of sputum induction in our population, our study has several potential limitations. First, it is a review series. While this might not influence safety and success rate analyses, it should be taken into consideration when interpreting the sputum cell counts as the asthmatic and nonasthmatic groups were heterogeneous. Consequently, the characteristics of the population studied may also limit the generalizability of the results. Second, although we obtained a good success rate, it should be noted that sputum induction requires a substantial amount of time to perform and process. Because considerable technical support and expertise are also required to process, stain, and interpret the samples, the procedure is only of value in specialized centers. The use of fluorocytometric analysis of induced sputum cells might shorten the process and provide complementary information on activated cells in the bronchial mucosa [31]. Third, although sputum induction is a safe method in children, 12% of our series developed mild symptoms. Further research into analyses of induced sputum cells, cytokines, and mediators may provide information on cellular and molecular mechanisms that may account for suboptimal responses to asthma treatment.

In conclusion, sputum induction is a relatively safe, noninvasive procedure in children that allows a direct assessment of airway inflammation.

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